REVIEW ARTICLE

How and when to measure anticoagulant effects of direct oral anticoagulants? Practical issues

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KEY WORDS

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ABSTRACT

Direct oral anticoagulants (DOACs) do not require dose adjustment based on laboratory testing. However, it might be necessary to measure their plasma concentrations in the following specific situations: 1) before thrombolytic therapy in patients with stroke; 2) before surgery or invasive procedure; 3) in case of adverse events (thrombosis or hemorrhage); 4) when immediate reversal of anticoagulation is needed; 5) in patients with extreme body weight; 6) when administering additional drugs potentially interfering with DOACs; and 7) when overdosage is suspected regardless of concomitant bleeding. Basic coagulation tests, such as prothrombin and activated partial thromboplastin time, should not be used as standalone tests to assess the levels of anticoagulation as they are not specific for DOACs and their results are dependent on the type of reagent used for testing. Plasma DOAC concentrations should be assessed by dedicated tests: dilute thrombin time or ecarin tests (for dabigatran) or anti–factor Xa assays (for anti–factor Xa inhibitors). Dedicated tests should be calibrated against their respective plasma calibrators at certified DOAC concentrations and results should be expressed as ng/ml. Caution should be exerted when interpreting the results of the most common hemostatic parameters such as antithrombin, proteins C and S, lupus anticoagulant, or individual coagulation factors, as they may be strongly affected by the presence of a DOAC. Whenever possible, these parameters should be measured 4 to 5 days after discontinuation of DOAC anticoagulation.

Introduction Direct oral anticoagulants (DOACs) have been licensed in many countries and are now used worldwide for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation and for the treatment or prevention of venous thromboembolism (VTE), conditions which, until recently, have been managed with vitamin K antagonists (VKAs). Currently, there are 4 licensed DOACs: dabigatran, a direct thrombin inhibitor, and rivaroxaban, apixaban, and edoxaban that are direct factor Xa inhibitors. The Food and Drug Administration (FDA) has licensed an additional anti–factor Xa inhibitor (betrixaban) for the prophylaxis of VTE in patients hospitalized for acute medical illness, who are at risk for thromboembolic complications due to moderate or severe restricted mobility and other risk factors.

DOACs have distinct advantages over VKAs, and according to the current trend, it is anticipated that a considerable number of patients will be switched from VKAs to DOACs within the next few years.[1-4] The characteristics that make DOACs more favorable than VKAs include a predictable anticoagulant effect, relatively wide therapeutic window, rapid onset and offset of action, and little or no interaction with other medications or food products. DOACs have been tested in randomized clinical trials and proved safe and effective when administered at a fixed dose (based on patient characteristics) without the need for dose adjustment based on laboratory testing. The main disadvantages of DOACs are their elimination from the circulation through the kidney or the liver, as well as properties that contraindicate their use in patients with severe renal insufficiency or chronic liver disease. Much debate has been done over the last decade on the role that clinical laboratories may have in the management of patients on DOACs. Regulatory authorities (FDA

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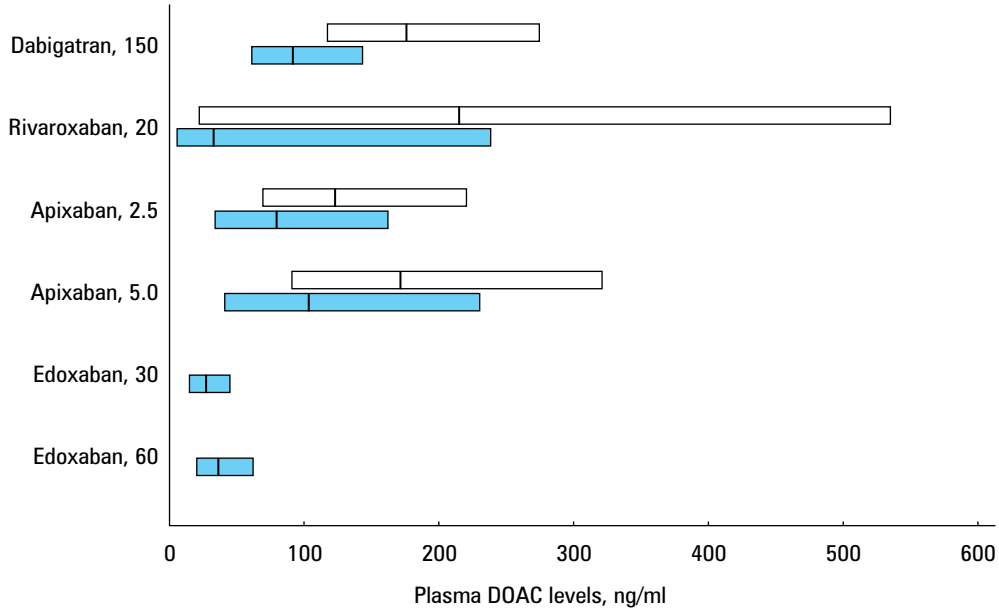

FIGURE 1 Expected concentrations (mean and 25th–75th percentiles) for patients on individual direct oral anticoagulants (DOACs; doses in mg), as reported by the technical annex of the European Medicine Agency[6-9] or Ruff et al.[10] Vertical lines denote mean values. White and blue bars denote peak and trough value, respectively.

and European Medicine Agency [EMA]) have not yet issued recommendations. Hence, clinicians prescribing these drugs rely on recommendations issued by scientific societies when available, but there is no uniform application. Furthermore, hospital administrators and laboratory operators are left without guidance to make decision on whether hospitals need to be equipped with these tests. As a consequence, DOAC testing is poorly used despite calls to do so. This article aims to review and discuss the situations where testing may help clinicians manage patients on DOACs.

When dealing with anticoagulants in the clinical laboratory setting, 2 concepts should be distinguished. The first one is "monitoring", which refers to dose adjustment based on results from laboratory testing that assesses the level of anticoagulation. This concept applies to heparin (mainly unfractionated and possibly low-molecular-weight heparin), VKAs, and other less commonly used drugs (eg, argatroban). The other crucial concept is "measuring", which means the assessment of anticoagulation level or the drug concentration achieved in blood after administration of a fixed dose. We are strongly convinced that the major obstacle to the adoption of DOAC testing is the confusion between these concepts. The fact that DOACs do not need dose adjustment based on laboratory testing does not necessarily mean that measuring blood concentrations is not useful in specific situations. Below, we review the situations when either "monitoring" or "measuring" would be useful.

Direct oral anticoagulants: monitoring The above DOACs were investigated in large randomized clinical studies on thousands of index patients (orthopedic surgery, atrial fibrillation, or VTE) and proved effective and safe in comparison with preexisting treatments (heparin or VKA). These studies were carried out assuming the "one-dose--fits-all" concept, and therefore DOACs have been administered at a fixed dose (mainly based on patient characteristics) without further adjustment by laboratory testing. However, post hoc analyses of the data from clinical trials revealed that some sort of dose adjustment would have been useful to improve the efficacy and safety of DOACs. For instance, Reilly et al,[5] after analyzing the data of the RE-LY study (Randomized Evaluation of Long-Term Anticoagulation Therapy), concluded that in some patients who were at the extremes of the dabigatran concentration range and had one or more risk factors (ie, old age, reduced creatinine clearance, or low body weight), better outcomes might have been achieved by adjusting the dose. FIGURE 1 shows a relatively large interindividual variability of plasma DOAC concentrations as derived from the EMA technical annexes of patients on dabigatran, rivaroxaban, apixaban, or edoxaban included in the registration studies[6-9] or published literature.[10] Furthermore, FIGURE 2 depicts variations in the probability of major bleeding and ischemic stroke according to dabigatran plasma concentrations among patients included in the RE-LY study.[5] The line describing the probability of ischemic stroke is relatively flat, indicating that dabigatran in this population is relatively effective over a wide range of plasma concentrations. Conversely, the line describing the probability of major bleeding is relatively steep, indicating that safety in this population is somewhat dependent on dabigatran concentration. There are 2 logical consequences that stem from these post hoc analyses. First, plasma dabigatran concentrations are relatively varied in the patient population despite the fact that patients take the same dose. These observations have been confirmed in subsequent real--life studies.[11] The reason for this interindividual variability is still unknown. Perhaps they are due to a combination of effects: variable drug clearance from the circulation (renal and/or liver function, although within normal limits, may

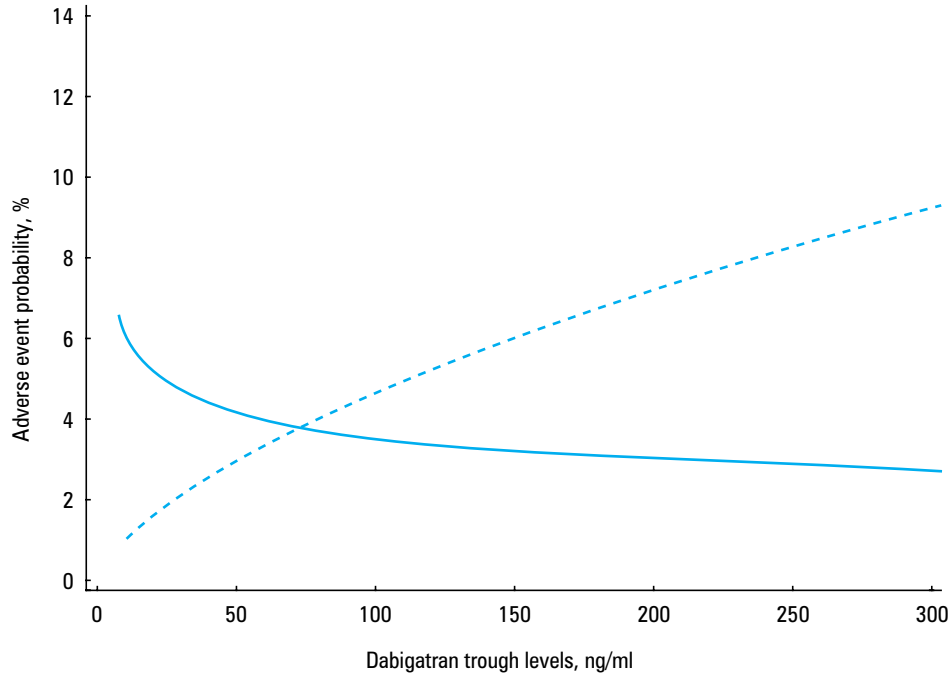

FIGURE 2 Relationship of the risk of ischemic stroke (solid line) or hemorrhagic events (dotted line) in patients of the RE-LY study. Redrawn from the data reported by Reilly et al.[5]

vary to some extent); variable effect of genetic polymorphisms on dabigatran metabolism; and other, still unknown, effects. Whatever the reason, the relatively large interindividual variability suggests that there might be room for dose adjustment. The second consequence is that dabigatran is relatively effective over a wide range of plasma concentrations, but it is not equally safe over the same range. Again, this suggests that some sort of dose adjustment would be useful to limit the risk of bleeding during treatment. Similar post hoc analyses have been carried out for other DOACs and cumulatively suggest that a subset of patients would probably benefit from some sort of dose adjustment based on the measurement of plasma DOAC concentrations. More recently, an investigation conducted during real-life management of patients with atrial fibrillation showed that patients who present with relatively low plasma DOAC levels are at risk of developing recurrent thrombosis, especially those who have the highest CHA_2DS_2-VASc score.[12] It should, however, be noted that notwithstanding the above considerations, there are no results from large randomized clinical studies to support the concept of dose adjustment based on laboratory testing, and until results from these studies become available, DOACs can be prescribed at fixed doses, based on patient characteristics.

Direct oral anticoagulants: measuring In specific circumstances, information on plasma DOAC concentrations may facilitate clinical decision making on patient management. These circumstances are discussed in order of relevance below.

Thrombolytic therapy Thrombolysis is the therapy of choice for patients with ischemic stroke.[13] However, it is associated with a nonnegligible risk of bleeding, which is presumably increased in patients who are on DOACs. Physicians at the stroke unit may therefore benefit from knowing the type and plasma concentration of DOACs to make appropriate therapeutic decisions: patients with relatively low levels may undergo thrombolysis and those with relatively high levels might undergo reversal of anticoagulation by administration of specific antidotes.

Surgical or invasive procedures It has been estimated that nearly 2% of the general population in Western countries are currently on oral anticoagulants. About 10% of these patients need surgical or invasive procedures during their life.[14] When the risk of periprocedural bleeding is deemed high, temporary discontinuation of anticoagulation is warranted. Recommendations on how to deal with the laboratory perioperative management of patients on VKAs are well established, and it is advised to test for the international normalized ratio soon before the procedure to confirm if circulating VKA levels are relatively low to minimize the risk of perioperative bleeding.[15] Conversely, recommendations for DOAC testing are sparsely reported,[16] and preprocedural DOAC testing is rarely advised.[17] The prevailing opinion is that patients undergoing surgical or invasive procedures should be evaluated for the risk of bleeding based on the individual characteristics and on the procedure to be performed. In high-risk patients or procedures, DOACs should be discontinued 2 to 3 days before the procedure, without mandatory laboratory assessment of DOAC concentrations. Whenever urgent surgery or invasive procedure is required and the risk of bleeding is deemed high, administration of antidotes (if available) is advised.

Very few recommendations advise laboratory testing as an option.[17] The main argument in favor of the no-testing strategy rests on the assumption

that owing to the relatively short half-life, it is anticipated that DOACs are cleared from the circulation if anticoagulation is stopped 2 to 3 days before the procedure provided that renal clearance is normal. However, there are no randomized studies to prove or disprove the safety of the strategy based solely on DOAC pharmacokinetics. Data from a relatively small observational study of patients on dabigatran concluded that the application of a standardized discontinuation protocol without preoperative dabigatran measurement is relatively safe.[18] However, post hoc laboratory analyses of blood samples obtained preoperatively showed that 20% of the patients included in the study had dabigatran levels that were still relatively high. The study was not powered to show if patients with relatively high dabigatran levels had increased rate of bleeding.[18]

Another study, PAUSE (Perioperative Anticoagulant Use for Surgery Evaluation),[19] is ongoing at a multicenter level and is aimed at evaluating the safety of the standardized discontinuation protocol. The study protocol also includes local DOAC testing.[19] The primary goal of the PAUSE study is to evaluate the safety of the interruption protocol, and the secondary goal is to assess the effect of the interruption protocol on the concentrations of residual circulating DOAC levels.[19] It is reasonable to believe that the study will eventually demonstrate that the interruption protocol is safe without DOAC testing. However, it is anticipated that the strategy will be valid for most patients, but the proportion of those who will not benefit from the strategy is difficult to predict. The question remains whether it is ethical to be concerned with the majority and forget about the minority of patients for whom testing would be beneficial. The disadvantage of testing (eg, costs, delayed interventions) would be negligible if compared with the burden of unwanted periprocedural bleeding events.

There are other arguments against DOAC testing before surgical or invasive procedures, as discussed below.

Renal function Assessing renal function is mandatory before starting treatment, as DOACs cannot be prescribed in patients with severe renal impairment. Although the Cockroft–Gault equation[20] is not completely adequate,[21,22] it is widely used for assessing creatinine clearance (CrCl) as a measure of renal function in candidates for DOAC treatment. However, it is not widely recognized that renal function may vary over time, especially in elderly patients.[23] Hence, to be on the safe side, CrCl should be measured soon before surgery or invasive procedure. Specific DOAC testing is undoubtedly a more direct indication of residual circulating DOAC levels than the surrogate measurement of CrCl.

Test availability Clinical laboratories in many countries are not yet equipped with specific tests for DOAC (see below), and a vast number of clinicians do not use them. Local hospital administrators do not include them in the list of routine laboratory parameters, and, consequently, local laboratories do not set them up. Furthermore, regulatory authorities have not yet issued recommendations on their use, and this is another reason for their poor use despite urgent need to do so.[24]

Test turnaround time To be useful, the results of testing should be promptly available. Dedicated tests for DOAC measurement can be set up in any of regular coagulometers that are standard equipment of average clinical laboratories. The methods are very simple and do not require more expertise than that required for testing the prothrombin time or antithrombin activity. A recent external quality assurance survey conducted in Italy showed that average clinical laboratories across the country performed DOAC assays for common quality control plasma with good accuracy and precision. The interlaboratory coefficient of variation (ie, 10%) compared favorably to that observed within the same exercise for the international normalized ratio.[25] Overall, DOAC testing is simple to run even in emergency situations, and the results may be available within 30 minutes.

Cutoff values The lowest DOAC level that allows a safe surgical or invasive procedure is unknown. Such data should be derived from clinical trials that are lacking and will probably never be conducted. Most recommendations advise that DOAC levels of less than 50 ng/ml can be considered relatively safe, and this cutoff should therefore be pragmatically adopted.

Adverse events In case of thrombosis or hemorrhage, clinicians may benefit from knowing if recurrent thrombosis or hemorrhage occurred when patients received adequate anticoagulation, or if they were underanticoagulated or overanticoagulated.

Reversal of anticoagulation When patients on anticoagulants present with life-threatening hemorrhage, anticoagulation should be promptly reversed by infusion of specific antidotes or coagulation factor concentrates. Idarucizumab has been registered by the FDA and EMA and can be used to neutralize dabigatran. Another antidote (ie, andexanet alfa) has been approved by the FDA for reversal of rivaroxaban or apixaban and is currently under examination by the EMA. Clinical trials have shown that the 2 antidotes are effective for anticoagulation reversal within minutes from administration in patients on dabigatran (idarucizumab), rivaroxaban, or apixaban (andexanet alfa).[26,27] The study protocol for both studies did not require DOAC measurement before antidote administration, and patients were treated solely because they presented with life-threatening hemorrhage while on DOACs. However, blood samples were collected at preinfusion,

and the post hoc DOAC measurement showed that nearly 25% of the patients in the idarucizumab study and 30% of those in the andexanet alfa study had relatively low concentrations of dabigatran or rivaroxaban/apixaban before antidote administration.[26,27] What follows is that a relevant proportion of patients would probably receive unnecessary antidote if DOACs were not measured before antidote administration.[28] Furthermore, recent observations have shown that patients treated with idarucizumab may occasionally present with rebound dabigatran levels a few hours after successful reversal.[29] Therefore, treating physicians would benefit from knowing plasma DOAC concentrations not only before but also after antidote administration.[30]

Extreme body weight Patients with extreme body weight have been excluded from clinical trials on DOACs. Therefore, it is still unknown whether the same dose is effective or safe for slim and obese patients alike. Considering that obesity affects nearly 20% of the general population and its prevalence is steadily increasing, a considerable proportion of overweight subjects worldwide will require anticoagulation. Testing DOACs in this population may help decide on the most appropriate dose for their treatment.

Drug-to-drug interactions Patients on oral anticoagulants often receive other treatments. Although the main effects of multiple treatment on DOACs have recently been reported,[17] the effect of some relatively common drugs is not yet well known. Testing DOACs before and a few days after the intake of drugs potentially interfering with DOACs would inform on whether there is a clinically relevant interaction.

Overdose DOAC testing is required whenever clinicians suspect that patients may be on overdose, regardless of whether bleeding complications are present.

Test selection for direct oral anticoagulants

Basic coagulation test Clinicians are familiar with the old and time-honored coagulation tests: prothrombin time (PT), activated partial thromboplastin time (APTT), or thrombin time (TT), and would more likely use them instead of the more specific and dedicated tests (see below) to assess the level of anticoagulation achieved by DOACs. Although these tests are (variably) affected by DOACs, their results are dependent on the type of drug used and on the composition of commercial reagents used for testing. For instance, there are DOACs that prolong (although variably) the APTT or TT (ie, dabigatran) or the PT (ie, rivaroxaban), but there are others (eg, apixaban or edoxaban) that do not. Furthermore, there are thromboplastins that prolong the PT of patients on rivaroxaban and others that do not.[31] Consequently, the indiscriminate use of the PT or APTT to assess anticoagulation levels for patients on DOACs could be misleading. For example, the observation of normal PT or APTT does not necessarily mean that there is no circulating drug if the reagent used for testing is insensitive. Conversely, abnormal PT or APTT would suggest (not affirm) that there is circulating drug, as the PT and APTT are global assays that are responsive not only to DOACs but also to abnormalities, such as liver disease, vitamin K deficiency, lupus anticoagulants, or mild congenital deficiencies of procoagulant factors. The exception rule is the regular (undiluted) TT. As mentioned above, this test is prolonged in the presence of dabigatran. However, the average plasma dabigatran concentration in a patient taking 150 mg twice daily makes the TT unclottable. Because of this excessive responsiveness, the TT cannot be used to measure the dabigatran concentration, but if it is within the reference range, it can exclude the presence of the drug.

Overall, we do not recommend the PT or APTT to evaluate anticoagulation in patients on DOACs unless the responsiveness of these tests to each of the DOACs has been established. This can be achieved by testing, with the local PT or APTT, a set of commercially available plasma calibrators at graded and certified DOAC concentrations to assess for the lowest DOAC concentration able to prolong the PT or APTT beyond the upper limit of the reference range.

Specific direct oral anticoagulant testing The gold standard for the measurement of DOAC concentrations is high-pressure liquid chromatography/mass spectrometry. However, this method is not easily available in most clinical laboratories, especially in emergency situations. Yet there are alternatives, which are described below and summarized in TABLE 1.

Diluted thrombin time The regular (undiluted) TT is excessively responsive to dabigatran. Appropriate dilution of the patient plasma into pooled normal plasma (usually 1:4) makes TT adequately responsive to dabigatran, with a clotting time prolongation linearly and dose-dependently related to a clinically relevant range of dabigatran concentrations. For example, 200 ng/ml of dabigatran (usually observed in a patient taking the dose of 150 mg twice daily) can prolong diluted TT twice over the baseline clotting time. The diluted TT test is commercially available from many manufacturers and can be conveniently used to quantify the dabigatran concentration.

Ecarin test Another commercial assay that can be used for dabigatran is based on ecarin. This is a snake venom extract able to convert factor II (prothrombin) into meizothrombin. The formation of meizothrombin is inhibited by dabigatran in a linear and dose-dependent manner, and the extent of inhibition can in turn be measured by a specific chromogenic substrate or clotting technique.

TABLE 1 Tests to measure plasma concentrations of direct oral anticoagulants

DOAC	Assays[a]
Dabigatran	Diluted thrombin time or ecarin clotting (or chromogenic) test
Rivaroxaban	Anti–factor Xa
Apixaban	Anti–factor Xa
Edoxaban	Anti–factor Xa

a Assays must be calibrated by using certified standards for each DOAC.

Abbreviations: DOAC, direct oral anticoagulant

TABLE 2 Reported effect of direct oral anticoagulants on the activity of the most common hemostatic parameters

Parameter	Effect
Antithrombin activity	Overestimation[a]
Protein C activity	Overestimation[b]
Protein S activity	Overestimation[c]
Coagulation factors	Underestimation
Fibrinogen	Underestimation in patients on dabigatran
Activated protein C resistance (APC ratio)	Underestimation[d]
Lupus anticoagulants	Difficult result interpretation
Factor XIII	Underestimation in patients on dabigatran

a Depending on the DOAC and on the method used for antithrombin measurement (see text for more details)

b Anticoagulant activity; the chromogenic activity not affected

c Anticoagulant activity; free antigen not affected

d With APTT-based methods

Abbreviations: APTT, activated partial thromboplastin time; others, see TABLE 1

Anti–factor Xa assays The general principle of these assays has been exploited for many years to measure the anti–factor Xa activity exerted by unfractionated or low-molecular-weight heparin. Appropriate dilutions of patient plasma into a suitable buffer are added with exogenous excess of factor Xa. Factor Xa is inhibited in a linear and dose-dependent manner by any of the anti–factor Xa inhibitors (rivaroxaban, apixaban, or edoxaban) present in plasma. The residual factor Xa is in turn measured by a specific chromogenic substrate. The absence of anti–factor Xa activity excludes the presence of clinically relevant plasma concentrations of anti–factor Xa inhibitors.

Plasma calibrators are commercially available for any DOAC. They are made of pooled normal plasma added with certified graded amounts of any DOAC. These plasma calibrators can be used locally to construct calibration curves from which plasma drug concentrations (expressed as ng/ml) can be derived by interpolation of optical density or clotting time. For proper result interpretation, accurate knowledge of the time elapsed from the last drug intake to blood drawing is mandatory.

The above methods are relatively easy to implement in any of the last-generation coagulometers available in clinical laboratories and do not require more expertise than that needed to run a PT or antithrombin activity tests. The between-laboratory performance is quite reassuring as shown by the good interlaboratory coefficient of variation observed in a recent nationwide proficiency program.[25]

Effect of direct oral anticoagulants on common hemostatic parameters Many clinicians order coagulation tests during oral anticoagulation. For example, the measurement of individual coagulation factors and thrombophilia parameters (such as antithrombin, protein C, protein S, activated protein C resistance, lupus anticoagulant, or factor VIII) could be occasionally ordered in patients who are on DOACs. It is important to be aware that DOACs may strongly interfere with the measurements of the above parameters, resulting in misinterpretation of the results. This is shortly described below and presented in TABLE 2. For a more detailed discussion, see Tripodi et al.[32]

Antithrombin Antithrombin activity might be considerably overestimated in a patient on dabigatran if testing is based on factor IIa (thrombin) inhibitory activity. Conversely, antithrombin activity in patients on rivaroxaban, apixaban, or edoxaban could be overestimated if the testing method is based on factor Xa inhibitory activity. The overestimation could result, for example, in misclassifying patients with congenital heterozygous deficiency as normal individuals.

Protein C and protein S Both naturally occurring anticoagulants, when measured for their anticoagulant activity, could be overestimated in a patient on DOACs. The chromogenic activity of protein C or the antigenic activity of protein S are not affected by DOACs.

Activated protein C resistance When the anticoagulant response to activated protein C is assessed by an APTT-based assay performed in plasma with and without the addition of exogenous activated protein C, the response could be underestimated in a patient on DOACs. The search for factor V Leiden mutation is not affected by DOACs.

Lupus anticoagulant The laboratory diagnosis of lupus anticoagulant is based on APTT and dilute Russel viper venom tests that require screening, mixing, and confirmation procedures. Tests and procedures are affected by DOACs, and the result interpretation is thus relatively difficult.

Coagulation factors Factor VIII is occasionally used as an additional parameter to assess thrombophilia. Its activity (as well as the activity of other procoagulant factors) can be variably underestimated in patients on DOACs. Similarly, fibrinogen and factor XIII can be underestimated in patients on dabigatran. Whenever possible, it is advised to test for the above parameters 4 to 5 days after discontinuation of DOAC anticoagulation.

Concluding remarks DOACs are the most obvious alternatives to VKAs for prevention of stroke and systemic embolism in patients with atrial fibrillation, as well as for treatment or prevention of VTE. Their use will be increasing within the next years. The management of a treated patient will be much better than in the past, but it will pose new challenges for many medical specialties, including internal medicine. Although there are hints suggesting that some sort of dose adjustment based on laboratory testing would be useful for selected patient subgroups, currently there are no clinical studies to recommend generalized adoption of laboratory testing and dose adjustment thereafter. However, it is increasingly appreciated, based on the literature and clinical practice, that laboratory testing aimed at measuring DOAC concentrations in specific situations is an important issue that deserves attention of regulatory authorities and hospital administrators. DOAC testing should be urgently made available to patients and clinicians.

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