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Review

Direct oral anticoagulants (DOACs): From the laboratory point of view

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ABSTRACT

Direct oral anticoagulants (DOACs) represent a new generation of drugs that have been increasingly used in the prevention and treatment of thromboembolic states. According to the mechanism of anticoagulant action, DOACs are divided into two groups: direct inhibitors of thrombin (dabigatran) and direct inhibitors of activated factor X (FXa) (rivaroxaban, apixaban, edoxaban, betrixaban). Compared to the vitamin K antagonists, DOACs are superior in terms of onset of action, pharmacokinetic and pharmacodynamics properties and fixed daily dose without the need for routine coagulation monitoring. Despite these advantages, there are clinical conditions in which laboratory measurement of DOACs should be performed. Although DOACs have an impact on screening haemostasis assays (prothrombin time, PT; activated partial thromboplastin time, aPTT; and thrombin time, TT), these tests are not appropriate for quantifying drug levels. Therefore, specific quantitative methods (LC-MS/MS as a gold standard method for all DOACs, coagulometric and chromogenic assays for dabigatran, and chromogenic anti-Xa assays with drug-specific calibrators for inhibitors of FXa) should only be used for determination of DOACs concentration. The aim of this review is to present all aspects of laboratory assessment of DOACs, including pre-analytical, analytical and post-analytical factors in the overall testing process with a special accent on the available specific quantitative methods for measurement of DOACs in circulation.

Keywords: direct oral anticoagulants (DOACs), anticoagulation, laboratory monitoring

INTRODUCTION

Direct oral anticoagulants (DOACs) represent a new generation of drugs that have been increasingly prescribed in the prevention and treatment of thromboembolic diseases in the last decade (1). According to the mechanism of anticoagulant action, DOACs are divided into

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two groups: direct thrombin inhibitor (DTI) or dabigatran (Pradaxa®, Boehringer Ingelheim, Germany) and direct inhibitors of activated factor X (FXa) that include rivaroxaban (Xarelto®, Bayer AG, Germany, and Janssen Pharmaceuticals, Belgium), apixaban (Eliquis®, Bristol-Myers Squibb, Italy, and Pfizer, USA), edoxaban (Lixiana®, Daiichi-Sankyo, Japan and Savaysa, USA) and betrixaban (Bevyxxa®, Portola Pharmaceuticals, USA) (2–4). All DOACs (except betrixaban) are licensed for the prevention and treatment of venous thromboembolism (VTE) and for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation (NVAF). The introduction of a particular DOAC drug into clinical practice for certain clinical indications was based on extensive clinical studies in which the efficacy and safety of their use were compared with the existing anticoagulant drugs, such as vitamin K antagonists (VKAs) and heparins (5–11). Betrixaban [*N*-(5-chloropyridin-2-yl)-2-(4-(*N*,*N*-dimethylcarbamimidoyl)benzamido)-5-methoxybenzamide, $C_{27}H_{26}ClN_5O_7$] is the only direct FXa inhibitor drug between DOACs approved by Food and Drug Administration (FDA) in 2017 (3, 12), with limited data on the effect on laboratory assays published so far. So, this review will primarily address the laboratory assessment of dabigatran, rivaroxaban, apixaban and edoxaban.

In comparison with VKAs, DOACs have demonstrated superiority in terms of faster onset and offset of action, fixed daily doses and lesser food and drug interactions (13, 14). These anticoagulant drugs inhibit both free and bound activated serine proteases, which is clinically important since both thrombin and FXa retain activity.

Due to these advantages, it was generally considered that treatment with DOACs does not require routine laboratory monitoring (14). On the other hand, there have been numerous debates about DOAC monitoring for more than a decade. Despite the benefits of DOACs, there are certain clinical conditions and specific populations in which laboratory measurement of DOACs in plasma should be performed either to assess the degree of anticoagulation or to exclude clinically relevant drug concentrations in terms to aid clinical decision-making. Thus, the results of clinical experiences dispute the fact that the treatment with DOACs completely excludes the need for laboratory diagnostics (15). Therefore, as the use of DOACs represents a new era of anticoagulant therapy in routine clinical practice, it is obvious that laboratories have a significant role in the management of patients on such treatment (12, 16, 17).

Since DOACs affect the coagulation cascade by diminishing fibrin formation, these drugs subsequently have an effect on the results of both screening and specialized haemostasis assays. The test results of individual screening assays are differently affected depending on the drug group. So prothrombin time (PT) test is more affected by the inhibitors of FXa whereas dabigatran as a direct thrombin inhibitor has a greater impact on the results of thrombin time (TT) and activated partial thromboplastin time (aPTT) tests. Further, the effect of DOACs on these screening coagulation assays depends also on the sensitivity of different commercial reagents for the same test (18). Therefore, screening coagulation plasma assays should not be considered appropriate either for quantifying drug levels or for reliable assessment of anticoagulant effect. On the other hand, knowing the impact of DOACs on the results of individual screening coagulation assay is a precondition for the correct interpretation of the test results.

In order to help in making clinical decisions in certain conditions that require quantitative determination of drug concentration, the introduction of DOACs into clinical practice has resulted in the development of specific coagulation methods for their quantitative measurement in plasma, such as coagulometric and chromogenic assays for dabigatran,

and chromogenic anti-Xa assays with drug-specific calibrators for direct FXa inhibitors. These quantitative methods should be used in all clinical conditions intended for the determination of DOACs concentration. Although not yet available in all coagulation laboratories for routine application, these quantitative assays may provide an accurate assessment of DOACs.

This review provides an overview of laboratory measurement of DOACs and the specific quantitative methods that should be used in all clinical conditions intended to determine the concentration of DOACs in circulation.

Direct thrombin inhibitor (DTI)

Dabigatran etexilate is an oral prodrug that is metabolized by esterases in the liver, gut and plasma into the active form dabigatran {ethyl 3-[[2-[[4-[(Z)-*N*′-hexoxycarbonylcarbamimidoyl]anilino]methyl]-1-methylbenzimidazole-5-carbonyl]-pyridin-2-ylamino] propanoate methanesulfonic acid, $C_{35}H_{45}N_7O_8S$} which acts as competitive and irreversible DTI of both free and fibrin-bound thrombin by binding to its active site (19). Firstly, it was licensed for thromboprophylaxis after orthopaedic surgery in 2008, then in 2011 for use in atrial fibrillation and finally in 2014 for deep vein thrombosis (DVT) and uncomplicated pulmonary embolism (PE), afterwards preventing stroke and systemic embolism, also in adults who have non-valvular atrial fibrillation and are considered to be at risk of stroke, and treating blood clots in veins and preventing them from occurring again (20). Dabigatran became the first oral blood thinner approved by the FDA for children in 2021 (21). The prescribed dose depends on the indication, patient's age, body mass index (BMI) and renal function (20). Dabigatran has low bioavailability (3–7 %) and is largely eliminated by the kidneys (80 %). Thus, the patient's renal function is of crucial importance for correct dosing and achieving anticoagulant effects without undesirable side effects (12). Peak plasma concentration is reached 1.5 to 3 hours after dose application, with a half-life in the circulation of 12 to 14 hours in patients with normal renal function. Reduction of renal function can affect plasma concentrations and creatinine clearance (CrCl) should be checked in all patients before starting treatment with dabigatran (22). Patients with CrCl 15–30 mL min^{-1} per 1.73 m^2 should be treated with reduced doses of dabigatran, whereas in patients with CrCl 30–50 mL min^{-1} per 1.73 m^2 routine monitoring of renal function should be performed (21). In general, DOACs should not be used in patients with severe renal impairment (CrCl < 15 mL min^{-1} per 1.73 m^2) and those on dialysis.

High body mass (> 120 kg) was associated with an increased risk of gastrointestinal bleeding but no differences in stroke, mortality or clinically relevant bleeding were found compared to subjects with body mass < 120 kg suggesting that dabigatran could be used in overweight patients (> 120 kg) with NVAF (23).

Further, it is important to note that dabigatran as a P-glycoprotein (P-gp) substrate implies drug interactions with certain P-gp inducers and inhibitors (20). Pharmacogenetics and drug-drug interactions could alter dabigatran concentrations in plasma, so, a better understanding of genetic background could contribute to personalized patient response to dabigatran (13).

Direct factor Xa inhibitors

Rivaroxaban [5-chloro-*N*-(((5S)-2-oxo-3-(4-(3-oxomorpholin-4-yl)phenyl)-1,3-oxazolidin-5-yl)methyl)thiophene-2-carboxamide, $C_{19}H_{18}ClN_3O_5S$] is an oral direct anti-FXa in-

hibitor (24). It is a highly selective and competitive inhibitor of both free FXa and FXa bound to the prothrombinase complex, thus, it is valuable in preventing thrombus extension. In 2008, European Medicine Agency (EMA) approved the use of rivaroxaban for thromboprophylaxis after orthopedic surgery. Three years later, rivaroxaban was approved for thromboprophylaxis of non-valvular atrial fibrillation and treatment of deep vein thrombosis (DVT), after that, in 2012, for treatment of pulmonary embolism (PE). The approval follows for thromboprophylaxis in acute coronary syndrome (ACS) in 2013 and for treatment and prevention of VTE recurrence in children in 2020. Regarding the pharmacokinetic properties of rivaroxaban, the drug is rapidly absorbed and has a high bioavailability.

However, bioavailability is related to the nourishment status of the patient, being high (80–100 %) in well-nourished people, due to the fact that high fat, high caloric food enhances absorption of rivaroxaban. Rivaroxaban binds to plasma proteins, mostly to albumin (92–95 %) (25). Due to hepatic metabolism, it should not be used in patients with hepatic disease associated with coagulopathy. Regarding drug interactions, rivaroxaban is a substrate of the P-gp transporter and is metabolized by the cytochrome P450 3A4 isoenzyme (CYP3A4) (13). Therefore, concomitant use of drugs which inhibit P-gp and/or CYP3A4 can significantly increase the concentration of rivaroxaban in plasma.

Apixaban [1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5-dihydropyrazolo[3,4-c]pyridine-3-carboxamide, $C_{25}H_{25}N_5O_4$] is another oral, direct, reversible and highly selective anti-FXa inhibitor of both free FXa and FXa bound in the prothrombinase complex and FXa bound to platelets (2, 26). Although it has no direct effects on platelet aggregation, indirectly it inhibits platelet aggregation induced by thrombin. It was approved by EMA for thromboprophylaxis in patients with hip or knee replacement in 2011, for thromboprophylaxis of atrial fibrillation (AF) in 2012 and for treatment of DVT and PE in 2014. Its half-life in circulation is approximately 12 hours and absorption is about 50 %. Extreme body mass can cause variability in the bioavailability of apixaban.

Approximately 60 % of its metabolism is through the faecal route, while only 25–29 % is eliminated *via* renal excretion (27). Apixaban is also a substrate for P-gp and CYP3A4 enzymes, so concomitant treatment with drugs metabolized by these enzymes can require dose adjustment (28). ABCB1 gene locus and its SNPs are implicated in altering plasma levels of apixaban.

Edoxaban [*N*1-(5-chloropyridin-2-yl)-*N*2-((1S,2R,4S)-4-(dimethylcarbamoyl)-2-(5--methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamido)cyclohexyl)oxalamide, $C_{24}H_{30}ClN_7O_4S$] is the direct and reversible FXa inhibitor (29). It is highly selective and inhibits free FXa, and that in the prothrombinase complex, thus reducing thrombin generation and decreasing the risk of thrombus formation. In 2011 edoxaban was approved for VTE prevention after hip or knee replacement surgery and hip fracture surgery in Japan, and in 2014 for the prevention of stroke and systemic embolism in patients with NVAF and for the treatment and prevention of recurrent VTE. FDA approved edoxaban in 2015 for the treatment of DVT and PE and reduction of the risks of stroke and systemic embolism in patients with NVAF. Edoxaban is rapidly absorbed reaching a peak concentration in circulation within 1 to 2 hours after oral administration. It has a half-life of 10 to 14 hours with a bioavailability of 62 %. It is renally excreted and its absorption is unaffected by food. Edoxaban is a substrate for P-gp transporter whereas a low fraction of this drug (< 10 %) is metabolized by CYP3A4 isoenzyme (30).

Clinical indications for assessment of DOACs

Although it is generally accepted that no routine laboratory monitoring is needed in patients treated with DOACs, cumulative evidence of case reports from patients with major bleeding and regulatory documents associated with supra-therapeutic levels of anticoagulants and bleeding are present. It was shown that there are special clinical conditions in which measuring the levels of DOACs in circulation is required (12).

The main conditions intended for determination of DOACs concentration in circulation may include the following clinical scenarios: before elective or urgent surgery or invasive procedures, in the case of adverse events (bleeding or thrombosis) during therapy, when making a decision on thrombolytic therapy in stroke patients, if suspicion of overdose or drug accumulation, when the need for the reversal of anticoagulation, when severe impaired renal function, if patients are taking other drugs known to affect the pharmacokinetics of DOACs, when extreme of body mass (< 50 kg and > 110 kg), in children (31–33). Further, despite the beneficial attributes of DOACs, monitoring the bleeding risk often represents a concern for clinicians prescribing DOACs, particularly in older patients (> 80 years old) and those with co-morbidities or taking other medications that may increase the risk of bleeding (34).

Since DOAC accumulation increases the risk of bleeding, measurement of its concentration may be useful to ensure an optimal treatment response without concomitant side effects. Further, measurement of DOAC concentration can be beneficial in patients experiencing a haemorrhagic or thromboembolic event during treatment with DOAC, in a patient with a suspected overdose, or in children. In emergent clinical situations, such as bleeding, thrombosis, urgent surgery and thrombolysis, laboratory assessment of DOACs is aimed to identify concentrations within or above the expected therapeutic range.

Management of urgent invasive procedures or surgery requires an assessment of the patient's haemostatic status in order to decide about the potential use of agents acting as a reversal of anticoagulation. The Subcommittee on Control of Anticoagulation of the International Society on Thrombosis and Haemostasis recommended that in patients with serious bleeding, a drug concentration > 50 ng mL^{-1} is likely to be sufficiently high to warrant administration of an antidote (35). In patients requiring an urgent surgical intervention that is associated with a high risk of bleeding, antidote administration should be considered if the drug concentration exceeds 30 ng $mL^{-1.}$

In patients with acute ischemic stroke requiring thrombolysis (36), the plasma concentration of 10 ng mL^{-1} for apixaban, 50 ng mL^{-1} for dabigatran or 100 ng mL^{-1} for rivaroxaban, has been proposed as a cut-off value for considering of thrombolytic therapy with a recombinant tissue plasminogen activator (37, 38). DOACs concentrations above specified cut-offs preclude the possibility of thrombolysis.

For accurate measuring DOACs concentrations in a perioperative setting, specific quantitative assays are required.

Peri-procedural management

In elective peri-operative settings, it is of importance to know if a DOAC is still present in the blood. Interruption of DOACs should not be based on their half-lives only, but also on the residual drug concentration. For the majority of patients undergoing elective

procedures, routine laboratory assessment of DOACs is not required if guidelines related to the time of DOACs interruption are respected (32, 38). However, in some clinical situations or patients, such as moderate renal impairment in dabigatran-treated patients, or the use of antiarrhythmic in anti-FXa-treated patients, could be associated with delayed DOACs elimination. Direct oral anticoagulants are interrupted 1 day before low bleed risk procedures and 2 days before high bleed risk procedures. Especially in patients with renal dysfunction, treated with dabigatran, drug concentration mostly depends on renal elimination. Longer interruption intervals may be needed (39). If possible, dabigatran should be discontinued 2–5 days before invasive or surgical procedures due to increased risk of bleeding (longer times should be considered for patients undergoing major surgery). Dabigatran should be restarted promptly after surgery.

Therefore, measurement of DOACs concentration in these and some other special clinical situations may help to guide the timing of invasive procedures (40–42). Further, interruption of the DOACs depends on the bleeding risk associated with the procedure and the thrombotic risk of the individual. If a procedure is associated with a minor risk of bleeding, only a short period of discontinuation is necessary. However, in any procedure associated with a major risk of bleeding or those patients with a high risk of thrombosis (who had a pulmonary embolism the month before the procedure), the DOACs should be discontinued several days before and may even require bridging with an alternate anticoagulant such as low molecular mass heparin.

Restarting the DOACs after the surgery or invasive procedure depends on the risk of bleeding and should be delayed until any sign of bleeding is present. If the bleeding risk is minimal, DOACs may be restarted 6 to 8 hours after surgery or procedure (38). However, if the bleeding risk is high, 2–3 days after surgery are recommended before restarting DOACs therapy.

Reversal of DOACs

In parallel with the introduction of DOACs in clinical practice, research has also focused on finding cures which act as specific antidotes for a particular group of these drugs, and whose application allows the fastest possible and most effective reversal of the anticoagulant effect of the drug in vitally endangered patients with bleeding (12, 43). For dabigatran, the specific antidote idarucizumab (Praxbind) (44) is available: a fragment of a human monoclonal antibody, that specifically binds with high affinity to dabigatran and its metabolites and thus neutralizes the anticoagulant effect of the drug. For drugs from the group of FXa inhibitors, a specific antidote, andexanet alpha, a recombinant human analogue of FXa, has been approved.

DIRECT ORAL ANTICOAGULANTS AND LABORATORY TESTING

Impact of DOACs on screening coagulation assays

DOACs are drugs that specifically affect a special segment of the coagulation cascade thus subsequently diminishing fibrin formation. Therefore, these drugs affect the results of most clot-based haemostasis assays including prothrombin time, activated partial thromboplastin time and thrombin time (2). In general, the effect of DOACs on the results

of individual screening coagulation assay depends on several key factors. At first, it is caused by their mechanism of action. Such, dabigatran as a direct thrombin inhibitor affects TT and aPTT, while it has a lesser or minor effect on PT results (45, 46). In contrast, DOACs acting as inhibitors of FXa have a more pronounced effect on the PT test, and minor or even no effect on the aPTT test, while the TT test is not affected at all. Mechanism of DOAC action, the impact of DOACs on screening coagulation assays and quantitative tests for DOACs are described in Fig. 1. As previously described, according to the mechanism of anticoagulant action, DOACs are divided into two groups: direct inhibitor of thrombin (dabigatran) and direct inhibitors of activated factor X (FXa) (rivaroxaban, apixaban, edoxaban, betrixaban). This picture provides a brief view of DOAC laboratory monitoring and specific quantitative methods which should be used in all clinical conditions intended for the determination of DOACs concentration.

Secondly, the result on the impact of individual DOAC drugs also depends on the reagent used, meaning that different commercial reagents exert variable sensitivity for the same screening assay and the impact of each DOAC. Further, due to their short half-lives in circulation (approximately 12–15 hours), DOACs show differently depending on the time of sampling after the last drug intake (peak *vs.* trough drug concentration). Rivaroxaban among all DOACs has the greatest effect on PT, only for certain sensitive PT reagents, which may not be as widely used as other, less sensitive PT reagents.

Regarding the international normalized ratio (INR), it was specifically developed for monitoring of VKA therapy and it is not an appropriate measurement unit for DOACs. The INR is suboptimal in assessing DOACs, having high reagent dependence and low sensitivity and specificity. It may provide information if laboratories recognize their limitations.

Therefore, the INR/ISI methodology used for VKAs is not suitable for the measurement of DOACs (15). INR reflects a mathematical calculation using a PT ratio as further adjusted with a correction factor called the international sensitivity index (ISI). As INR and

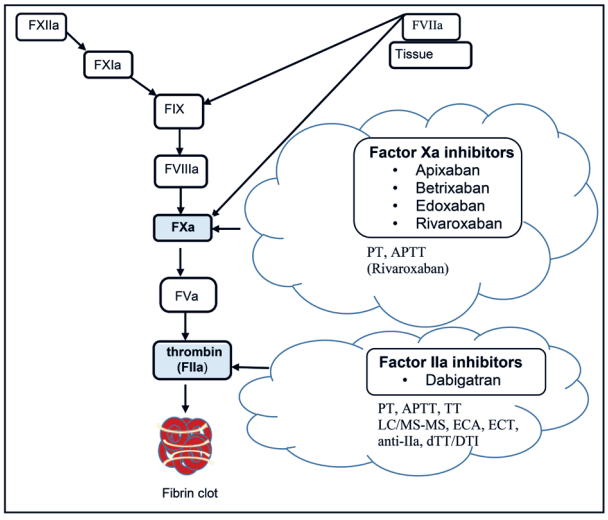

Fig. 1. Metabolism of DOAC drugs and laboratory monitoring.

ISI are based on VKA sensitivity, the PT should not be expressed as INR in patients treated with DOACs (47). Although efforts to standardize PT methods by creating an ISI for rivaroxaban, analogous to the ISI for VKAs (48) have been published, this practice has not been widely embraced and has not been demonstrated to be applicable to apixaban or edoxaban PT measurements.

For dabigatran, PT is not sufficiently affected, even in conjunction with aPTT for ruling out the presence of clinically relevant levels. A normal aPTT excludes levels of dabigatran above the therapeutic range but does not exclude the presence of dabigatran in the therapeutic range (45). In contrast, a normal TT excludes the clinically relevant concentration of dabigatran (> 30 ng mL^{-1}) whereas a prolonged TT could suggest either the presence of clinically relevant or even trivial levels of dabigatran, because TT is highly affected by dabigatran (dabigatran concentration even below 30 ng mL^{-1} significantly prolong TT and at concentrations above 50 ng mL^{-1}, TT result is usually greater than the upper measurement limit, depending on the reagent used) (46).

As for DTI, even for DOACs belonging to direct inhibitors of FXa, there is remarkable variability in the sensitivity of different commercial reagents. Further, among all FXa inhibitors, rivaroxaban is a drug with the highest effect on PT followed by edoxaban, whereas apixaban does not affect PT (45). Rivaroxaban and edoxaban affect PT at both through and peak values in most patients, but high variability between reagents prevents inclusion in the quantification of FXa inhibitors.

aPTT is non-linearly prolonged with increasing concentrations of dabigatran and rivaroxaban. However, aPTT is less affected by FXa inhibitors than DTI. Further, the sensitivity of reagents for aPTT is different.

It has been shown that combined, both normal PT and aPTT, measured with sensitive reagents may exclude dabigatran concentrations above 50 ng L^{-1} but fails to detect the presence of rivaroxaban at a concentration of 50 ng mL^{-1} and apixaban of up to 200 ng mL^{-1} (15).

Among all FXa inhibitors, apixaban has the least impact on screening coagulation assays. Therefore, these assays are of no value even for qualitative assessment of apixaban at on-therapy doses. For edoxaban, the impact on PT and aPTT is only modest and variable thus making these assays unsuitable for clinical assessment of the anticoagulant effect. For most reagents, PT will be prolonged only at peak concentrations (49).

Therefore, the low sensitivity and specificity of the PT and aPTT to DOACs suggest that these assays are not appropriate to quantify the concentration of DOACs (15). On the other hand, the knowledge of the impact of DOACs on screening coagulation tests is crucial for avoiding misinterpretation of the test results. It has to be emphasized that results of both PT and aPTT within the reference range do not exclude therapeutic concentrations of DOACs.

Although not being a test that has clinical significance in patients treated with DOACs, fibrinogen is another screening coagulation assay that could be affected by these drugs, but the results vary depending on the reagent used. Thus, some commercial reagents manifest false decreased fibrinogen values in patients treated with DOACs (50, 51). In fact, measuring fibrinogen in patients taking dabigatran can give falsely low results, but with marked variations between different reagents (51, 52).

Dabigatran has no impact on D-dimer assays (52). However, since in patients treated with dabigatran inhibition of thrombin is present, suppression of D-dimer levels could be

Table I. Effects of DOACs on the results of screening coagulation assays

Assay	Direct thrombin inhibitor	Inhibitors of FXa	Comment	Ref.
PT	↑	↑	Most sensitive to rivaroxaban.	50–52
			Poorly reflects the intensity of anticoagulation.	
			Normal PT (with sensitive reagent) excludes the above on-therapy rivaroxaban levels but does not exclude the presence of rivaroxaban in the on-therapy level.	54–56
			Rivaroxaban is the most sensitive to PT, following edoxaban.	
			For apixaban, depending on the reagent, PT may be normal in the presence of on-therapy levels.	
aPTT	↑↑	↑↑	Most sensitive to dabigatran.	
			Poorly reflects the intensity of anticoagulation.	54, 58
			Normal aPTT excludes the above on-therapy dabigatran levels, but does not exclude the dabigatran on-therapy range.	
TT	↑↑↑	No	Normal TT excludes the presence of dabigatran. It could be useful in the perioperative setting.	54, 87
			Useful only to exclude the presence of dabigatran.	
			Prolonged TT can suggest both low and on-therapy levels of dabigatran.	
Fibrinogen	↓/No	No	Mostly not affected by DOACs.	52
			Some reagents give falsely lower values in patients on dabigatran.	
D-dimer	No	No		53

aPTT – activated partial thromboplastin time, PT – prothrombin time, TT – thrombin time

expected. Dabigatran is associated with a greater reduction in D-dimer without the pronounced reduction of FVIIa seen with warfarin (53). These different effects on the coagulation system might explain the better efficacy and less intracranial bleeding observed with dabigatran compared with warfarin.

Table I gives an overview of the effects of individual DOACs on screening coagulation parameters. In general, all assays which are affected respond in a dose-dependent manner, with peak concentrations having a stronger influence than trough concentrations in plasma.

IMPACT OF DOACS ON SPECIALIZED COAGULATION ASSAYS

Considering that DOACs interfere with fibrin formation and consequently affect the results of screening coagulation assays, the assumption that they impact the results of other specialized assays based on the coagulometric measuring principle is understandable. Such, assays for individual coagulation factor measurement, as well as assays intended for

thrombophilia screening including antithrombin (AT) activity, clot-based protein C (PC) and protein S (PS) activities, activated protein C resistance (APCR) and lupus anticoagulant (LA) tests are all affected in the presence of DOACs. Therefore, it is very important to be aware of the impact of DOACs on these specialized assays, especially tests for thrombophilia testing, in order to prevent false interpretation and mismanagement of patients (54).

In accordance with the relative impact on PT and aPTT, coagulation factors assays are significantly affected by DOACs, with the aPTT-based factors (VIII, IX, XI, XII, XIII) most affected by dabigatran, whereas PT-based measurements (assessment of clotting factors II, V, VII and X) are most affected by rivaroxaban, following edoxaban and apixaban. Therefore, the activity of each factor can be falsely underestimated due to the presence of a particular DOAC. However, to decrease activity level below 50 % required concentration is still greater than 100 ng mL^{-1} for rivaroxaban and even 500 ng mL^{-1} for apixaban (55, 56). In line with the aforementioned sensitivity of aPTT and PT reagents, DOACs also significantly influence inhibitor's screening based on these assays, and most likely drive to a false conclusion (47). DOACs affect clot-based methods for PC and PS, leading to a drug concentration-dependent increase in the results and inaccurate conclusions. Additionally, a falsely normal result in a patient with PC or PS deficiencies can be overcome with other functional assays with a chromogenic endpoint, such as protein C-activity, which do not require the generation of Xa or IIa and do not show any interference (57, 58). The presence of DOAC also has an impact on APCR testing in terms of false increase, therefore, leading to misinterpretation of assay results (58). In APCR assay, the clotting time can be prolonged by the presence of all DOACs, with an unpredictable effect on the normalized ratio of measured clotting times (in seconds) with and without an additive protein C activator.

Further, there is a linear correlation between all DOAC levels and the dilute Russell viper venom time (DRVVT), with moderate precision, but it showed high sensitivity (95 %) and specificity (90 %) for clinically significant DOAC levels. The DRVVT detects clinically significant levels of DOACs and, in conjunction with the dilute thrombin clotting time (TCT), may be used as a screen for the presence and type of DOAC (59). Normal DRVVT--DOAC results ruled out concentrations > 30 ng mL^{-1}, except for apixaban. The DRVVT--DOAC assay could be a useful screening test in an emergency setting (60).

Both, LA (lupus anticoagulant) antibodies screen and confirm assays (dilute Russell viper venom time test confirm, dRVVTc) with the addition of excess phospholipids as part of a thrombophilia screening, can be prolonged in the presence of all DOACs, although the effect of DOACs on these assays cannot be predicted (45, 56).

The activity of AT may be determined by measuring the inhibitory effect on either thrombin or FXa for the target enzyme. Depending on the target enzyme used, the presence of thrombin inhibitors or FXa inhibitors will result in falsely higher AT levels. Thus, methods based on anti-FIIa inhibition are affected by dabigatran, whereas those based on anti-FXa inhibition are affected by rivaroxaban and apixaban in a direct dose-response manner (45, 51, 56).

In general, all affected specialized assays respond in a dose-dependent manner, with peak concentrations having a stronger influence than trough concentrations. It is important to keep in mind that false-positive or false-negative results are possible in patients receiving DOACs and can lead to huge diagnostic errors.

Therefore, it should always be kept in mind that treatment with DOACs represents an important pre-analytic factor in thrombophilia testing. Whenever possible, it is necessary to postpone phenotypic analyses at least 5 days after therapy discontinuation (57). Exceptions are molecular diagnostics of FII G20210A mutation and mutation of FV Leiden (FVL) because DOACs do not influence them.

Alternatively, *in vitro* removal of DOAC compounds from plasma prior to coagulation testing has been reported (61–63). DOAC-Stop (adsorbing agent, Hematex Research, Hornsby, Australia), DOAC-Remove (activated carbon, 5-Diagnostics, Switzerland), both reportedly were able to neutralize all DOACs with minimal effect on haemostasis tests. The impact of DOACs on laboratory assays used for thrombophilia testing (*e.g.*, AT, PS, PC, LA, and APCR) is a well-known issue and may cause false-positive and false-negative results. The correct interpretation of tests that are performed in patients taking DOACs is mandatory to prevent misclassification and the subsequent clinical consequences. Therefore, DOAC--Stop is the first remedy able to remove DOACs from a plasma sample, a simple way to overcome the interference of DOAC on coagulation tests and should facilitate the interpretation of thrombophilia screening tests in patients taking DOACs.

SPECIFIC COAGULATION ASSAYS FOR DOAC CONCENTRATION MEASUREMENT

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

The liquid chromatography-mass spectrometry method is coupling physical separation by liquid chromatography with mass analysis by mass spectrometry. Thus, this method, being highly specific and sensitive, is able to reliably determine DOACs concentrations at very low levels. Accordingly, LC-MS/MS represents the gold standard method for the DOAC quantitation (12). A range of concentrations for DOACs covers from 5 to 500 ng mL^{-1}, thus making the method suitable for both peak and trough DOACs concentrations analyses. LC-MS/MS method is accurate in the entire range of DOACs' concentrations in plasma. However, despite this advantage, technical complexity and inaccessibility in most laboratories make this method inaccessible in everyday practice (4).

Furthermore, considering that other factors such as pro- and anticoagulation agents affect the haemostatic status of the patient, functional assays may reveal a more accurate overall status of haemostasis (2). Moreover, functional coagulation assays adopted on automated coagulometers can obtain a turnaround time within 60 minutes thus making them convenient in emergency situations.

Specific methods for dabigatran measurement

Unlike LC-MS/MS method, drug-calibrated coagulometric or chromogenic methods can be adopted to automated coagulation analysers, thus being available in the majority of the modern clinical laboratories performing hemostasis testing (Table II).

For laboratory assessment of dabigatran, direct thrombin inhibitor (DTI), there are available both coagulometric methods [dilute thrombin time (dTT) and ecarin clotting test (ECT)] and newer chromogenic methods [ecarin clotting assay (ECA) and anti-FIIa].

Diluted thrombin time. – The high sensitivity of TT to dabigatran led to the development of a calibrated dTT test using dabigatran standards to calculate the dabigatran plasma

Table II. Specific quantitative methods of screening coagulation assays

Drug		Method		Ref.
		Coagulometric	Chromogenic	
Direct thrombin inhibitor	Dabigatran	DTT	ECA	64, 65
		ECT	Anti-FIIa	64
Direct FXa inhibitor	Rivaroxaban	–	Anti-FXa calibrated with rivaroxaban	71
	Apixaban	–	Anti-FXa calibrated with apixaban	66
	Edoxaban	–	Anti-FXa calibrated with edoxaban	66

ECA – ecarin chromogenic assay, DTT – dilute thrombin time; ECT – ecarin clotting time

concentration. The use of diluted plasma allows the measurement of dabigatran at a wide range of concentrations. A diluted TT test can measure a wide range of dabigatran concentrations in plasma and shows a good correlation with LC-MS/MS method (64).

Thrombin time is prolonged by the influence of dabigatran but not by the factor Xa inhibitors. A therapeutic level of dabigatran measured by dTT is in the range of 40 to 100 seconds, although results may vary between laboratories due to the combination of different reagents and coagulometers (65). Limitations of this method could be present in the case of switching therapy from heparins or related drugs to dabigatran because these drugs also have an effect on the dTT test resulting in falsely elevated levels of dabigatran.

Ecarin-based assays. – These methods are based on the ability of ecarin, a metalloprotease isolated from snake *Echis carinatus*, to convert the prothrombin to a meizothrombin which can be diminished in the presence of a direct thrombin inhibitor. Among ecarin-based methods intended for quantitative measurement of dabigatran two groups differ: coagulometric (ecarin clotting time, ECT) in which time to form fibrin is measured, and chromogenic ecarin assay in which chromogenic substrate is measured. Since the ECT measures the time required for clot formation, deficiencies of prothrombin (FII) and fibrinogen may affect its result. ECA, as a chromogenic method calibrated with specific calibrators, is affected neither by fibrinogen nor prothrombin deficiencies. In the ECA assay patient sample is pre-diluted with a buffer containing prothrombin, thus alleviating the limitation of the prothrombin factor present in the ECA test. Further, as the ECA is not a clotting assay, conversion of fibrinogen to fibrin is not measured and fibrinogen level in plasma does not affect the result. Studies have shown a linear relationship between both ECT and ECA results and dabigatran concentration and a good correlation with LC-MS/MS measurements (64, 65). Additionally, insensitivity to heparins makes ecarin-based assays valuable in the case of concomitant use of heparin (46).

Anti-IIa. – Chromogenic anti-activated factor IIa (anti-FIIa) assay is based on measurement of thrombin inhibition after adding thrombin reagent to plasma sample (66, 67). The test uses drug-specific calibrators and shows a good correlation with LC-MS/MS when measuring DTI plasma trough and peak concentrations.

Specific methods for the measurement of FXa inhibitors

Anti-FXa. – All methods intended for the determination of FXa inhibitors are based on the anti-FXa method principle, *i.e.*, on the inhibition of FXa by drug presence in plasma (rivaroxaban, apixaban, edoxaban), in a linear and dose-dependent manner, followed by measurement of residual FXa activity using specific chromogenic substrate. Commercially available plasma calibrators that are made of pooled normal plasma spiked with increasing amounts of a particular DOAC drug utilised for the construction of calibration curves from which plasma drug concentrations can be derived (17, 68). Results from several studies show the high sensitivity of these methods toward the presence of FXa inhibitors. However, attention should be paid to expected concentrations since assay sensitivity depends on methodology and assay used. Namely, uncertain results for rivaroxaban can be obtained if plasma concentration is lower than 30 ng mL^{-1}; this can be overcome using an adapted method though it may reduce the measurement range. Limited data support the accuracy of DOAC-specific coagulation assays around the current safe-for-treatment threshold of 30 ng mL^{-1} (69). The stability of the reagents varies from manufacturer to manufacturer and the quantification range is usually well above 20 ng mL^{-1}.

In general, anti-FXa chromogenic assays using specific calibrators provide accurate measurement of anti-FXa inhibitors in plasma above 30 ng mL^{-1} (70). Chromogenic anti-FXa methods calibrated with specific drugs are still not widely available in laboratories. However, the anti-FXa chromogenic assay used to monitor heparin therapy is able to reliably exclude clinically relevant concentration (< 30 ng mL^{-1}) of direct FXa inhibitors but are affected by high inter-assay variability and limited range of linearity and, therefore, should not be used to quantify direct anti-FXa inhibitors (71).

However, LC-MS/MS method is simply feasible, but only in a specialized laboratory. The method is easy-to-use for the simultaneous determination of all dabigatran, apixaban and rivaroxaban by LC-MS/MS within three minutes with a concentration range of 1 to 500 ng mL^{-1} without dilution. In the normal practice of the coagulation laboratory, it is advisable to use specific tests for DOAC determination as screening. The LC-MS/MS method is suitable as an arbitration method for serious conditions. This methodology has a high degree of specificity and sensitivity, with a limit of detection (*LOD*) and a limit of quantitation (*LOQ*) between 0.025 and 3 ng mL^{-1}, depending on the drug and technology (4). Bruckner *et al.* (72) developed and validated an analytical method for the determination of DOAC by HPLC–MS/MS. It simultaneously determines three DOACs with limits of quantification of 0.57 ng mL^{-1} for dabigatran, 0.79 ng mL^{-1} for rivaroxaban, and 0.22 ng mL^{-1} for apixaban. The HPLC-MS/MS is used as a referential method for assessing the suitability of other methods but not for routine determination. For detection in serum, *LOD* is 0.5–1.0 ng mL^{-1}, and *LOQ* is 1.9–3.6 ng mL^{-1}. The all above-mentioned methods are performed also for other biological samples, such as stomach content, urine, and venous blood.

MEASUREMENTS OF DOACS IN OTHER BIOLOGICAL SAMPLES

DOACs can also be measured in samples other than plasma, such as urine, serum and whole blood. It is generally assumed that serum and plasma can be interchangeably used for therapeutic drug monitoring of DOACs. Apixaban and rivaroxaban concentrations are significantly higher in serum than in plasma. The difference is more pronounced with

rivaroxaban and larger between serum and citrate-plasma than between serum and EDTA-plasma. Higher factor X activity in serum may explain the observed concentration differences. The choice of the matrix is, thus, important when interpreting therapeutic drug monitoring results and in research involving analyses of direct oral anticoagulants. The authors recommend citrate-plasma as the preferred matrix (73).

Regarding measuring DOAC concentrations in urine, it is important to point out that the measurement is much quicker than in plasma samples and it takes approximately 10 minutes to obtain a result with the DOAC Dipstick. However, because the DOAC Dipstick result is interpreted visually, it is observer-dependent and may be invalid if the urine colour is abnormal (74). These potential errors can be eliminated by using the reader (DOASENSE GmbH, Germany). The DOAC Dipstick has other advantages over conventional blood assays. For example, the test pads do not react with heparin, nadroparin, fondaparinux and coumadin. The thrombin inhibitor pads can also detect r-hirudin and argatroban at high concentrations, so these may be confused with dabigatran when interpreting the test result but this is highly unlikely to occur.

Testing of DOACs in urine samples may be useful in specific clinical conditions such as acute renal failure or emergency surgical interventions, unexpected bleeding or thrombotic events during DOACs treatment, in elderly patients or suspicion of overdose or intoxication. Point-of-care (POC) assays in urine are based on the development of different colours in the presence or absence of particular DOACs (75). During recent several years, urine DOAC screening tests are available as a rapid qualitative and semi-quantitative assessment of recent DOAC exposure. Harenberg *et al*. (76) have published recommendations regarding the use of a urine dipstick device which was shown to be sensitive and specific enough to determine the presence of both FXa and DTI inhibitors in urine samples. A study by Margetic *et al*. (77) has confirmed that the DOAC dipstick can be used to exclude the presence of clinically relevant levels (> 30 ng mL^{-1}) of anti-FXa and anti-FIIa inhibitors in patient urine. A positive DOAC dipstick test result suggests that DOACs are present in the circulation in concentration which should be quantified in plasma, as the next step in clinical decision-making.

The measurement of DOAC in serum samples is also possible and can be beneficial in specific clinical situations when additional blood sampling for plasma is not feasible. Testing in serum samples uses the same quantitative methods as in plasma and can be performed at automated coagulometers (78). However, it has been shown that dabigatran concentrations are about two-thirds lower in the serum samples than in the plasma due to the consumption of dabigatran by thrombin during *in vitro* blood clotting. That effect does not occur for rivaroxaban and apixaban (79).

Besides the above-mentioned POC test, other global assays [*e.g.*, thrombin generation assay (TGA), prothrombinase induced clotting time (PiCT), thromboelastography (TEG), thromboelastometry (TEM), and activated clotting time (ACT)] have been tested for various DOACs (12). Viscoelastic hemostatic assays (VHAs) are whole blood point-of-care tests that have become an essential method for assaying hemostatic competence in liver transplantation, cardiac surgery, and most recently, trauma surgery involving hemorrhagic shock. It has taken more than three-quarters of a century of research and clinical application for this technology to become mainstream in these three clinical areas (80). Within the last decade, thromboelastography (TEG® 5000, Haemonetics Corporation, USA) and rotational thromboelastometry (ROTEM® delta, Instrumentation Laboratory, USA), have been

supplanted not only by cartridge systems (TEG® 6S, Haemonetics Corporation, USA, and ROTEM® sigma, Tem International, Germany), but also by more portable point-of-care bedside testing iterations of these legacy devices [*e.g.*, Sonoclot® (Sienco Inc., USA), Quantra®, (HemoSonics, USA) and ClotPro® (enicor, GmbH, Germany)].

In urgent clinical situations, such as trauma, urgent surgery or before thrombolysis, rapid quantification of direct oral anticoagulant plasma drug levels is needed (81). Using the ClotPro® analyser, two novel viscoelastic tests for the detection of clinically-relevant plasma drug levels in trauma patients were implemented (81). The ecarin clotting time was used to assess the plasma concentration of dabigatran and Russell's viper venom clotting time to determine the plasma concentration of direct factor Xa inhibitors. Strong positive correlations between plasma drug levels and clotting time values were demonstrated in the specific ClotPro® assays. Cut-off values for detecting clinically-relevant drug levels showed high levels of sensitivity and specificity. There are specific reagents for the ClotPro® analyzer that are sensitive to DOACs (RVV test and ECA test) (82).

Despite their easiness of use and short turn-around-time, POC devices have many disadvantages such as lack of standardization, high cost, lack of being sufficiently studied and not sensitive enough to exclude clinically relevant concentrations of DOACs in the perioperative setting. Therefore, its use should be restricted to specific clinical contexts.

FACTORS IN DOACS MEASUREMENT

Pre-analytical factors

Considering that the credibility of laboratory test results is closely related to the quality of the sample, the importance of pre-analytical factors including stability of the sample, as well as sample type, administered DOAC drug and time of blood collection is obvious. Regarding sample type, platelet-poor plasma prepared with a buffered solution containing sodium citrate at a concentration of 0.109 mol L^{-1} (3.2 %), can be used for quantitative and qualitative clot-based and chromogenic assays (62). Sample stability depends on the particular drug and storage temperature.

The stability of dabigatran in plasma at room temperature is up to 24 hours with the remark that for TT testing it is reduced up to 4 hours after drawing blood. Stability can be extended up to 14 months by freezing at –20 °C. Rivaroxaban and apixaban are stable for at least 8 hours at room temperature and 48 hours when stored at 5 °C to up to 30 days when frozen at –20 °C (16, 67). Gosselin *et al.* (16) have shown that there are no clinically significant changes in the results after up to three freeze-thaw cycles (Table III).

In order to obtain an accurate insight into the anticoagulant activity of the drug and the hemostatic status of the patient, it is important to follow the pharmacokinetics of the DOAC and collect blood at the appropriate time after the last drug administration. As previously mentioned, DOACs have relatively short half-lives in circulation (10 to 15 hours) and their maximum plasma concentrations are reached in 1 to 4 hours after the last intake, depending on the drug. Therefore, it is of crucial importance to know when the last DOAC dose was taken relative to the time of blood sampling. Since assessment of trough drug levels presents lower inter-variability and better correlation with adverse events (bleeding or thrombosis) in comparison with assessment of peak concentrations, it is recommended

Table III. The most important preanalytical factors in laboratory assessment of DOACs

Preanalytical factor	Comment	Ref.
Time of sampling	The impact on screening assays and plasma concentration varies relative to the last drug intake.	16
	Peak concentration is reached at 1 to 4 hours after drug intake and depending on the drug and trough concentration is reached before the next drug dose.	84
Sample type	Plasma prepared in 3.2 % sodium citrate is recommended for quantitative and qualitative clotting and chromogenic assays.	85
	LC-MS/MS can use both serum and plasma.	
Sample stability	For dabigatran, sample stability in plasma is 24 hours at both room temperature and in the refrigerator (5 °C) and 14 months when frozen at < –20 °C	83
	When using the TT test stability is 4 hours.	86
	For rivaroxaban and apixaban samples, stability is at least 8 hours at room temperature, 48 hours at 5 °C and at least 30 days at –20 °C.	
	For edoxaban, there is no sufficient data on stability for functional anti-FXa assays.	
	Plasma samples for dabigatran that could not be tested within 24 hours of collection should be frozen as well as plasma samples for anti-FXa DOACs that cannot be tested within 8 hours after collection.	
Multiple freeze-thaw cycles	Three cycles had no effect on the measurement of rivaroxaban and edoxaban using functional chromogenic anti-FXa assays or MS methods.	83
	Data for apixaban and dabigatran are conflicting.	86

to perform analysis at the trough drug concentration in all non-urgent clinical situations (12). It can be achieved if blood sampling is done 12 hours after the last dose taken for drugs given twice a day, and 24 hours for drugs given once daily. Despite these recommendations, in emergencies, in which it is impossible to influence the time of sampling, for an accurate interpretation of the results, still it is necessary to know when the last dose of the drug was taken (77).

Analytical factors. – The guidance document from ICTH addresses all phases of laboratory DOAC measurements, including the analytical phase (gold standard method, screening and quantifying methods) which is already mentioned (16).

Further, data suggest that very low levels of DOACs in plasma are associated with thromboembolic events suggesting that laboratory measurement of DOACs concentration may improve patient management in special situations. However, since therapeutic ranges are still not clearly defined, it is difficult to titrate the dose. Therefore, knowing the time between the last drug dose and blood sampling is always required for all hemostasis assays in DOAC-treated patients. Laboratories should also know the sensitivity of their own reagent/analyser combinations. Additionally, the International Council for Standardization in Haematology (ICSH) provided recommendations for the proper validation or verification of the performance of laboratory assays prior to implementation. Prior to

performing method validation or verification, a plan (protocol) should be written that describes how the validation will be conducted and the acceptance criteria. Method validation studies should include precision, accuracy, linearity, determination of *LLOQ* (lower limit of quantitation), *LLOD* (lower limit of detection), and reportable range, and may include stability studies as well as comparison with the gold standard (LC-MS/MS).

All quantitative chromogenic methods for DOACs are automatable and can be adopted to different automated coagulometers in order to be easily implemented in coagulation laboratories (12). Besides the knowledge about the sensitivity of specific quantitative methods for DOAC assessment, laboratory experts should be familiar with important characteristics of available quantitative methods including reagent stability, measurement range and time required for assay performance. Reagent stability data indicate that they are stable for 8 weeks (2 months) after reconstitution if stored at 2–8 °C (83). The reportable range of quantitation is 20–500 ng mL^{-1}, which is adequate for assessing both, peak and trough concentrations. Given that the implementation of quality control is necessary to ensure the overall quality of the system, it is recommended to perform daily internal quality control (IQC) using two levels (low and high concentrations) of the control samples (82–85). In order to verify the accuracy of the system, there are also external quality assessment (EQA) providers available. Besides assuring the quality performance of the laboratory which provides DOAC testing, participating in EQA schemes also helps in the harmonization of the testing results globally.

Post-analytical factors

The concentration of all DOACs should be reported in ng mL^{-1}. At present, there are no clearly defined therapeutical ranges, but expected both trough and peak levels, depending on dosing regimen and clinical indication are available and always should be cited with each reported test result for DOACs (12, 16, 84).

CONCLUSIONS

Although DOACs have a significant effect on the result of screening coagulation tests, these assays are not suitable for assessing the anticoagulant effect of these drugs. On the other hand, knowledge of DOAC's impact on the results of these assays is a precondition for their proper interpretation and use. In all clinical conditions requiring DOAC assessment, specific quantitative methods should be applied.

DOACs also significantly influence the results of numerous specialized coagulation assays, among which those for thrombophilia screening are of crucial importance for avoiding incorrect interpretation and prevention of misdiagnosis in patients treated with DOACs. Both, laboratory medicine professionals and treating physicians should have a thorough understanding of the limitations of screening coagulation assays that are until now often used for qualitative assessment of DOACs. They should collaborate in establishing protocols for laboratory assessment of DOACs.

In laboratories that do not have the capacity to implement specific quantitative methods for DOAC determination, at least specific POC tests for qualitative assessment of DOACs should be implemented.

At present, it is considered that neither the dose nor dosing intervals should be changed in response to the results of coagulation parameters alone, especially the screening ones. Instead, a change of a type of DOAC drug, or a switch to a VKA, could be considered in the case that laboratory parameters suggest consistent overdosing or underdosing of a specific DOAC. Furthermore, when prescribing DOACs, for correct test result interpretation, it should always be taken into account that hepatic and renal function have an effect on the circulating concentrations of these drugs, as well as certain drug-drug interactions.

Abbreviations, acronyms, symbols. – ABCB1 – ATP-binding cassette sub-family B member 1, ACS – acute coronary syndrome, ACT – activated clotting time, AF – atrial fibrillation, APCR – activated protein C resistance, APTT – activated partial thromboplastin time, AT – antithrombin, BMI – body mass index, CKD – chronic kidney disease, CrCl – creatinine clearance, CYP3A4 – cytochrome P450 3A4 isoenzyme, CYP450 – cytochrome P450, DOAC – direct oral anticoagulants, dRVVT – dilute Russell viper venom time, DTI – direct thrombin inhibitor, DVT – deep vein thrombosis, ECA – chromogenic ecarin assay, ECT – ecarin clotting time, EQA – external quality assessment, FII – prothrombin, FVL – factor V Leiden, FXa – activated factor X, INR – international normalized ratio, INR/ISI – the international normalised ratio (INR)/international sensitivity index (ISI), IQC – internal quality control, ISI – international sensitivity index, ISTH – International Society on Thrombosis and Hemostasis, NVAF – non-valvular atrial fibrillation, LA – lupus anticoagulant, *L(LOD)* – lower limit of detection, *L(LOQ)* – lower limit of quantification, PC – clot-based protein C, PE – pulmonary embolism, P-gp – P-glycoprotein, PiCT – prothrombinase induced clotting time, POC – point of care, SNP – single nucleotide polymorphism, PS – protein S, PT – prothrombin time, TGA – thrombin generation assay, TEG – thromboelastography, TEM – thromboelastometry, TT – thrombin time, VKA – vitamin K antagonists, VTE – venous thromboembolism.

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