Position Paper on laboratory testing for patients on direct oral anticoagulants. A Consensus Document from the SISET, FCSA, SIBioC and SIPMeL

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Abstract

Although direct oral anticoagulants (DOAC) do not require dose-adjustment on the basis of laboratory test results, the measurement of their anticoagulant effect is useful in special situations. This position paper issued by the Italian Scientific Societies that are mainly involved in the management of patients on DOAC is aimed at providing guidance to care-givers on which tests should be used and the situations in which testing is useful. The guidance is based on the data from the literature so far available and/or on consensus among experts.

Keywords: DOAC, anticoagulation, thrombosis, consensus.

Introduction

Direct oral anticoagulants (DOAC) represent a new class of oral anticoagulants which, unlike vitamin K antagonists (VKA), directly target activated coagulation factors[1]. Randomised clinical trials carried out over the last decade have shown that these drugs are effective and safe compared with VKA for treating or preventing venous thromboembolism or systemic embolism in patients with non-valvular atrial fibrillation[1]. Although DOAC do not require dose-adjustment on the basis of results of laboratory tests, the measurement of their anticoagulant effect may be needed in special situations. Spectrometry/high-pressure liquid chromatography is the standard approach for assessing DOAC plasma concentration, but these techniques are impractical in the general clinical laboratory. Global coagulation tests such as the time-honoured prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT), or dedicated tests such as the dilute thrombin time (dTT), anti-factor IIa (anti-FIIa), ecarin tests or anti-factor Xa (anti-FXa) assays have been developed for laboratory use over recent years. The aims of this position paper are to describe the tests that are presently available to assess plasma DOAC concentration and to provide guidance on their usefulness for the management of anticoagulated patients. This document represents a consensus statement of the Italian Society on Thrombosis and Hemostasis (Società Italiana per lo Studio dell'Emostasi e della Trombosi, SISET), the Italian Federation of Thrombosis Centres (Federazione Centri per la diagnosi della trombosi e la Sorveglianza delle terapie Antitrombotiche, FCSA), The Italian Society of Clinical Biochemistry and Clinical Molecular Biology (Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica, SIBioC) and the Italian Society of Clinical Pathology/Laboratory Medicine (Società Italiana di Patologia Clinica e Medicina di Laboratorio, SIPMeL).

The drugs

The DOAC currently licensed in the vast majority of Western countries are dabigatran (Pradaxa®), a pro-drug converted upon absorption into the active molecule that specifically targets free- and clot-bound thrombin, rivaroxaban (Xarelto®), apixaban (Eliquis®) and edoxaban (Lixiana®), which are direct FXa inhibitors. The main characteristics of these drugs are presented in Table I.

The tests
Global tests
Prothrombin time

The PT is the basic test of coagulation that measures the time (in seconds) for plasma to clot upon activation

Table I - Main characteristics of the direct oral anticoagulants currently licensed in Western countries.

Drug	Dosage to be prescribed (mg)	Cleared from circulation by	Time to peak after intake	Time to trough after intake
Dabigatran	[a]150 or 110 b.d.	Kidney (80%)	Approx. 2 h	Approx. 12 h
Rivaroxaban	[b]20 or 15 o.d.	Kidney (33%) and/or liver	Approx. 2 h	Approx. 24 h
Apixaban	[c]5 or 2.5 b.d.	Kidney (27%) and/or liver	Approx. 2 h	Approx. 12 h
Edoxaban	30 or 60 o.d.	Kidney (50%) and/or liver	Approx. 2 h	Approx. 24 h

[a]In the USA the lower dose to be prescribed is 75 mg b.d.; [b]15 mg b.d. in the first 3 weeks for treatment of venous thromboembolism; [c]10 mg b.d. in the first week for treatment of venous thromboembolism; b.d.: twice daily; o.d.: once daily.

with tissue thromboplastin and calcium chloride. The PT is a global test responsive to FVII, FX, FV, FII and fibrinogen, and may also be prolonged in response to circulating anticoagulants (e.g., lupus anticoagulants), high concentrations of unfractionated heparin or severe vitamin K deficiency. It is implicit from the above description that the PT can be prolonged by any of the DOAC that target FXa or thrombin, but also by other defects. The PT can be measured in a clinical laboratory with a large variety of commercial thromboplastins, which because of the source of tissue factor (tissue extract [rabbit, ox brain or human placenta], recombinant, etc.) and phospholipid composition, possess varied responsiveness to DOAC and or VKA[2,3]. The responsiveness to VKA does not invariably parallel that observed for DOAC[3]. Commercial thromboplastins that are very responsive to VKA, but not to DOAC, are not, therefore, infrequent[3]. The consequences stemming from the above observations are the following:

- any information derived using a given thromboplastin when testing patients on DOAC cannot necessarily be generalised to other thromboplastins, even if they have the same origin (extracted or recombinant);
- the international normalised ratio (INR) derived from a PT test that has been developed and validated for patients on VKA is not necessarily valid for patients on DOAC. Specific calibrations for the PT, with results expressed as INR, valid for rivaroxaban, have been proposed[3,4], but not yet implemented;
- whenever the PT is used for patients on DOAC, results should be expressed as a clotting time or ratio of patient-to-normal. The latter should be preferred as it is intuitive (the higher the ratio the greater the prolongation of the clotting time) and serves also to minimise (to some extent) analytical variability;
- when interpreting PT results in patients on DOAC, it should be considered that the test might be prolonged because of defects of coagulation other than the drug taken by the patient.

Activated partial thromboplastin time

The APTT is the basic test of coagulation that measures the time (seconds) for plasma to clot upon activation of the contact system of coagulation with particulate (kaolin or silica) or soluble (ellagic acid) substances, phospholipids as platelet substitutes and calcium chloride. The APTT is a global test responsive to all coagulation factors except FVII and FXIII, and is often prolonged in response to circulating anticoagulants (e.g., lupus anticoagulants) or to unfractionated heparin, and (less frequently) to low molecular weight heparin. Implicit from the above description is that the APTT test can be prolonged by any of the DOAC targeting FXa or thrombin, but also by other defects. Like the PT, the APTT can be measured in a clinical laboratory using a variety of commercial preparations that, because of the source of their reagents and phospholipid composition, show varied responsiveness to DOAC[5-11]. It is not, therefore, uncommon that commercial APTT reagents are variably responsive to DOAC. Furthermore, normal or shortened APTT due to high FVIII levels or other causes could attenuate or mask the effect of DOAC. The consequences stemming from the above observations are the following:

1- any information derived using a given APTT reagent when testing patients on DOAC cannot necessarily be generalised to others, even if they are apparently from the same origin;
2 - whenever the APTT is used for patients on DOAC, results should be expressed as ratio of patient-to-normal clotting time;
3 - when interpreting results of the APTT in patients on DOAC, it should be considered that the test might be prolonged because of defects of coagulation other than those stemming from the drug taken by the patients.

Thrombin time

The TT is the basic test of coagulation that measures the time (seconds) for plasma to clot upon activation with thrombin and calcium chloride. The TT is a global test responsive to fibrinogen (both low and dysfunctional fibrinogen) and to the presence of inhibitors of fibrin formation. The test is invariably prolonged in response to unfractionated

2

heparin. Implicit from the above description is that the TT can be prolonged by any drug that targets thrombin (e.g., argatroban, hirudin, dabigatran[12]), but also by other defects. The TT can be measured in a clinical laboratory using a variety of commercial preparations that, because of the source (human or bovine) and concentration of thrombin, show varied responsiveness to thrombin inhibitors. The consequences stemming from the above observations are the following:

1 - any information derived for a given TT reagent when testing patients on thrombin inhibitors cannot necessarily be generalised to others;
2 - whenever the TT is used for patients on thrombin inhibitors, the results should be expressed as ratio of patient-to-normal clotting time;
3 - when interpreting the results of the TT in patients on thrombin inhibitors, it should be considered that the test might be prolonged because of defects of coagulation other than those stemming from the drug taken by the patients.

Specific tests

There are specific tests useful to assess the plasma DOAC concentration which can be used according to the drug under evaluation.

Dilute thrombin time

The dTT has the same characteristics as its parent TT, but in the former, the patient's plasma is diluted in pooled normal plasma[13]. This dilution is mainly aimed to decrease the excessive responsiveness of the TT to thrombin inhibitors (especially dabigatran). The dTT can be easily set up in an ordinary coagulometer. It can be carried out even in emergency settings, with little expertise, and results can be expressed as ng/mL by interpolation of the patient's clotting time from a dose-response curve, constructed by testing plasma calibrators at fixed concentrations of the inhibitor being tested. Certified plasma calibrators and controls with target specific values for dabigatran are commercially available.

Anti-factor IIa assay

This is a chromogenic test that reflects the ability of plasma to inhibit thrombin (IIa). The test is carried out by mixing suitable amounts of patient's plasma with excess amounts of thrombin (IIa). Residual thrombin is then measured by a synthetic chromogenic substrate specific for thrombin. By definition, the smaller the residual thrombin, the greater the inhibitor concentration. The anti-FIIa assay can be carried out even in emergency settings, and results can be expressed as ng/mL by interpolation of the patient's chromogenic activity from

a dose-response curve constructed by testing plasma calibrators at fixed concentrations of the inhibitor under evaluation. Plasma calibrators for dabigatran are commercially available.

Ecarin tests

Ecarin is a snake venom extract capable of directly converting prothrombin (FII) into (meizo) thrombin, skipping the coagulation cascade upstream of prothrombin. The test can be conveniently run in two versions[14]. In the first, ecarin is mixed with the patient's plasma and the generated (meizo)thrombin is measured by a specific chromogenic substrate and the ensuing chromogenic activity. In the second, the generated (meizo)thrombin is measured by recording the clotting time of the mixture. Both the chromogenic activity and the clotting time depend on the concentrations of any of the thrombin inhibitors under evaluation. Results can be expressed as ng/mL of the relevant drug by interpolation of the chromogenic activity or clotting time from a dose-response curve. Plasma calibrators for dabigatran are commercially available and can be used to prepare local calibration curves.

Anti-factor Xa assay

This is a chromogenic test that reflects the ability of plasma to inhibit FXa[15]. The test can be carried out by mixing suitable amounts of patient's plasma with excess amounts of FXa. Residual FXa is then measured by a specific synthetic chromogenic substrate. By definition, the smaller the residual FXa, the greater the inhibitor concentration. The anti-FXa assay can be carried out even in emergency settings, with little expertise, and results can be expressed as ng/mL by interpolation of the patient's chromogenic activity from a dose-response curve constructed by testing plasma calibrators at fixed concentrations of the inhibitor under evaluation. Plasma calibrators for rivaroxaban, apixaban or edoxaban are commercially available and can be used to test the respective drugs.

Tests to identify the type of anticoagulant drug

Physicians in emergency departments need to know which drug is being taken by patients in order to make decisions on immediate anticoagulant neutralisation (antidotes or coagulation factor concentrates). This is critical when a patient is unconscious and has no personal cards reporting the type and dose of the drug being taken. This issue will become increasingly important as the number of drugs licensed for treatment increases over time. Simple, bedside assays[16] should be implemented to help physicians in emergency departments to make quick decisions.

Table II - Recommendations on tests to be used for direct oral anticoagulants.

Drug	PT	APTT	TT	dTT	Ecarin	Anti-FXa
Dabigatran	[a]No	[b]No	[c]Yes	Yes	[d]Yes	No
Rivaroxaban	[e]No	[f]No	No	No	No	Yes
Apixaban	[f]No	[f]No	No	No	No	Yes
Edoxaban	[f]No	[f]No	No	No	No	Yes

PT: prothrombin time; APTT: activated partial thromboplastin time; TT: thrombin time; dTT: dilute thrombin time; anti-FXa: anti-factor Xa activity.
[a]: Poorly responsive to increasing drug concentration and reagent-dependent; [b]: Responsive to increasing drug concentration and reagent-dependent; [c]: Strongly responsive even to low levels of the drug. If normal, it may be used to rule out significant circulating drug levels; [d]: Clotting or chromogenic; [e]: Moderately responsive to increasing drug concentration, but reagent-dependent; [f]: Poorly responsive to increasing drug concentration and reagent-dependent.

Recommendations

Based on the experience so far accumulated, the following recommendations can be made (see also Table II).

1 - Whenever possible refrain from using PT/APTT as standalone tests to assess the DOAC anticoagulant effect. These tests may be abnormal because of these drugs, but their prolongation is not unequivocally associated with the concentration of the drug and may produce misleading results[2,3,5-11]. There may be occasions (depending on the reagent used for testing) in which the tests are within normal limits even when there are clinically significant plasma concentrations of the drugs[11]. Alternatively, there may be occasions in which test results are beyond the upper limit of the normal range, yet the drug concentration is relative low[11]. The PT (if measured using certain thromboplastins) is more responsive to rivaroxaban than to dabigatran, apixaban or edoxaban. The APTT (if measured with certain reagents) is more responsive to dabigatran, but much less to rivaroxaban, apixaban or edoxaban. Whenever possible, laboratories should assess the responsiveness of their locally used PT/APTT reagents to DOAC by testing certified calibrators for each of them to identify the lowest concentration capable of prolonging the clotting time beyond the upper limit of the local normal range.

2 - dTT, ecarin tests and the anti-FIIa assay are the tests of choice to measure the plasma concentrations of dabigatran. Results must be expressed as ng/mL by interpolation of the clotting time or chromogenic activity from a calibration curve locally constructed by testing certified calibrators at increasing dabigatran concentrations.

3 - Given its excessive responsiveness to dabigatran[12], a TT within normal limits most likely excludes the presence of clinically relevant dabigatran concentrations, although a TT ratio (patient-to-normal) of 7.0 has been observed for plasma with relatively small dabigatran concentrations i.e., 40 ng/mL (Tripodi, *unpublished observation*).

4 - Anti-FXa assays should be used to measure the plasma concentrations of anti-FXa drugs, rivaroxaban, apixaban or edoxaban. Undetectable anti-FXa activity probably excludes clinically relevant drug concentrations.

Situations in which testing is useful

The dose of DOAC does not need to be adjusted on the basis of results of laboratory tests. This probably discouraged many laboratories from implementing testing in this setting until recently. Conclusive data on whether and when laboratory testing is needed are, therefore, lacking. However, there is increasing consensus among experts that laboratory measurements of the anticoagulant effect of DOAC may be useful in special circumstances[17-20], and guidance has been provided on this issue by scientific societies[18-20]. The following considerations are based mainly on the scanty data that are presently available and on common sense, keeping in mind the main precept of bioethics *"primum non nocere"* (first do not harm). Accordingly, we advise as follows:

1 - dedicated tests for DOAC should be urgently set up in all clinical laboratories and made available to clinicians[21];

2 - regulatory authorities should urgently approve their use for patient management albeit in special conditions[21];

3 - guidelines on how and when to do DOAC testing should be urgently implemented in local hospitals.

In this document, we discuss the potential usefulness of testing.

Initiation of treatment

Baseline laboratory evaluation (e.g., blood count, PT, APTT, liver function tests and [estimated] creatinine clearance [CrCl][22]) are mandatory for any patient before starting anticoagulation.

Surgical or invasive procedure

DOAC are characterised by a quick onset of action and short half-life. Hence, discontinuation of treatment a few days before an intervention (here called *"pharmacokinetic strategy"*) should limit the probability of having excessive circulating drug levels

4

that would increase the risk of bleeding. Safe adoption of this strategy requires evaluation of the risk of bleeding associated with the procedure and accurate knowledge of the time of the last intake of drug and gastrointestinal absorption. Furthermore, since the elimination of DOAC (especially dabigatran) is heavily dependent upon renal function, that must be assessed by estimation of the CrCl[22]. Because of unpredictable variations (especially in the elderly), CrCl should be estimated shortly before the procedure but nevertheless gives a surrogate indication of the residual circulating drug. Furthermore, CrCl may not be correlated with DOAC concentrations in plasma[23]. Laboratory testing for drug concentration carried out with dedicated tests would, therefore, be a more direct and valuable indicator of residual drug. There are comments in favour of the so-called *"laboratory strategy"*[24-26], but also others in favour of the *"pharmacokinetic strategy"*[27]. However, conclusive studies on this issue are lacking. Notably, one Canadian study showed that 80% of patients on dabigatran undergoing surgical/invasive procedures, who discontinued DOAC with a standardised protocol a few days pre-procedure had (post-hoc) APTT values that were within the normal range or negligible DOAC levels as measured by dTT.[28] The study was, however, underpowered to estimate the risk of post-operative haemorrhage. The authors concluded that the *"pharmacokinetic strategy"* is worth pursuing without significant risk for patients[28]. However, it is debatable whether one should overlook the possible risk incurred by the remaining 20% of patients in whom there were relevant drug concentrations in plasma at the time of the invasive procedure. There are arguments against the *"laboratory strategy"*. First, alarming values beyond which one should be worried are not precisely known. Given the limited experience with DOAC treatment in addition to the common practice of dismissing laboratory testing, alarming values have not yet been determined. It is, however, reasonable to assume that provisional cut-off values could be those that are smaller than the lower limit of detection of most assays (i.e., 30-40 ng/mL) or higher than 500-600 ng/mL, bearing in mind that the bleeding risk varies depending on the procedure being carried out. Second, the turnaround time for laboratory testing needs to be relatively short in order for testing to be useful. All dedicated DOAC tests are relatively simple to be set up and run, require little expertise and results can be available in less than 30 minutes.

Before thrombolytic therapy

It has been estimated that up to 2% of individuals with non-valvular atrial fibrillation treated with DOAC may develop acute ischaemic stroke each year, thus requiring thrombolytic therapy, which is associated with a >5-fold increased rate of intracranial bleeding[29]. Laboratory testing for DOAC would identify those patients in whom the risk of bleeding overcomes the benefit of thrombolytic therapy.

Adverse events

Patients may develop thrombotic or haemorrhagic adverse events while on anticoagulant treatment. On these occasions, treating physicians benefit from knowing if the patient is under- or over-anticoagulated. Hence, laboratory testing would be required.

Over-anticoagulation

Laboratory testing is useful whenever over-anticoagulation is suspected, even in the absence of overt clinical events.

Antidotes

Antidotes have been or are being developed both for dabigatran and anti-FXa drugs. Preliminary data from clinical trials showed that administration of these agents is effective and safe for patients admitted to emergency departments because of life-threatening haemorrhage[30,31]. The protocol adopted for those trials did not include measurement of DOAC concentration before the administration of antidotes. Post-hoc laboratory testing on plasma samples collected before administration showed that in 25-30% of the patients, the pre-infusion DOAC concentrations were relatively low[30,31]. Laboratory testing (if promptly available) would, therefore, be important for the treating physician to make decision on the proper use of antidotes[32]. A recent study showed that rebound effects of dabigatran are possible in some patients after neutralisation achieved by the recommended dose of idarucizumab[33]. It is, therefore, possible that in specific situations repeated doses of the antidotes are needed; hence it is useful to measure DOAC not only before, but also after administration of the antidotes.

Additional drugs

Additional drugs for which the interference with DOAC is unknown. Drug interference for DOAC is much less relevant than for VKA. However, there may be unknown effects for certain common drugs. Laboratory testing before and a few days after the initiation of new drugs may provide important information. DOAC testing may also be warranted in patients with an acute illness (e.g., pneumonia with dehydration/vomiting or diarrhoea).

In over- or under-weight subjects

The "one-dose-fits-all" strategy might not be generally applicable[34]. This aspect has not been

addressed by the registration clinical trials, as patients with extreme body weight were excluded. To learn more on this aspect, laboratory testing may be useful in patients who are obese or very slim.

Compliance

Laboratory testing could be potentially useful to assess adherence to treatment. However, in consideration of the short half-life of DOAC (8-15 hours), a dose missed a few days earlier than testing might not be detected.

Timing of testing

DOAC are characterised by a quick onset of action and short half-life. They reach peak levels in plasma approximately 2 hours after ingestion and trough levels after 12 or 24 hours for those drugs that are administered twice or once daily, respectively. The implicit consequence is that interpretation of testing for DOAC requires accurate knowledge of the time elapsed between administration of the last dose and blood drawing. Another important practical issue is the clinical relevance of peak or trough levels. Although scanty data are available, a recent study that measured dabigatran concentration for patients enrolled in the RE-LY trial showed that the association between bleeding events and drug levels is stronger at trough levels than at peak levels[35]. Similar information has been reported for edoxaban[36].

Reporting results

Results should be reported as drug concentration (ng/mL). It should be realised that there are no accurate DOAC plasma levels as determined with the current dedicated tests in patients on steady state. Laboratory professionals are, therefore, advised to report drug concentrations and to inform physicians on the presumed expected values in treated patients. Data from the technical annex of the respective drugs released by the European Medical Agency (EMA) may be taken as provisional information, when available[37-40]. In this respect, mean concentration (range) values to be expected at steady state have been reported for dabigatran, rivaroxaban and apixaban and are presented in Table III. Data on edoxaban were not reported in the EMA technical annex[40] and those in the literature are very limited. Ruff *et al*.[41] reported expected concentrations at trough after administration of 30 or 60 mg o.d. in a large study including 6,780 patients of the ENGAGE AF-TIMI trial (Table III).

Effects of direct oral anticoagulants on the most common haemostatic parameters

Besides modifying the global tests of haemostasis (PT, APTT and congeners, see above) DOAC may variably modify many of the most common parameters of haemostasis when they are measured as activity; antigens are not affected. It is important that prescribing physicians are aware of these interferences when interpreting test results (see also Table IV).

Antithrombin

Plasma antithrombin inhibitory activity is measured using thrombin or FXa as target enzymes. The method requires addition of excess amounts of either enzyme

Table III - Expected peak and trough levels at steady state reported for each direct oral anticoagulant by the technical annexes issued by the European Medical Agency for dabigatran, rivaroxaban and apixaban[37-39] or by the literature for edoxaban[40].

Drug and dose (mg)	Peak (ng/mL)		Trough (ng/mL)	
	Mean	*Range*	*Mean*	*Range*
Dabigatran 150	175	117-275	91	61-143
Rivaroxaban 20	215	22-535	32	6-239
Apixaban for prevention of VTE: elective hip or knee replacement				
2.5 b.d.	77	41-146	51	23-109
Apixaban for prevention of stroke and systemic embolism				
2.5 b.d.	123	69-221	79	34-162
5 b.d.	171	91-321	103	41-230
Apixaban for treatment of DVT, treatment of PE and prevention of recurrent DVT and PE				
2.5 b.d.	67	30-153	32	11-90
5 b.d.	132	59-302	63	22-177
10 b.d.	251	111-572	120	41-335
Edoxaban 30	n.a.	n.a.	27	15-45
Edoxaban 60	n.a.	n.a.	36	19-62

VTE: venous thromboembolism; DVT: deep vein thrombosis; PE: pulmonary embolism; n.a.: not available; b.d.: twice daily.

Table IV - The expected effect of direct oral anticoagulants on the most common haemostatic parameters (see text for more details).

Parameter	Expected effect
Antithrombin activity	Overestimation
Protein C[a], protein S[a] activity	Overestimation
Coagulation factors	Underestimation
Fibrinogen	Underestimation[b]
Activated protein C resistance (APC ratio)	Overestimation
Lupus anticoagulants	Difficult result interpretation
Factor XIII	Underestimation[b]

[a]: Anticoagulant activity; the chromogenic activity is not affected;
[b]: In patients on dabigatran.

to plasma and subsequent measurement of the residual enzymatic activity. By definition, the greater the residual enzymatic activity, the lower the antithrombin activity. If the patient is taking dabigatran and the target enzyme used for antithrombin measurement is thrombin, antithrombin activity will be overestimated, as thrombin activity will be inhibited not only by antithrombin, but also by dabigatran. The same overestimation will occur when the drug taken by the patient is an anti-FXa and the target enzyme used for testing is FXa. The extent of overestimation could be such to mask the diagnosis in patients with congenital heterozygous antithrombin deficiency[5-7,10,42]. To avoid these undesirable effects, laboratory testing for antithrombin in patients on dabigatran should be performed with methods using FXa as the target enzyme. Conversely, methods using thrombin as the target enzyme should be used for patients on anti-FXa drugs.

Measurements of protein C and protein S activity

DOAC may give rise to overestimation of the activity of protein C or protein S when measured by anticoagulant methods[7,10]. DOAC will increase the plasma clotting time, thus mimicking increased protein C or protein S inhibitory activity. The effect may be reagent-dependent as shown in a recent study on apixaban, in which three commercial platforms were compared[10]. There was no effect when protein C inhibitory activity was measured as chromogenic activity[7].

Coagulation factors

Underestimation of the clotting activity of individual coagulation factors is expected in patients taking DOAC[10, 43]. FVIII activity when measured with chromogenic assays might be underestimated in patients taking anti-FXa drugs. Finally, the presence of dabigatran or the anti-FXa drugs (rivaroxaban or apixaban)[44] in the tested plasma could lead to false-positive FVIII inhibitor Bethesda titres.

Fibrinogen

Fibrinogen activity is usually measured by recording the clotting time upon addition of thrombin to plasma (Clauss method). It is, therefore, not surprising that fibrinogen may be underestimated in patients taking dabigatran. The effect is, however, method-dependent[5]. Laboratories should check with the manufacturer for any possible interference before measuring fibrinogen in patients on DOAC.

Activated protein C resistance

Activated protein C (APC) resistance when measured with the APTT-based methods with and without activated protein C and results expressed as the APC ratio may be overestimated in patients on DOAC[5,6].

Lupus anticoagulant detection

Lupus anticoagulant (LA) is searched for by means of phospholipid-dependent tests such as the APTT or the dilute Russell viper venom test. These tests are variably affected by DOAC (see above), and the interpretation of LA detection in patients taking these drugs may be problematic[7,10,45]. It should be mentioned that there are reports in the literature on special procedures used to try to overcome the interference of DOAC on LA testing[46-48]. However, the reagents and methods employed are neither widely available nor standardised and cannot, therefore, be recommended for general use to search for LA in patients on DOAC. When the laboratory diagnosis of LA is strictly needed, temporarily switching the patient from DOAC to low molecular weight heparin (if the brand used locally does not interfere with LA testing) could be a valuable alternative.

Factor XIII

Functional FXIII can be underestimated in patients on dabigatran[49].

Heparin

The measurement of heparin activity in plasma is based on the same principle as that used for the anti-FXa drugs. Hence, the presence of heparin may erroneously mimic the effect of anti-FXa drugs. To overcome this interference some commercial anti-FXa assays for DOAC have added heparin neutralisers. The laboratory should check with the manufacturer for the presence of such neutralisers in the method used locally.

Recommendations

1 - Clinicians prescribing tests and laboratory professionals should be aware that the most common haemostatic parameters may be affected by DOAC.
2 - Whenever possible testing should be performed after discontinuation of the treatment (4-5 days).

3 - If this is not feasible, results should be interpreted with caution (see above).

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