2021 Update of the International Council for Standardization in Haematology Recommendations for Laboratory Measurement of Direct Oral Anticoagulants

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Abstract

In 2018, the International Council for Standardization in Haematology (ICSH) published a consensus document providing guidance for laboratories on measuring direct oral anticoagulants (DOACs). Since that publication, several significant changes related to DOACs have occurred, including the approval of a new DOAC by the Food and Drug Administration, betrixaban, and a specific DOAC reversal agent intended for use when the reversal of anticoagulation with apixaban or rivaroxaban is needed due to life-threatening or uncontrolled bleeding, andexanet alfa. In addition, this ICSH Working Party recognized areas where additional information was warranted, including patient population considerations and updates in point-of-care testing. The information in this manuscript supplements our previous ICSH DOAC laboratory guidance document. The recommendations provided are based on (1) information from peer-reviewed publications about laboratory measurement of DOACs, (2) contributing author's personal experience/expert opinion and (3) good laboratory practice.

Keywords
► diagnosis management
► guidance
► direct oral anticoagulants
► laboratory

Introduction

In 2018, the International Council for Standardization in Haematology (ICSH) published a consensus document providing guidance for laboratories on measuring direct oral anticoagulants (DOACs).[1] Since that publication, several significant changes related to DOACs have occurred, including the approval of a specific DOAC reversal agent (intended for use when the reversal of anticoagulation with apixaban or rivaroxaban is needed due to life-threatening or uncontrolled bleeding), andexanet alfa (Andexxa in the United States and Ondexxya in the European Union) from Portola Pharmaceuticals Inc.[2,3] Betrixaban (Bevyxxa, Portola), the fourth direct factor Xa (FXa) DOAC was approved for use in the United States but has since been discontinued by the manufacturer and will not be addressed. In addition, this ICSH Working Party recognized areas where additional information was warranted, including patient population considerations and updates in point-of-care testing (POCT). The information in this manuscript supplements our previous ICSH DOAC laboratory guidance document.[1] The consensus recommendations provided are based on (1) information from peer-reviewed publications about laboratory measurement of DOACs, (2) contributing author's personal experience/expert opinion and (3) good laboratory practice.

Patient Selection for DOAC Testing

As with the first ICSH DOAC publication, whether or not patients should be tested is beyond the scope of this document.[1,4–6] However, laboratory staff should be aware of emerging publications conveying potential advantages of measuring DOAC levels (►Table 1). In addition to previously

Table 1 Indication for testing of direct oral anticoagulants (DOACs) according to the level of evidence for non-urgent situations

Indication	Rationale	Practical consideration	Source of information
Non-urgent situations			
Advanced age	Increased rate of bleeding events with age and increased susceptibility of bleeding events with DOAC accumulation	If done, plasma DOAC concentrations should be measured at trough, just before the next pill or capsule intake after 5 or more intakes to ensure the DOAC has reached its steady state. Plasma DOAC concentration should be in the range of concentrations observed in other populations	Post hoc analyses of safety outcome from phase 3 clinical trials and post-marketing observational studies. *NB: Data are lacking to show the benefit of adjusting the dose based on individual pharmacokinetic (PK) evaluation, but these data suggest that the optimal drug level varies with age*
Severe renal failure and dialysis dependence	Increased levels of DOAC reflected by increased C_{MAX} and AUC, especially for dabigatran, rivaroxaban and edoxaban. Apixaban seems less affected based on PK studies. Bleeding risk and bleeding-related death were increased significantly in these population compared with warfarin *NB: AHA, ACC, HRS and EHRA guidelines all refrained from supporting use of dabigatran, rivaroxaban and edoxaban in patients with chronic kidney disease (CKD) or on dialysis. Only warfarin and apixaban seems to be safer in these populations*	If done, plasma DOAC concentrations should be measured at trough, just before the next pill or capsule intake after 5 or more intakes to ensure the DOAC has reached its steady state. Plasma DOAC concentration should be in the range of concentrations observed in other populations	Post hoc analyses from phase 3 clinical trials and post-marketing observational studies *NB: Data are lacking to show the benefit of adjusting the dose based on individual PK evaluation, but these data suggest that the optimal drug level varies with renal function*
Prior interventions with high bleeding risk	To be on the safe side, intervention categorized as being at high bleeding risk should be done in patients with no or undetectable DOAC concentration. Using the PK approach would not ensure all patients will have cleared completely the DOAC as many variables could interfere with the elimination of DOACs. As some of the factors used to set up the PK approach also rely on surrogate biomarkers (e.g., serum creatinine or liver function), the most obvious and rationale solution could be the measurement of DOAC concentrations	Plasma DOAC concentration should be measured within a few hours before the intervention and planned surgical intervention should proceed when the level is considered low enough. Plasma DOAC concentration should be in the range of concentrations observed in other populations	Post-marketing observational studies *NB: there are currently no prospectively validated data with hard clinical endpoints on cut-off values of any coagulation test to guide the timing of elective or urgent surgery*

Table 1 (*Continued*)

Indication	Rationale	Practical consideration	Source of information
Non-urgent situations			
Body mass index (BMI above) 40 kg/m^2	For patient with BMI above 40 kg/m^2, if a DOAC is chosen, obtaining a peak and trough DOAC concentration estimate after at least 5 doses may be of interest to ensure the plasma concentrations are roughly within the range published for other patients *NB: It remains unclear whether adequate DOAC concentrations are achieved to be clinically effective. The majority of post hoc analyses showed reassuring data for patients up to 40 kg/m^2 but further data are needed in extreme obese*	If done, plasma DOAC concentrations should be measured at trough, just before the next pill or capsule intake and at peak after 5 or more intakes to ensure the DOAC has reached its steady state. Plasma DOAC concentration should be in the range of concentrations observed in other populations.	PK studies and expert opinion
Drug interactions	Numerous drug interactions have been described and investigated by the manufacturers, sometimes requiring dose adaptations. However, unknown drug interactions as well as multiple drug interactions may interfere with drug levels to a degree, which may have a clinical relevance. Evaluating DOAC levels in these conditions may identify drug accumulation or clearance	If done, plasma DOAC concentrations should be measured at trough, just before the next pill or capsule intake after 5 or more intakes to ensure the DOAC has reached its steady state. Plasma DOAC concentration should be in the range of concentrations observed in other populations	PK studies, case reports and post-marketing observational studies

indicated clinical situations (usually urgent situations) where DOAC measurements may be useful,[1,6] evidence is accumulating between drug exposure and clinical outcome (►**Table 2**).[7–11] Additional data, albeit low grade, may support other situations and patients who may benefit from DOAC assessment.[12,13] Included are patients with advanced age,[14,15] severe renal failure and dialysis dependence,[15,16] as well as patients with acute bleeding, to determine appropri-

ate reversal strategies and associated dosing required.[17–19] Some have also suggested DOAC measurements in patients the day prior to undergoing interventions with high bleeding risk (e.g., complex endoscopy, spinal or epidural anesthesia, thoracic surgery, abdominal surgery, major orthopaedic surgery or neurosurgery),[5,20–23] although it should be noted that this approach of measuring DOACs is currently not supported by clinical evidence and the relevance of the

Table 2 Indication for testing of direct oral anticoagulants according to level of evidence for urgent situations

Indication	Rationale	Practical recommendation	Source of information
Urgent situations			
Acute bleeding and determination of appropriate reversal strategies	Measuring the anticoagulant effects or plasma drug levels of DOAC can help determine their contribution to bleeding or to determine when it is safe to perform an urgent or unplanned intervention. Assessing potential rebound effect after administration of reversal agents *NB: Delaying antidote administration until coagulation test results are available may be detrimental in DOAC-treated patients with life-threatening bleeding, such as intracranial bleeding or in those requiring emergency surgery for life-threatening conditions such as a ruptured aortic aneurysm*	Measurement of plasma DOAC concentration should be done as soon as possible *NB: Recommendations for antidote administration are based on plasma DOAC concentrations. In patients with serious bleeding, a DOAC concentration > 50 ng/mL is considered sufficiently high to warrant antidote administration, whereas in those requiring an urgent intervention associated with a high risk of bleeding, antidote administration should be considered if the DOAC concentration exceeds 30 ng/mL*	Case series and expert opinions. Post hoc analyses from phase 3 clinical trials and case series

current threshold is questioned.[24] Specifically, although the "Perioperative Anticoagulant Use for Surgery Evaluation" (PAUSE) study reported acceptable bleeding rates with their clinically defined anticoagulant interruption strategies and defined thresholds, that is, analyses were done for residual DOAC levels $\geq$30 ng/mL and $\geq$50 ng/mL,[25,26] it is not known what DOAC level would be considered "safe" to undergo a surgical procedure or intervention and with the vast majority of patients, a wait time period appears to be safe.[25,26] With limited data on patients with a body mass index (BMI) >40 kg/m^{2}, DOAC pharmacokinetic and/or pharmacodynamic measurements in this population may be considered.[27,28] In addition, many elderly patients with non-valvular atrial fibrillation may acutely develop decompensated heart insufficiency with increase of liver enzymes, decreased intestinal blood flow and develop an unpredictable pharmacokinetic profile which may lead to an increased bleeding risk. DOAC measurements may be useful in detecting DOAC overexposure and bleeding risk, DOAC underexposure and thrombotic risk, and identifying previously undescribed and described drug–drug interactions, although this needs to be confirmed in larger cohorts.[29–31] It should be noted that paediatric patients may have lower DOAC levels than adults,[32] and modifications of anti-Xa methods may be required. In addition, discrete age-partitioned and age-appropriate reference intervals are likely needed for coagulation test in the paediatric population.[33]

Consensus Recommendations

- *This ICSH Working Party recognizes there are insufficient data to date for providing dose-adjustment recommendations based on DOAC levels alone. Nevertheless, DOAC measurements may identify potential excessive clearance or drug accumulation and could be used in situations where the benefit of such measurement is likely to outweigh the risk, for example, in non-urgent situations.*
- *Several categories of patients may benefit from DOAC level measurements to ensure they are within the concentration range observed in pharmacokinetic investigations during drug developments.*
- *If a DOAC measurement has been requested for urgent purpose, results should be provided within 30 minutes to aid in acute clinical decision-making.*
- *This ICSH Working Party encourages laboratories to provide DOAC measurements per clinical need. DOAC results must be used (and interpreted) in the context of patient history, DOAC type, DOAC dose, last dose and potential impact on clinical management (e.g., surgical intervention, bleeding, reversal strategies).*

DOAC and Laboratory Testing

The first ICSH laboratory DOAC guidance document already detailed test procedures or methods for quantifying DOACs such as the ecarin clotting time (ECT), dilute thrombin time (dTT) or anti-Xa measurements (►Fig. 1).[1] More methodological details can also be found elsewhere.[34–36] Of particular note, the ECT used in the dabigatran trials and the ECT range

cited in prescriber information are based on an ECT reagent concentration of 6 IU/mL.[35]

Interference of DOAC on Coagulation Assays

It has been widely shown that DOACs may interfere with coagulation testing, even at low DOAC concentrations.[37] Thus, even trough collections aimed to minimize DOAC concentration may be inadequate to completely eliminate drug interference in certain assays.[37] To ensure an undetectable DOAC concentration, a delay of 3 days or more (depending on DOAC, renal function and clinical situations) between the last intake and testing could be necessary. A longer delay is likely necessary for lupus anticoagulant (LA) testing with dilute Russell viper venom time (dRVVT) tests, due to the interference that may still be present when DOAC concentration is below the lower limit of quantification of the anti-Xa-based method (anti-Xa). However, due to high inter-individual DOAC variability and potential thrombotic risk, a wait period of 3 days may not be a suitable alternative unless bridging therapy (e.g., low-molecular-weight heparin [LMWH]) is considered.[38] Alternatively, in vitro removal of DOAC compounds from plasma prior to coagulation testing has been reported and may be more suitable.[39–47] DOAC-Stop (adsorbing agent, Hematex Research, Hornsby, Australia) and DOAC-Remove (activated carbon, 5-Diagnostics, Basel, Switzerland), both reportedly able to neutralize all DOACs with minimal effect on haemostasis tests, have been recently commercialized.[39–47] However, care should be taken, especially in LA testing, since in the reported studies, complete reversal did not occur in every sample and reversal varies among the different DOACs.[42,43,45,48,49] Some differential effects may be observed between use of DOAC-Stop and use of DOAC-Remove since these products are not identical or necessarily interchangeable.

Additionally, a slight procoagulant effect of DOAC-Stop has been shown in thrombin generation assays (TGAs) that use an intermediate concentration of tissue factor (i.e., around 5 pM). This procoagulant effect seems to be related to slight reduction in tissue factor pathway inhibitor (TFPI).[50,51] The elimination of DOAC presence in plasma using filters like the DP-Filter (5-Diagnostics) or the DOAC-Filter (Diagnostica Stago, France) showed promising results.[39,52] However, potential unintended filtration of coagulation proteins seen with other filtering mechanisms (e.g., von Willebrand factor) may occur, but the interference of DOAC-Stop or DOAC-Remove on these other coagulation proteins has also been found (e.g., interference on TFPI), impacting mainly TGA.[51] Lastly, new products are currently under evaluation that demonstrates low to no DOAC interference for LA detection.[53,54]

Interference of DOAC on Platelet Aggregation and Fibrinolysis Assays

Sokol et al demonstrated a reduction in thrombin-induced platelet aggregation with rivaroxaban and apixaban, a result different from a previous investigation with rivaroxaban.[55,56] This requires further investigations and
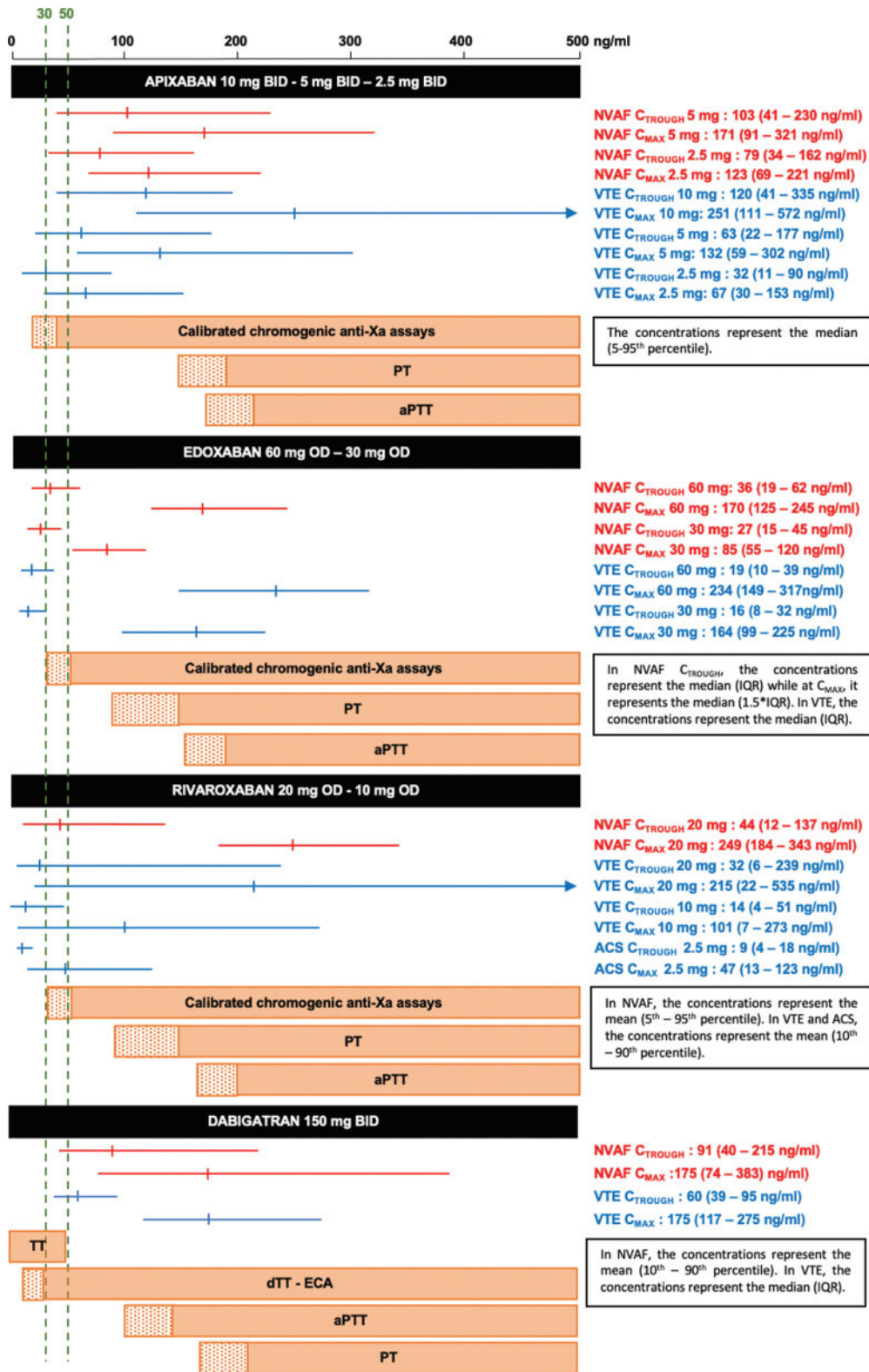
Fig. 1 Laboratory testing for direct oral anticoagulant (DOAC) and expected plasma concentrations after therapeutic doses. *Orange boxes* represent ranges of applicability of the corresponding test. *Dashed orange boxes* represent the zone in which the variability may change due to different reagent sensitivities. Note that only reagents considered as sensitive/reactive were considered. Plasma concentration ranges are extracted from the European Summary of Product Characteristics for all indications of apixaban[128] and dabigatran[129] and for VTE and ACS indications of rivaroxaban,[130] from Mueck et al for rivaroxaban in NVAF,[131] and from Ruff et al,[7] Weitz et al[132] and Verhamme et al[133] for edoxaban. (ACS, acute coronary syndrome; APTT, activated partial thromboplastin time; C_{MAX}, maximum plasma concentration during the dosing interval; C_{TROUGH}, minimum plasma concentration during the dosing interval; dTT, diluted thrombin time; ECA, ecarin chromogenic assay; IQR, interquartile range; NVAF, non-valvular atrial fibrillation; PT, prothrombin time; TT, thrombin time; VTE, venous thromboembolism.)

confirmations. As expected, a similar effect has been reported by Shimizu et al with dabigatran.[57] However, the interference with platelet aggregation is most likely an indirect effect of DOACs driven by the inhibition of thrombin generation.[58] Additionally, it has been shown that dabigatran, rivaroxaban, and apixaban enhance fibrinolysis, but this depends on the presence of thrombomodulin in the test system.[59–62] As such, caution should be used when performing and interpreting the results from any coagulation-related test from a DOAC-treated patient.[37]

Management of Heparin Bridging in DOAC Treated Patients

DOAC-treated patients may suffer an acute event that requires bridging with unfractionated heparin (UFH) or LMWH. For UFH bridging of dabigatran, only the anti-Xa activity should be considered suitable to measure UFH effect, as activated partial thromboplastin time (APTT) and (dilute) thrombin time will be prolonged by both drugs.[63] In the case of direct FXa inhibitors, alternatives to APTT or anti-Xa measurements are required, since both anticoagulant types affect these tests, leading to supra-therapeutic anti-Xa values.[64] Testing options to address this could include (1) an UFH-calibrated thrombin time test or (2) neutralizing the DOAC effect in vitro using aforementioned neutralizing products. In studies using drug-enriched plasma, DOAC-Stop extracts DOACs efficiently with no effect on heparin-type anticoagulants, but it binds argatroban and hirudin-type anticoagulants.[45] To date, data on the efficacy of UFH monitoring in the presence of such compounds or using thrombin time calibration curve are lacking.

DOAC and Thrombin Generation Assays

Global tests such as the TGA have been described as promising to assess the pharmacodynamic profile of anticoagulants.[65,66] Given the known DOAC thrombin generation profiles, the concentration thresholds proposed in the literature may provide highly different anticoagulant activities in a particular

patient and TGA may be seen as another way of expressing and assessing the degree of anticoagulation in DOAC-treated patients (►Fig. 2).[24,66–71] The ST Genesia (Diagnostica Stago, Asnières sur Seine Cedex, France), an automated analyzer for thrombin generation testing has the potential for a wide implementation in routine laboratories. Preliminary observations showed that thrombin generation testing is affected by all anticoagulant drugs, suggesting that this assay could be useful in assessing DOAC activity, but this deserves further confirmation in larger cohorts to validate this approach since to date, the role of TGA for clinical decision-making in DOAC-treated patients is not clear.[68,70–72]

Limitations of Laboratory Testing

Previously, the ICSH DOAC Working Party provided provisional guidance for the effect of DOACs on commonly ordered coagulation assays.[1] The limitations for assessing DOAC presence, pharmacokinetics or pharmacodynamics using screening or global assays, or other coagulation tests are still present, although the use of neutralizing systems appears promising. DOAC-neutralizing systems have not been fully evaluated for all tests or test platforms and their use and interpretations must employ a degree of caution.[1] Local verification of in vitro neutralizing agents (activated charcoal or filters) to assure (1) adequate DOAC neutralization by using sensitive techniques and (2) no deleterious effect on the test method is required prior to clinical use.

Consensus Recommendations

- *Caution should be used when performing and interpreting the results from any coagulation test result from a DOAC-treated patient.*
- *In vitro use of DOAC-neutralizing agents must be used with caution and must be locally verified prior to clinical use.*
- *Select thrombophilia test methods (e.g., clot-based measurement of protein C or LA) can show interference at low DOAC concentrations. Use of DOAC-neutralizing products*
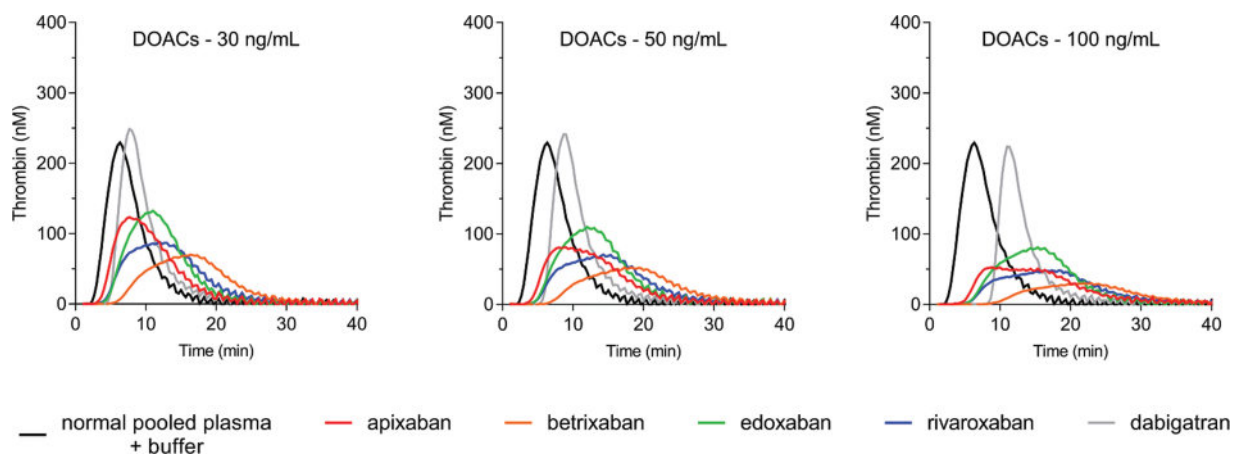

Fig. 2 The thrombogram parameters from thrombin generation test and representative changes at relevant concentrations of direct oral anticoagulants (DOACs). Note: Thrombin generation was triggered by 5-pM tissue factor with 4-μM phospholipids in absence of exogenous thrombomodulin or exogenous activated protein C.

may not completely achieve DOAC reversal; thus results from some assays may refrain factitiously elevated despite neutralization.

- *DOAC removal systems appear to be a suitable in vitro means of neutralizing DOAC from plasma to minimize drug interference in coagulation testing, although it is unclear whether these DOAC removal systems are interchangeable with DOAC neutralization products.*
- *Laboratories should have a procedure for adequately assessing and differentiating anticoagulation effect when bridging therapy is required.*
- *Although preliminary results are encouraging, there is currently no sufficient evidence to recommend TGAs to guide clinical decisions in a DOAC-treated patient.*
- *Laboratories should be aware of limitations of laboratory testing for DOAC measurements and/or effect of DOACs on coagulation assays.*

DOAC Reversal Agents

Andexanet Alfa

Since the initial publication of the guidance, a specific reversal agent, andexanet alfa, was approved in the United States for rivaroxaban and apixaban when reversal of anticoagulation is needed due to life-threatening or uncontrolled bleeding, and has also been approved by the Committee for Medicinal Products for Human Use in Europe for the same application.[3,73] There are two dosing strategies (low and high dose), as a bolus followed by a continuous infusion. This reversal agent will reduce the levels of direct FXa inhibitors, as measured by calibrated chromogenic anti-Xa activity.[74] There is a transient rise in prothrombin F1 + 2 fragments and D-dimer value after andexanet alfa is given, and an increase in thrombin generation, which may be related to the observed concomitant TFPI inhibition.[2,3,75,76] Whether this observation is due to the inhibition of TFPI or whether it is clinically important is not yet known and deserves some caution.

Idarucizumab

Idarucizumab is a specific reversal agent for dabigatran and is indicated in adult dabigatran etexilate–treated patients with dabigatran etexilate when rapid reversal of its anticoagulant effects is required such as in emergency surgery or urgent procedures and in life-threatening or uncontrolled bleeding. It has been approved for the same indications in both the United States and Europe based on the results from the REVERSE-AD study, which showed the efficacy and safety of idarucizumab to reverse the anticoagulant effect of dabigatran in dabigatran-treated patients who experienced serious bleeding or required urgent invasive procedures.[77–79] Although the product has been on the market for more than 3 years, only a small case series and four large studies evaluated its safety and efficacy in a real-world setting.[80–84] No specific dabigatran monitoring is currently recommended before reversal or during follow-up according to the prescribing information as approved by the regulatory authorities.[77,78]

DOAC Reversal Agents and the Laboratory

As andexanet alfa reduces the DOAC level after bolus and/or infusion, but DOAC levels recover following cessation of infusion, it can be speculated that post-infusion coagulation tests may be affected (for rivaroxaban, the residual drug level after andexanet alfa treatment was ∼40% from pre-treatment levels, a concentration that can still affect coagulation tests).[85] Evaluating post-infusion rivaroxaban or apixaban anti-Xa measurements is not supported by current Food and Drug Administration (FDA) recommendations as they indicate that the likelihood of using anti-FXa activity as a surrogate endpoint to predict a clinical benefit of haemostasis is not evident.[3] However, pre-treatment DOAC measurements may be warranted[86] to determine whether the low- or high-dose regimen should be used, as well as providing the potential to avoid unnecessary patient exposure to reversal antidotes.[87] However, this cannot be detrimental to the patients and should not delay the administration of reversal agents, especially in DOAC-treated patients with life-threatening bleeding, such as intracranial bleeding or in those requiring emergency surgery for life-threatening conditions such as a ruptured aortic aneurysm. In such context, rapid POC device with appropriate clinical performance is highly needed to guide the best strategy for patient's management.

It should be noted that the current dosing recommendations of andexanet alfa are based on both the dose and the time since the last intake of apixaban and rivaroxaban.[2,3] However, in an unconscious patient, such information cannot always be obtained. The plasma concentration of apixaban or rivaroxaban could be of interest in this context, but the definition of specific thresholds based on these plasma concentrations at the time of the admission is not yet available. Otherwise, specific tests are required in the unconscious patient to discriminate between the type of anticoagulant (IIa or Xa inhibitor) and could be useful to follow the efficacy of andexanet alfa administration. Several POC devices are currently under investigation that may prove useful in this setting (see the section on POC device below). Importantly, commercially available anti-FXa assays measure FXa inhibitors using drug-specific calibrators and controls. However, there are limitations when these assays are used for measuring DOAC concentration in andexanet alfa patient samples. One of the limitations is the large sample dilution in the assay set-up, which causes dissociation of the inhibitor from the andexanet alfa-inhibitor complex (due to the reversible binding equilibrium of the andexanet alfa inhibitor), resulting in an erroneous elevation of the anti-FXa activity following andexanet alfa administration. Therefore, some anti-Xa assays may have to be modified to be utilized if chromogenic anti-Xa assays are used to evaluate the degree of reversal of andexanet alfa.[88]

For dabigatran reversal, a single dose of idarucizumab (Praxbind, Boehringer Ingelheim) will bind up to 1,000 ng/mL of the drug, but there appears to be a rebound or dissociation effect after 12 to 24 hours. As such, measurements of dabigatran may predict the need for secondary dosing of this reversal agent.[19] In a retrospective study, it has been shown that the

assessment of dabigatran levels before introducing the reversal therapy could predict the haemostasis effectiveness and the potential rebound in dabigatran levels after idarucizumab injection and that specific dabigatran threshold (i.e., 264 ng/mL as reported in this study) may be of interest to predict haemostatic ineffectiveness, dabigatran rebound, and outcomes after reversal.[80] Idarucizumab has no known impact on coagulation parameters by itself.

Other agents that have been used for DOAC reversal include three- or four-factor prothrombin complex concentrates (PCCs) or activated PCCs.[89–94] These non-specific reversal agents are expected to have an impact on coagulation screening tests but not on anti-Xa- or anti-IIa-based assays, but data are currently limited with DOAC reversal strategies. The amount of PCC needed to stop DOAC-induced bleeding may depend on the residual DOAC concentration at the time PCC is administered.[95] However, although clinical bleeding may be sufficiently controlled with a single dose of PCC, the impact of DOAC on some laboratory tests may not be completely abolished as the relationship between residual DOAC level as measured by laboratory testing and the risk of uncontrolled bleeding is currently unclear.[18]

Consensus Recommendations

- *For andexanet alfa, due to its pharmacodynamic profile, the use of anti-Xa techniques for the evaluation of post-infusion rivaroxaban or apixaban anti-Xa activity is not supported.*
- *Post–andexanet alfa treatment, testing of apixaban and rivaroxaban concentrations is affected by anti-Xa methods that use high sample pre-dilutions causing factitiously elevated FXa DOAC results.*
- *For idarucizumab, measurements of dabigatran may predict the need for secondary dosing of this reversal agent since the presence of idarucizumab does not seem to interfere with dabigatran.*
- *It is currently unclear how to best assess the reversal efficacy of specific antidotes (i.e., andexanet alfa or idarucizumab) using laboratory tests and requires further investigation.*
- *PCC administration should not be monitored by measurement of DOAC concentrations that will not be modified.*
- *Assessment of DOAC reversal by global or specialized laboratory assays is method dependent and may be misleading.*

DOAC Point-of-Care Testing

The widespread use of DOACs and the need for urgent determination in aforementioned specific clinical situations have spurred several investigators and manufacturers to pursue POCT technologies for measuring (or quantifying) DOAC effect.[96–98] Included are microfluidic technologies[98–101] and surface acoustic wave (SAW) technologies.[102] Although the preliminary findings are promising, shortcomings include use of an animal model,[101] or in vitro enriched DOAC blood,[99] data from a small series of patients[99,100] and only a limited number of DOACs assessed.[99,101,102] In addition, these methods appear to be several years from actual clinical implementation, as none have undergone the rigors of in vitro device (IVD) clinical trials.

The TEG 6s NOAC assay is a cartridge currently undergoing clinical trials which can be used for qualitative DOAC assessment.[103–105] The four-channels, single-use NOAC cartridge contains kaolin in channel 1, ecarin in channel 2, FXa in channel 3, and abciximab in channel 4, with channels 2 and 3 providing differentiation in DOAC effect of prolonged clotting times. In a small series of patients receiving dabigatran, rivaroxaban, or apixaban, the receiver operating characteristic (ROC)analysis yielded a sensitivity of 94 and 92% for channel 2 (dabigatran) and channel 3 (direct FXa inhibitors), respectively.[105] Since the last publication,[1] Harenberg et al published recommendations regarding the use of a urine dipstick device which was shown to be sensitive and specific to determine the presence of both FXa and factor IIa inhibitors in urine samples. The evaluation of the DOAC dipstick test in emergency medicine and other patient groups is currently ongoing. This device allows qualitative determination of direct thrombin or FXa inhibitors and may aid in generating algorithms for clinical decision-making in a bleeding patient or for a patient requiring urgent surgical intervention in conjunction with laboratory plasma-based assays.[106,107] However, cautious and informed use of this urine DOAC screening method is required, as there is no direct relationship between plasma and urine DOAC concentrations despite the excellent sensitivity and specificity of the device. In any case, if DOAC is detected in the urine by the dipstick device, it should be confirmed with more specific testing to confirm the presence of DOAC in the blood.

Although not specifically a POCT, dried blood spot (DBS) technology may be a suitable alternative to traditional blood collection for non-emergent assessment of DOACs.[98,108] This method would allow for at-home collection using finger stick blood collection onto filter paper, which is then sent via postal service to a laboratory that can provide a quantitative DOAC level determination using tandem mass spectrometry. However, it must be emphasized that mass spectrometry testing using DBS must also be validated using DBS-collected samples. In addition, the haematocrit level of blood may cause systematic bias in analyte measurement in DBS samples, and it is also a practical challenge to train and ensure appropriate DBS collection procedures being performed by in-home patients since inappropriate DBS collection can cause significant variability in assay measurement. However, volumetric absorptive microsampling (VAMS), a recent microsampling technique used to obtain dried specimens of blood, promises to bring some significant advantages over DBS, related to sampling volume accuracy, haematocrit (HCT) dependence, pre-treatment and automation.[109] We also must emphasize that the lack of availability of liquid chromatography with tandem mass spectrometry (LC-MS)/MS in smaller laboratories, long turnaround time, cost and labour-intensive sample preparation restrict the use of this strategy in most laboratories. However, if the testing is not urgent, the VAMS collection device can be sent to a reference laboratory which can provide standardized and validated DOAC analyses overcoming the potential geographical limitations.[110]

Consensus Recommendations

- *Tests and technologies of various POCT devices may provide totally different type of results.*
- *Global coagulation POCT like SAWs and thromboelastometry are promising for identifying the drug on board, but their usefulness to evaluate the degree of anticoagulation is still unclear and further investigations are warranted.*
- *Rapid urine testing may rapidly identify the DOAC type taken, which may assist clinical decision-making.*
- *DBS and VAMS technology may be of interest to perform pharmacokinetic investigations without suffering from geographical limitations and rapid access to specialized laboratories.*

External Quality Control

Most international external quality control (EQA) programmes now have established EQA exercises for DOACs and demonstrate a wide implementation of specific DOAC testing in certain regions of the world.[111–118] Nevertheless, in regions where the regulatory authorities have refused the approval of these specific kits, access to drug measurements may be limited to specialized laboratories. In addition, only few undertake the in-house validation of these techniques refraining the clinicians to ask for these specific drug measurements. This is detrimental to the patients, especially knowing the limitations of routine coagulation tests for DOAC testing which are used instead. These routine tests showed a poor analytical and clinical performance in the different clinical settings where DOAC measurement may be beneficial.[6,119]

Some international EQA programmes have also undertaken and published studies looking at DOAC interference in haemostasis tests.[111–118] Some have also undertaken studies looking at neutralizing the interference of DOACs in haemostasis tests.[120] Although differences were seen between the various methodologies, reliable and reproducible DOAC levels were measured overall. A 5-year overview of experience for the quality performance of DOACs over a large concentration range showed a good correlation between the different methodologies. Although no international calibration standards were available, the overall coefficients of variation (CVs) were small for dabigatran, rivaroxaban and apixaban, and were also comparable to the CVs (range: 3–14%) for the international normalized ratio derived from the same years.[113]

The outcome for the various methodologies in the EQA surveys could be used to establish clinical decision rules adapted for specific reagents. This is especially relevant in the ranges approaching clinical decision limits. Laboratories are strongly encouraged to participate in EQA programmes that adequately address the pharmacodynamics and pharmacokinetics of DOACs, as well as the identification of DOAC sources of interference in other coagulation assays.

Consensus Recommendations

- *Laboratories are strongly encouraged to participate in EQA programmes that assess DOAC effects on screening tests and quantitative measurements, as well as their interference in other coagulation assays.*
- *Collecting information on DOAC testing availability and performance around the world is necessary to help various working parties to provide guidelines.*

Future Perspectives

emergence of DOACs and their increased use as well as the introduction of anticoagulants in future will provide a challenge for clinical laboratories. It is likely that DOAC use will increase as clinical trials are currently in the process for paediatric use. Dabigatran use in paediatric patients with VTE demonstrated non-inferiority to standard treatment.[121] Rivaroxaban use in paediatric patients (Einstein-Jr clinical trial, NCT02234843) is completed and awaiting approval for use in cerebral venous thrombosis[122] and catheter-related VTE.[123] Summary of the use of rivaroxaban in the paediatric population is also available elsewhere.[124] Apixaban is being evaluated in VTE reduction in paediatric patients with congenital heart disease[125] and acute lymphoblastic.[126] Edoxaban is currently under investigation for use in paediatric patients at risk of thromboembolic complications due to heart disease (www.clinicaltrials.gov; NCT 03395639). Other DOAC clinical trials include use for VTE prevention in patients with cancer (clinicaltrials.gov; NCT03240120; NCT03692065), post-bariatric surgery (clinicaltrials.gov; NCT03522259; NCT02406885), SARS-CoV-2 infections (clinicaltrials.gov; NCT04757857; NCT04650087; NCT04542408) and others will likely increase the use of DOACs once efficacy has been established.

In addition to the increase in use of DOACs in multiple settings with unclear expected "on-therapy" ranges and drug detection requirements, other technical considerations and concerns for the clinical laboratory would be the other anticoagulants under investigation.[127] As these drugs effect in vivo anticoagulation, it is likely their ex vivo effect will also add another layer of complexity and concern for the clinical coagulation laboratory. The hope and promise of POC methods with increased sensitivity and specificity for novel anticoagulants, including DOACs, may alleviate some burden on the laboratory.

What is known about this topic?

- Direct oral anticoagulants are used worldwide for several thromboembolic indications.
- The 2018 ICSH document provided haemostasis-related guidance for clinical laboratories.
- This study addressed all phases of laboratory DOAC measurements.

What does this paper add?

- This guidance updates the 2018 edition with a particular focus on antidotes, POCT and global coagulation tests

Conflict of Interest
Among the authors, J.D. is the CEO and founder of QUALIblood s.a., a contract research organization manufacturing the DP-Filter, is a co-inventor of the DP-Filter (patent application number: PCT/ET2019/052903) and reports personal fees from Daiichi Sankyo, Mithra Pharmaceuticals, Stago, Roche and Roche Diagnostics outside the submitted work. E.L.-L received lecture fees and consulting fees from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb-Pfizer, Daiichi Sankyo, Portola, CSL Behring, Leo and Aspen. She received external funds for conducting a clinical contract study from Bayer and Daiichi Sankyo, for a research project that she initiated from Bayer AG, Bristol-Myers Squibb-Pfizer, Daiichi Sankyo and CSL Behring. E.J.F. and S.M.B. have no conflict of interest. I.G.T. received consulting fees from Bayer, Boehringer Ingelheim and Bristol-Myers Squibb-Pfizer. R.C.G. reports personal fees from Diagnostica Grifols, Siemens Healthcare Diagnostics and Diagnostica Stago, and has provided expert testimony on dabigatran and rivaroxaban testing.

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