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Review Article

How the Hemostasis Laboratory Can Help Clinicians to Manage Patients on Oral Anticoagulants

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Abstract. Oral anticoagulants are widely used to treat or prevent cardiovascular diseases in millions of patients worldwide. They are the drugs of choice for stroke prevention and systemic embolism in patients with non-valvular atrial fibrillation and prosthetic heart valves, as well as for treatment/prevention of venous thromboembolism. Oral anticoagulants include vitamin K antagonists (VKAs) and direct oral anticoagulants (DOACs). The hemostasis laboratory plays a crucial role in the management of treated patients, spanning from dose adjustment based on laboratory testing that applies to VKAs to the measurement of drug concentrations in special situations that apply to DOACs. This article aims to overview how the hemostasis laboratory can help clinicians manage patients on oral anticoagulants. Special interest is devoted to the international normalized ratio, used to manage patients on VKAs and to the measurement of DOAC concentrations, for which the role of the laboratory is still not very well defined, and most interferences of DOACs with some of the most common hemostatic parameters are not widely appreciated.

Keywords: Vitamin K antagonists; Direct oral anticoagulants; International normalized ratio; Hemorrhage; Thrombosis.

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Introduction. Oral anticoagulants are widely used for the treatment and prevention of cardiovascular diseases. Although there is no accurate information, it can be estimated that around 2% of the general population in Western countries is currently on oral anticoagulants. They are mainly used for the prevention of ischemic stroke and systemic embolism in patients with non-valvular atrial fibrillation and for treatment/prevention of venous thromboembolism. Oral anticoagulants are also used to prevent thrombosis in patients with mechanical heart valves and those with antiphospholipid syndrome. Among the currently used oral anticoagulants, one may consider the time-honored vitamin K antagonists (VKAs) and the more recent direct oral anticoagulants (DOACs). The present article aims to overview the role of the hemostasis laboratory in the management of patients on oral anticoagulants. The information reported herein is based on data from the literature and the personal opinion of the authors.

Vitamin K Antagonists (VKAs). These drugs have been (and are still) used as anticoagulants since their mechanism of action was elucidated. In the early 1920s, massive deaths from hemorrhage were observed among cattle in the Northern part of the United States and Canada. Those deaths were soon ascribed to the ingestion of sweet clover that, during storage and subsequent fermentation, developed anticoagulant substances called coumarins, which were responsible for hemorrhage and animal deaths. Years later, coumarin substances, possessing the same characteristics as those derived from fermented sweet clover, were synthesized and used first as rat killers and then as anticoagulants in humans. Meanwhile, the principle of action of coumarins was fully elucidated, and it is now known that they act through the inhibition of vitamin K that, in normal conditions, is the mediator for the post-ribosomal carboxylation of such procoagulant factors as factor (F) VII, FIX, FX and FII (named vitamin K-dependent coagulation factors). Curiously, the same mechanism of action is also operative for two of the most important anticoagulant factors [i.e., protein C (PC) and protein S (PS)]. The carboxylation of the vitamin K-dependent coagulation factors is instrumental in making them adhere to phosphatidyl-serine, a negatively charged phospholipid expressed at the surface of activated platelets, thus helping to speed up thrombin generation and fibrin formation at the site of vessel wall injury. The elucidation of the mechanism of action of coumarins has been instrumental in the adoption of vitamin K as an antidote for these drugs, which were later called VKAs. Currently, the two most commonly used VKAs are warfarin (Coumadin®) and acenocoumarol (Sintrom®). They differ essentially for the half-life, which is relatively shorter for acenocoumarol than for warfarin. However, it was soon realized that VKAs cannot be administered at a fixed dose because their pharmacokinetic is not favorable, and the effective/safe dose is unpredictable. As a matter of fact, VKA dosage varies between individuals and also within the same individual at different time points. This variation is due to the interference of VKAs with food and other drugs that are concomitantly taken. VKAs reach their peak activity on average about one week after administration, and their effect is reduced markedly only several days after stopping treatment.

The prothrombin time (PT). This state of affairs led over the years to the use of PT as the test of choice for dose adjustment. The results of the PT depend, however, on the thromboplastin used for testing, and it was therefore soon realized that the application of PT in clinical practice would have been difficult because local laboratories may use different thromboplastins and hence may give different results when the PT is measured for the same patient. This would make dose adjustment of VKAs inherently difficult.

The international normalized ratio (INR). Starting from the 1980's, a system of thromboplastin calibration was initiated and refined.[1] It prescribes that local thromboplastins are calibrated against a common international standard for thromboplastin, prepared and distributed by the World Health Organization (WHO). The guidelines issued by WHO[2] require that local thromboplastins undergo a relatively simple calibration process, whereby PTs (seconds) for patients stabilized on VKAs and for a group of healthy subjects are measured with the thromboplastin being calibrated and with the WHO international standard for thromboplastin. Paired PTs are then plotted in a double-log scale, and after checking for linearity, an orthogonal regression line is drawn through the data points. The slope of the regression line represents the responsiveness of the thromboplastin being calibrated relatively to the WHO international standard for thromboplastin **(Figure 1)**.

The slope of the line is called international sensitivity index (ISI) and is used to convert PT results in seconds obtained with the local thromboplastin to those that would have been obtained using the WHO international standard for thromboplastin. Hence, the INR is the common scale whereby results of the PT, when used for patients on VKAs, do not depend on the thromboplastin used for testing. The conversion of PT into INR is easily obtained by the following equation:

$$\mathbf{INR = [PT_{patients}/MNPT]^{ISI}}$$

where the PT is the value in seconds for patients and the MNPT represents the geometric mean PT of 20 or more healthy subjects.

The development and refinement of the INR have been instrumental in performing clinical trials aimed at defining the most effective and safe therapeutic intervals. We now know that patients on VKAs are considered to be adequately anticoagulated when their INR falls most of the time between 2.0 and 3.0, a range that minimizes the risk of both hemorrhage and thrombosis. Patients

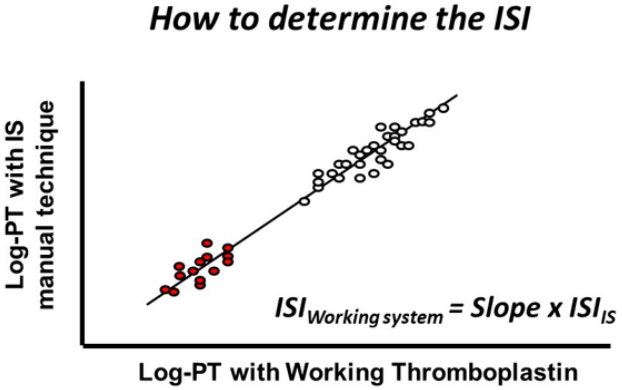

Figure 1. Schematic calibration of thromboplastins. Red and white dots represent the prothrombin time (seconds) for healthy subjects and patients stabilized on vitamin K antagonists. IS, the international standard for thromboplastin. ISI, international sensitivity index.

initiating VKAs should undergo laboratory screening, including a complete blood cell count and baseline coagulation tests such as the PT and activated partial thromboplastin time (aPTT). These are needed to identify patients with thrombocytopenia or carriers of mild hereditary defects of one or more coagulation factors that, although not causing bleeding problems in normal conditions, could put the patient at risk following the addition of anticoagulants.

Limitations of the INR. There are limitations of the INR that may impact patients' management. First, the INR (by definition) harmonizes PT results across laboratories, but only for patients on VKAs. In fact, the ISI of thromboplastins is determined using plasma for patients stabilized on VKAs and is therefore valid only for these patients. When PT is used for other clinical conditions, it should be expressed as clotting time (seconds) or simple ratio (patient-to-normal clotting time). Second, strictly speaking, the INR is valid only in the range of values from 1.5 to 4.5. In fact, plasmas from patients used for calibration are selected to have an INR comprised in that interval. This means that there is no assurance that there is dose-response linearity above or below this range. In practice, an INR above 4.5 has only an indicative value, and there is no assurance that (for example) an INR of 8.0 is different from an INR of 10.0. Hence, making a decision on the reversal of anticoagulation based on INR values higher than 4.5 should be done with caution and knowledge of the performance of the local reagents. Third, the INR may be influenced by conditions other than VKAs, such as partial deficiencies of coagulation factors or the presence of lupus anticoagulant (LA), which might prolong the PT and hence increase the INR. Specific studies have been performed to assess the effect of LA on PT-INR, and it is now known that there is no effect with the majority of commercial thromboplastins.[3] As a matter of fact, the PT is insensitive to LA unless thromboplastins are considerably diluted. Fourth, the INR is not valid at the beginning of treatment with VKAs, as in this phase, vitamin K-dependent coagulation factors are depressed at a different rate, depending on their half-life. However, since there is no other valid method to monitor VKA dosages, the INR is also used in the initiation phase of treatment. Finally, most of the commercial thromboplastins are added with chemicals, such as polybrene or enzymes (heparinase), that make them insensitive to heparins up to 1.0 UI/mL. This addition is needed to circumvent the effect that heparins may have on the prolongation of the PT-INR when anticoagulant therapy for venous thromboembolism is started and heparins and VKAs are concomitantly administered. On these occasions, the presence of heparins would make dose-adjustment of VKAs and attainment of the INR therapeutical interval difficult to achieve.

Direct Oral Anticoagulants (DOACs). DOACs are a consolidated reality in the armamentarium available to doctors for the treatment and antithrombotic prophylaxis of cardiovascular diseases. It is estimated that at least 70% of patients with non-valvular atrial fibrillation, who are treated for the prevention of ischemic stroke, or those treated for venous thromboembolism and its recurrence, are currently on DOACs, and only the remaining 30% are treated with VKAs.[4,5] However, there are still clinical conditions in which DOACs are not adequate. For example, VKAs are still the drugs of choice in the prevention of thrombosis in patients with mechanical heart valves and for the prevention of thromboembolic events in patients with antiphospholipid syndrome.[6] The reason for the success of DOACs rests mainly in their greater simplicity and manageability of use since they can be prescribed at a fixed dose, based on the patient's characteristics, and unlike VKAs, do not require dosage adjustment based on laboratory testing. Additionally, there are other direct-acting anticoagulant drugs (e.g., anti-FXI) in clinical trials that may have, at least from a theoretical standpoint, further advantages. It is, therefore, predictable that the DOACs will replace VKAs in the medium to long term, although the latter still have a non-negligible role.

Although DOACs do not require dose adjustment by laboratory testing, it would be wrong to think that they do not require laboratory support at all.[7,8] In the following paragraphs, we consider the role of the hemostasis laboratory in the management of patients on DOACs. Patients initiating DOACs should undergo laboratory screening, including a complete blood cell count and baseline coagulation tests such as the PT and aPTT. These are needed to identify patients with thrombocytopenia or carriers of mild hereditary defects of one or more coagulation factors that, although not causing bleeding problems in normal conditions, could put the patient at risk following the addition of DOACs.[9] Assessment of the creatinine clearance before initiation of treatment is paramount since DOACs are excreted through the kidney (**Table 1**).

The drugs, their characteristics, and mode of action. DOACs exert their antithrombotic action through the direct inhibitory effect against an individual coagulation factor. This mode of action is markedly different from that of VKAs, which exert antithrombotic function through the impairment of the carboxylation process of vitamin K-dependent coagulation factors. The function of DOACs also differs conceptually from that of heparins, which exert their function by indirectly inhibiting the procoagulant factors through the mediation of antithrombin.

The mode of action of DOACs determines some of their important characteristics. For example, DOACs have a much faster anticoagulant action than VKAs.

Table 1. Key features of DOACs.

Drug	Target Factor	Elimination	Time to peak	Time to trough
Dabigatran (Pradaxa®)	Thrombin	Kidney (80%)	2h	12h
Rivaroxaban (Xarelto®)	Factor Xa	Kidney (33%), Liver	2h	24h
Apixaban (Eliquis®)	Factor Xa	Kidney (27%), Liver	2h	12h
Edoxaban (Lixiana®)	Factor Xa	Kidney (50%), Liver	2h	24h

Peak concentration in plasma and, therefore, DOAC action is reached about two hours after administration. At the same time, the trough value is recorded after 12 or 24 hours, depending on whether the drug is taken twice or once daily (**Table 1**).

On the other hand, VKAs reach their peak activity on average about one week after administration, and their effect is markedly reduced only several days after stopping treatment. These differences become crucial in clinical practice. For example, DOACs are more manageable than VKAs, especially when a temporary discontinuation of treatment is needed for surgery and/or invasive procedures, which are deemed at potential bleeding risk. DOACs are eliminated from the circulation through the kidney and/or liver, with Dabigatran having the highest renal excretion (**Table 1**). Because of the above characteristics, it is recommended to assess for renal and liver function in individual patients before and during treatment to avoid possible accumulation of the circulating drug and the consequent increase in bleeding risk. Renal function is generally assessed by estimating creatinine clearance (CrCl) with an empirical formula (Cockcroft-Gault) that considers some biochemical parameters such as serum creatinine and patient variables such as body weight, age, height, and gender. In patients with CrCl <30 mL/min, Dabigatran is contraindicated and anti-FXa drugs should be prescribed with caution (they are contraindicated for CrCl <15 mL/min). Liver function can be evaluated by the measurement of liver enzymes.

Currently, there are four DOACs available: Dabigatran (Pradaxa®) is the only drug with antithrombin action, while Rivaroxaban (Xarelto®), Apixaban (Eliquis®) and Edoxaban (Lixiana®) inhibit FXa **(Table 1 and Figure 2)**.

All drugs are indicated for the prevention of ischemic stroke in patients with non-valvular atrial fibrillation and for treatment/prevention of venous thromboembolism, excluding those patients with mechanical heart valves and those with triple-positive antiphospholipid syndrome (concomitant positivity for anticardiolipin, anti-β2-GPI, and LA).[6] Clinical trials performed on large patient series have demonstrated that the efficacy and safety of DOACs are not inferior (or even superior) to those of VKAs when used at fixed doses based on patients' characteristics.

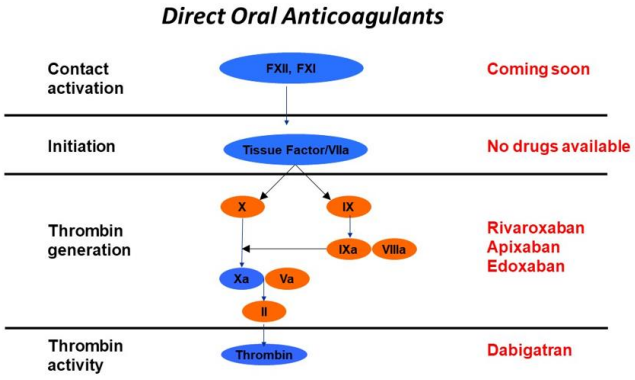

Figure 2. Schematic representation of direct oral anticoagulants acting at different steps of the coagulation scheme. Target factors are identified in blue.

Which role of the hemostasis laboratory in the era of DOACs. Owing to the favorable pharmacokinetic and dose-response predictability, DOACs have been designed for fixed-dose use with particular regard to patient's characteristics. There are at least two dosages for each medication, whereby the needs of most patients can be considered. At these doses, the rate of adverse events (hemorrhagic and thrombotic) that emerged from the studies, although limited, were measurable and, therefore, the question of whether some sort of dose adjustment based on laboratory testing may be useful to minimize the risk is still a matter of debate. For example, a review of clinical and laboratory data from the pivotal registration trial of Dabigatran has demonstrated that a dose adjustment in those patients with extreme plasma concentrations could have spared a number of adverse events.[10] In addition, real-life observational studies have documented wide variability of the plasma concentration for each DOAC[11] despite being taken at the same dose. The above observations suggest that the fixed dose is not necessarily applicable to all patients indiscriminately. Recently, an observational collaborative study (Measure And See, MAS) showed that a single measure of DOAC concentrations a few weeks after initiation of therapy may predict adverse events. In fact, some of the patients who had relatively low DOAC levels showed a greater propensity to develop thrombotic relapses more frequently during follow-up.[12]

Although these observations show that the fixed dose is not applicable to all patients, regulatory authorities and experts are reluctant to change this rule in favor of dose adjustment. Most believe that the benefits likely achieved with dose adjustment would not be offset by the reduction of adverse events and would further complicate therapy management in millions of patients.

Given that dose adjustment is not applicable to all patients and that DOACs will continue to be prescribed at a fixed dose, the fact remains that plasma concentration measurement is useful in some circumstances that we consider below. The personal

Table 2. Situations for which DOAC measurement is advised.[8]

Recommended					
Adverse events (thrombosis or hemorrhage)		Surgery, invasive procedures	Treatment with thrombolytic drugs	Overdose	Antidotes administration
Useful					
Interfering drugs	Extremes of body weight	Chronic anticoagulation			

opinions of the authors and other experts[8] suggest dividing these situations into two categories: those in which DOAC measurement is recommended and those in which it is considered useful (**Table 2**). Cumulatively, the number of subjects to be included in DOAC measurement would be relatively small and would not entail excessive burdens for the national health system or problems in the practical management of patients.

When DOAC measurement is recommended.

Adverse events (thrombotic or hemorrhagic) during therapy. In patients with adverse events, especially when lack of adherence to therapy or patient errors can be excluded, it is paramount to establish the reasons for adverse events in order to exclude that it is due to an excess (hemorrhage) or a defect (thrombosis) of the drug.

Surgery and/or invasive procedures. If surgery or invasive procedures deemed at increased bleeding risk are not programmable, DOAC measurement is needed to assess whether residual drug concentration in plasma is such to minimize the risk of bleeding. DOAC measurement may also be required for elective surgery/invasive procedures, and in such cases, the patient may be instructed to discontinue therapy 2-3 days before surgery. If renal function is normal, this protocol of discontinuation, given the short half-life of DOACs, may be adequate to ensure their complete elimination from circulation. However, to be on the safe side, it would be necessary to know (at least) the value of serum creatinine. Very often, this value is in the patient's records but could date back weeks or months earlier, and there is no certainty if this value is constant over time, especially in the elderly, in whom sudden and unpredictable changes may occur. It would then be necessary to proceed with an emergency serum creatinine measurement. However, it is still being determined what the advantage could be compared to the direct measurement of the residual drug, if one considers also that there is no a clear relationship between serum creatinine levels and DOAC plasma concentration.[11]

Treatment with thrombolytic agents. Despite proper treatment, some patients on DOACs for non-valvular atrial fibrillation may be referred to emergency departments because of ischemic stroke. In these instances, the therapy of choice is thrombolytic treatment in an attempt to lyse the thrombus. However, thrombolytic agents are burdened with a discrete risk of bleeding, which becomes even more important if there are relatively high concentrations of DOACs in the circulation. That is why DOAC measurement is paramount in this circumstance.

Overdose. In the suspicion of voluntary or accidental overdose, even in the absence of bleeding, DOAC measurement is needed for obvious reasons.

Administration of antidotes. There are at least two antidotes for DOACs: Idarucizumab, which neutralizes Dabigatran, and Andexanet alfa, which neutralizes anti-FX drugs. The two antidotes have been evaluated in randomized multicenter trials, which have shown efficacy and safety in patients referred to emergency departments for active bleeding. However, the respective study protocols did not prescribe the preventive measurement of DOACs, and antidotes were administered on the presumption that patients were bleeding because of an excess of DOACs. Post-hoc measurement on plasma samples collected during the studies reported that about 1/4 of the patients had received the antidote in the absence of significant amounts of circulating DOACs.[13,14] Therefore, based on these results, it is advisable to measure the DOACs before administering antidotes in order to optimize their use and treat patients more appropriately. Observational studies have also reported that occasionally patients treated with Idarucizumab showed complete and immediate neutralization of the drug, but after a few hours the concentration of circulating Dabigatran returned to levels similar to those measured before administration of the antidote.[15] This phenomenon is probably explained by the leakage of Dabigatran into extra-vascular spaces, where it cannot be reached and neutralized by the antidote because of its relatively high molecular weight. When the concentration of plasma Dabigatran is reduced by the action of Idarucizumab, the drug released into the extra-vascular spaces is recalled to circulation by osmosis, and its concentration increases. The patient may then need a second dose of antidote. This rebound phenomenon suggests that, in order to optimize the use of Idarucizumab and for patient safety, the plasma concentration of Dabigatran should be measured before and after administration of the antidote.

When DOAC measurement is potentially useful.

Interfering drugs. DOACs interfere with other drugs much less than VKAs, but occasional potentiation or depotentiation of their activity by other drugs cannot be excluded. When there is a suspicion of potential interference, the measurement of DOACs before and after the addition of the additional drug can provide very useful information.

Patients with extremes of body weight. The fixed dose is theoretically valid for the type of patients enrolled in clinical trials. Even though extremes of body weight were not among the exclusion criteria, these patients were not sufficiently represented in clinical trials. Therefore, an *ad hoc* evaluation of the efficacy and safety of DOACs in this type of patient is not possible. *Post-hoc* observational studies have yet to solve this problem completely, and whenever doubts do exist about the optimal dose in overweight or underweight patients, the measurement of plasma DOAC concentration could provide useful information.

Chronic anticoagulation. Given the variability in the plasma concentration of DOACs recorded in clinical trials and real-life observations, it is conceivable that individual patients may have plasma levels of DOACs that are her/his own characteristics. Although *ad hoc* studies are lacking, it is reasonable to assume that these concentrations may be constant over time for the same individual. Therefore, measurement of concentrations at the achievement of the steady-state of chronic anticoagulation (e.g., 4 weeks after initiation) and verification of the constancy/variation of this value in subsequent periods, could give important information on DOAC plasma levels, whose knowledge could be useful in particular circumstances. Measurement could also be occasionally useful in the elderly who do not have the assistance of caregivers to check for adherence to the therapy.

Which test for which DOAC. Although PT/aPTT may be more or less prolonged in patients taking DOACs, their response is variable and depends on the composition of the reagents. Therefore, PT/aPTT in these patients can give only a partial answer that could also be misleading in many respects. In fact, these tests may be prolonged for reasons other than the presence of DOACs (e.g., variable concentration/activity of individual coagulation factors). Hence, the PT/aPTT in patients on DOACs are not advisable. There are dedicated tests for DOACs that are commercially available and are relatively simple to perform in general clinical laboratories on ordinary coagulomaters even in emergencies. These tests can be calibrated with standards at known and certified concentrations for each drug, with results expressed in ng/mL.

Dilute thrombin time (dTT). The traditional thrombin time is over-sensitive to the presence of Dabigatran, so the test is uncoagulable even when Dabigatran is at relatively low concentrations. Minor modifications have been made to this test relating to the dilution of the sample, which allows adequate sensitivity to Dabigatran and can be used successfully for its measurement.

Ecarin test. This test is similar to the dTT but exploits the ability of ecarin (reptile venom) to activate FII. The thrombin, which is thus generated, is inhibited by Dabigatran, and the measurement of residual thrombin (by means of coagulation or chromogenic technique) allows for measurement of the concentration of the drug to be extrapolated from a dose-response calibration curve.

Anti-FXa activity tests. These are the same tests used for the measurement of heparins, which allow us to evaluate the ability of plasma to inhibit FXa added in excess to the plasma under test. They are based on FXa-specific chromogenic substrates and can be successfully used for the measurement of any of the anti-FX drugs (Rivaroxaban, Apixaban and Edoxaban).

When to perform DOAC measurement. Given the pharmacokinetics of DOACs, the time elapsing between the last drug intake and blood sampling is essential for the interpretation of results. Peak plasma concentration is reached approximately two hours after taking the drug and trough values after 12 or 24 hours, depending on whether the drug is administered twice or once daily.

Therapeutic intervals. The values obtained in the plasma of subjects treated with DOACs are poorly defined, due to the fact that there are few studies and because of the wide inter-individual variability, despite the fact that all patients take the same daily dose. It is, therefore, more useful to refer to the expected values that are reported in the technical annex of the drugs or in the literature, examples of which are shown in **Table 3**. In cases where DOAC measurement is performed, it is advisable to report the results as plasma concentrations expressed in ng/mL and the relative expected values.

Interference of DOACs, or VKAs with the most common hemostasis tests. Because DOACs are anticoagulant drugs, they interfere with the tests commonly used to evaluate hemostasis. However, some interferences are not so obvious from a theoretical standpoint and might lead to erroneous and dangerous conclusions. The consequences of DOAC interference are briefly discussed in the following paragraphs and reported in **Table 4**.

Table 3. Expected values of DOACs in patients under treatment. They refer to those reported in the European Medicines Agency (EMA) technical annex for Dabigatran, Rivaroxaban, and Apixaban and to those in the literature for Edoxaban.[16] VTE venous thromboembolism; DVT deep vein thrombosis: PE pulmonary embolism.

Medication (condition)	Test	Expected values (peak, ng/mL)	Expected values (trough, ng/mL)
Dabigatran 150 mg	Dilute Thrombin Time (dTT)	117-275	61-143
Rivaroxaban 20 mg	Anti-FXa Activity	22-535	6-143
Apixaban 2.5 mg bd (VTE prevention in orthopedic surgery)	Anti-FXa Activity	41-146	23-109
Apixaban 2.5 mg bd (stroke prevention)	Anti-FXa Activity	69-221	34-162
Apixaban 5 mg bd (stroke prevention)	Anti-FXa Activity	91-321	41-230
Apixaban 2.5 mg, bd (treatment of DVT, PE, and prevention of DVT and PE)	Anti-FXa Activity	30-153	11-90
Apixaban 5 mg bd (treatment of DVT, PE, and prevention of DVT and PE)	Anti-FXa Activity	59-302	22-177
Apixaban 10 mg bd (treatment of DVT, PE, and prevention of DVT and PE)	Anti-FXa Activity	111-572	41-335
Edoxaban 30 mg	Anti-FXa Activity	55-120	15-45
Edoxaban 60 mg	Anti-FXa Activity	125-245	19-62

Table 4. Possible interference of DOACs in the measurement of common hemostasis parameters

Parameter	Expected Interference
Antithrombin	Overestimation
Protein C (functional)	Overestimation
Protein S (functional)	Overestimation
Coagulation factors	Underestimation
Resistance to activated protein C	Overestimation
Fibrinogen	Possible underestimation in the presence of Dabigatran
FXIII	Possible underestimation in the presence of Dabigatran
Lupus anticoagulant	Difficult Interpretation of results (see text for more details)

Antithrombin. DOACs can significantly interfere with the measurement of antithrombin activity. For example, if antithrombin measurement is performed using thrombin as the target enzyme and the drug present in the patient's plasma is Dabigatran, antithrombin activity is falsely increased because Dabigatran inhibits the thrombin added in excess to perform the measurement.Similarly, if the target enzyme for antithrombin measurement is FXa and the drug in the patient's plasma is an anti-FXa (Rivaroxaban, Apixaban, or Edoxaban), the drug will inhibit the excess of FXa added to perform the measurement, and thus the measured activity of the antithrombin is falsely increased.

In the cases described, interference can be avoided by using as a target enzyme for the measurement of antithrombin the one that is not inhibited by the drug present in the patient's plasma. In other words, if the drug used for treatment is Dabigatran, the method for measuring antithrombin should use FXa as the target enzyme. Conversely, if the drug used for treatment is Rivaroxaban, Apixaban, or Edoxaban, the method for measuring antithrombin needs thrombin as the target enzyme.

Protein C (PC) and protein S (PS). As mentioned, PC and PS are vitamin K-dependent coagulation factors and are, therefore, reduced in patients on VKAs. DOACs prolong the coagulation tests on which functional assays of PC and PS are based. Therefore, the measured activity values for these proteins may be overestimated in the presence of DOACs. PC values measured by chromogenic assays and PS measured by immunoassay are not affected by the presence of DOACs.

Fibrinogen. Fibrinogen is usually measured by coagulation methods after addition of thrombin (Claus method) to the patient's plasma. In these cases, thrombin used as a reagent is inhibited by Dabigatran, leading to an underestimation of fibrinogen concentration. Interference from Dabigatran can be minimized by using (as a reagent) thrombin at high concentrations (e.g., 100 U/mL).

Factor XIII (FXIII). FXIII, when measured as functional activity, may be underestimated if the patient is treated with Dabigatran. This is due to the fact that the FXIII must be activated by thrombin prior to measurement. Therefore, the presence of Dabigatran leads to the inactivation of part of the thrombin, and this inevitably results in an underestimation of the FXIII activity.

Measurement of individual coagulation factors. The presence of one of the DOACs may give rise to artificially reduced levels of individual coagulation factors, which are based on PT or aPTT in combination with plasmas deficient in the factor to be measured, but also in case of some chromogenic tests (e.g., FVIII).

Lupus anticoagulant (LA). DOACs, but also VKAs, interfere with LA detection.[17,18] The explanation rests on the fact that both anticoagulants and LA prolong the clotting time of the aPTT and dRVVT tests used for LA

detection. Therefore, it becomes difficult (if not impossible) to correctly interpret the results of LA testing when used in anticoagulated patients. The golden rule prescribes that the search for LA be performed before initiating anticoagulant therapy or after its withdrawal for an adequate period. For practical reasons, this is not always possible, and often, patients' blood samples are sent to the laboratory with the request to detect LA when the patient has already been started on anticoagulation. In these cases, the laboratory approach is dependent on the anticoagulant drug administered.

If the anticoagulant drugs are VKAs, LA diagnosis is complicated because there is, at the moment, no completely reliable strategy to perform the LA search other than discontinuation of treatment for the time needed to clear drugs from circulation. If this is not possible, the most commonly used strategy is the dilution of patient plasma in normal plasma (1:1 ratio) in the belief that this dilution leads to a correction of the clotting time prolongation due to VKAs. This is not always the case and mostly depends on the composition of the reagent used for testing; because of this, false-negative or false-positive values may be expected. In addition, due to dilution (1:2), the potency of LA is reduced by 50% and, therefore, weak LA may be lost at diagnosis. Alternatives to make diagnosis of LA for patients on VKAs are the combined use of such snake venoms as Taipan and Ecarin. They are able to activate FII directly but have different phospholipids requirements. Studies have shown that their use may be useful to detect LA in anticoagulated patients.[19]

If patients are taking DOACs**,** LA diagnosis should not be performed because the patient's plasma would almost certainly be (false) positive for LA. Recent data from the literature show that in a population of LA-negative patients, more than 80% tested positive when the drug was Rivaroxaban, and the test used to diagnose LA was dRVVT.[20]

In these cases, however, some alternatives allow LA diagnosis. Activated carbon chemicals (DOAC-Stop®, DOAC-Remove®) have been developed and are commercially available, which, when mixed with a patient's plasma, after short incubation, adsorb DOACs onto their surface. LA can be measured on the supernatant after centrifugation without significant interference.[21,22] These substances have been variously studied to evaluate their ability to adsorb DOACs and have demonstrated a good capacity.[20] Studies related to their diagnostic capacity for LA have yielded varying results.

In some cases, activated carbons, in addition to DOACs, also adsorb other plasma substances on their surface, which could modify the procoagulant strength of the plasma, making the diagnosis of LA complicated. However, at present, activated charcoals are the only valid means of performing LA diagnostics in DOAC patients. They do not affect VKAs.

Conclusions. Clinical registration studies were designed to evaluate whether a fixed dose of DOACs, without dose adjustment based on laboratory testing, was effective in treating and preventing thrombotic recurrence while ensuring adequate hemostasis to avoid bleeding events. This design was strongly taken into consideration because a positive result would have been a significant advantage of the DOACs compared to VKAs, which need dosage adjustment (based on the INR). History has shown that the fixed dose intuition was valid and DOACs are now used with this regime, although real-life observations show that some patients could benefit from dose-adjustment. However, this does not mean that the plasma concentration of DOACs should never be measured, and there are, in fact, conditions and patients for whom the measurement of DOACs would be of extreme value to clinicians. Therefore, it is the responsibility of clinical laboratories to set up tests that are commercially available and relatively simple to perform at a time that allows them to be performed even in an emergency.

Conflicts of interest. AT received speakers' fees from Stago, Roche, BioMarin, Werfen. AP received honoraria for participating as a speaker at educational meetings organized by Sanofi.

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