Clinical pearls: Laboratory assessments of direct oral anticoagulants (DOACS)

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Summary
Direct oral anticoagulants (DOACS) are used for stroke prevention in patients with atrial fibrillation as well as for prophylaxis and treatment of venous thromboembolism. Clinicians who treat, or may encounter, patients with DOAC exposure, should be aware of the limitations of coagulation testing in this setting, and seek counsel from their laboratory to understand the effects of DOACS on coagulation results. Generally, assays that employ clot based principles, or methods that require thrombin or Factor Xa activation or substrates may be affected by the presence of DOACS. The clinical laboratory should have an algorithmic testing plan for adequately assessing the presence of all DOACS and readily provide this information to clinicians. We describe Clinical Pearls for DOAC assessment using common and esoteric coagulation testing.

Schlüsselwörter
Dabigatran, Rivaroxaban, Apixaban, Edoxaban, direkte orale Antikoagulanzien

Zusammenfassung
Direkte orale Antikoagulanzien (DOAK) werden zur Schlaganfallprophylaxe bei Patienten mit Vorhofflimmern und zur Prophylaxe sowie Behandlung von venösen Thrombembolien eingesetzt. Ärzte, die Patienten unter DOAK-Therapie behandeln oder mit diesen konfrontiert werden, sollten sich über die Grenzen der Bestimmung der Gerinnungswerte unter diesen Bedingungen im Klaren sein. Sie sollten den Rat ihres Labors einholen, um die Wirkungen der DOAK auf die Gerinnungswerte richtig einzuschätzen. Generell können Assays, die auf funktionellen Clotting-Tests basieren, oder Methoden, die Thrombin oder Faktor Xa als Substrat verwenden bzw. deren Aktivierung erfordern, von DOAK beeinflusst werden. Das klinische Labor sollte über einen algorithmischen Testplan verfügen, um die Präsenz aller DOAK in geeigneter Weise zu beurteilen und den Ärzten diese Informationen umgehend zur Verfügung stellen zu können. Wir beschreiben klinische Perlen für die DOAK-Untersuchung anhand der üblichen Gerinnungstests wie auch selteneren Verfahren.

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Thromboembolic risk is increased in patients with atrial fibrillation, those undergoing orthopedic procedures, trauma, cancer, and other causes. Without adequate pharmaceutical prophylaxis, these patients may have arterial or venous thromboembolic events, including stroke, deep vein thrombosis and pulmonary embolism (1, 2). While the administration of heparin for short-term, and vitamin K antagonists for long term anticoagulation in these patients have not been entirely been supplanted, newer agents have been in use for nearly two decades, specifically low molecular weight heparin for short term anticoagulation, and the direct oral anticoagulants (DOACS) for long term anticoagulation (3).

One common theme with these DOACS is their predictable pharmacokinetics and pharmacodynamics (in most patient populations) and the promise of little to no routine laboratory monitoring (4–7). However, it is mandatory that the clinical laboratory has the capacity to either detect or quantify these anticoagulants or has access to a reference laboratory that can do so (8). In certain populations with known DOAC exposure (e.g. bleeding patients, emergent surgery, trauma, etc) the capacity to measure DOAC concentration in blood may assist the clinician in managing these patients (9). Additionally, in those facilities that may provide emergent services, patients with unclear medication history, or inability to provide a medication history (e.g trauma, stroke, etc), the capacity to screen for recent DOAC exposure may also guide management (e.g. lytic therapy in stroke patient, antidote administration). The notion of "routine" monitoring of patients on DOACS remains controversial and is outside the scope of this manuscript. We aim

to summarize the laboratory methods that can either detect the presence of, or quantify, DOACS in an effort to aide clinicians.

The DOACS

Direct oral anticoagulants are small molecules that selectively inhibit the active sites of activated factor X (direct FXa inhibitors) or thrombin (direct thrombin inhibitors), both of which are critical enzymes in the common pathway of coagulation (▶Tab. 1). Dabigatran is a direct thrombin inhibitor while rivaroxaban, apixaban, edoxaban and betrixaban inhibit activated factor X. Betrixaban, a direct factor Xa inhibitor, has recently FDA approved for thromboprophylaxis in adult hospitalized patients. There is limited laboratory data published to date on this drug. Other new direct oral anticoagulants anti-IIa (AZD0837) and anti-Xa (letaxaban, darexaban, eribaxaban) are under pre-clinical or dose-finding investigation (9).

DOACS are used predominantly for thromboprophylaxis in atrial fibrillation and treatment of venous thromboembolism. DOACS are administered at fixed doses. They have a wider therapeutic window than vitamin K antagonists and a better bleeding profile (10).

> Unlike heparins which are indirect inhibitors, DOACS inhibit both free and bound activated factors.

As a class, DOACS have rapid onset (2–4 hours) and are short acting with a plasma elimination half-life of about 12 hours in those with normal renal function. Due to their predictable pharmacodynamics and kinetic properties routine measurement for titration of dose is not required. Pharmacokinetics tends not to be influenced by age, gender, and body weight even if dose reduction is proposed for edoxaban in patients with low body weight (≤60 kg). They vary in their bioavailability, liver metabolism and renal excretion rate although all DOACS have some element of renal excretion (4, 11). They are all acted on by the hepatic and gastrointestinal transport protein, P-glycoprotein (P-gp) (4, 12). P-gp serves as a mechanism for intestinal excretion. Hepatic clearance is mediated through cytochrome (CYP) 3A4, CYP 2J2 as well as non-cytochrome dependent mechanisms, except for dabigatran which is not metabolized by CYP-dependent reactions. The administration of drugs that inhibit both P-gp and CYP 3A4 (such as ketoconazole of HIV protease inhibitors) can lead to drug accumulation.

> DOACS do not demonstrate interaction with food, although it is recommended that rivaroxaban should be taken during meals.

Dabigatran is administered as dabigatran etexilate which is a prodrug converted to dabigatran via hydrolysis by esterase. It is generally administered twice daily at 110 or 150 mg depending on renal function as 80 % is renally excreted (4, 13). Rivaroxaban is administered once daily and elimination occurs through renal and non-renal routes. Apixaban is administered twice daily and edoxaban once per day. Apixaban and edoxaban have multiple elimination mechanisms. Given the short half-life of DOACS, compliance with therapy is essential.

Tab. 1 Pharmacodynamic and pharmacokinetic properties of DOACS (9–14).

	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
target	thrombin	FXa	FXa	FXa
prodrug	yes	no	no	no
active metabolite	no	no	no	yes
antidote	FDA and EMA approved	FDA approved	FDA approved	not FDA/EMA approved
administration	once or twice daily	once daily	twice daily	once daily
renal excretion	80 %	30–40 %	20–30 %	30–40 %
half-life	14–17 h	7–11 h	10–14 h	9–11 h

FXa: activated form of coagulation factor X; FDA: Food and Drug Administration; EMA: European Medicines Agency

Laboratory testing and DOAC anticoagulation

Early communications from highly regarded laboratory or hemostasis organizations and journals included a number of generic statements relating routine screening tests, the prothrombin time (PT) and activated partial thromboplastin time (aPTT) with DOAC presence, sensitivity, or concentration (13–15). Specifically, these statements included the following general information: The PT is more sensitive to anti-Xa DOACS than the aPTT, and conversely dabigatran tends to affect the aPTT more so than the PT, and that the sensitivity to DOAC concentration depends on the reagent used in the assay (13, 15).

While this general information appropriately alluded to limitations and exceptions of the PT and aPTT, these limitations seem to be lost in translation in guidelines from other organizations. Publications from neurosurgical, surgical, anesthesia, critical care related to DOACS and screening coagulation tests tend to suggest the role of the PT and aPTT with and without caveats related to differences noted in reagent sensitivity between manufacturers (16–19). Based on our own institutional experiences, there is low likelihood that clinicians who practice outside the laboratory (e.g. surgeons, emergency department physicians, etc…) are familiar with the specific reagents used in the clinical laboratory, or reference laboratory that provide service to their patients. As such, a general promulgation of misleading information about the utility of these screening tests abounds.

It must be emphasized, that the PT and aPTT are tests that may be useful for screening purposes including assessing patients for factor deficiency, treatment efficacy (e.g. warfarin and other coumarin agents), treatment reversal (e.g. vitamin K,

(a)PCC or FFP), and presence of inhibitors. However, other tests such as the dilute thrombin time (dTT) (20), ecarin based methods (21, 22) (either ecarin clotting time (ECT) or the chromogenic variant (ECA)), and adapted anti-Xa assay (23–25, 60), are more specific to drug monitoring and assessment.

When calibrated with the appropriate DOAC, these methods have demonstrated acceptable or equivalent measurements when compared to accepted means for assessing drug concentration (e.g. mass spectrometry).

Drug calibrated dilute Russell's Viper venom time has demonstrated a linear response to DOAC concentration, both direct thrombin and FXa inhibitors, but conflicting lower level of quantitation data requires further investigation (26–28). Importantly, commercial calibrators and controls should be used specifically with the test for which they are designed and they cannot be used to calibrate or assess the sensitivity of global assays such as the PT and the aPTT. However, the combination of the screening and drug quantitation laboratory assays would optimize the assessment of a patient with known and unknown medication history (8).

Other methods for assessing DOAC anticoagulation include thromboelastometry (TEG or ROTEM), and urinary measurements of excreted metabolites (29–31). Both offer some potential utility in emergent situations. However, other causes for prolonged R time (TEG) or CT time (ROTEM) and the lack of correlation of urinary DOAC results with blood concentrations are potential limiting factors.

> Thus, quantifying DOAC exposure is technically possible but there are currently no established, "therapeutic" ranges. However, "on-therapy" ranges are available from the large phase-2/3 studies and can be taken into account in daily practice (32, 33).

In addition, experts in the field have proposed specific plasma drug concentration cut-offs to reduce the risk of hemorrhagic complications in the perioperative setting while others have proposed thresholds to guide the administration of reversal agents and these uses would require laboratory measurement (19, 34).

Clinical pearls with PT and DOACS

- Most PT reagents demonstrate a linear, drug dependent concentration effect with most DOACS (8, 35–40).
- All PT reagents demonstrate variable degree of sensitivity to DOACS (8, 35–40).
- PT is practically insensitive to apixaban (8, 41–43).
- Within a single manufacturer, PT reagents are generally more sensitive to anti-Xa DOACS than dabigatran, except for apixaban (5, 44).
- Use of commercial calibrators or controls to assess local sensitivity to DOAC may not be optimal for all reagent systems as commercial calibrators or controls may not have the appropriate matrix for a plasma-based assays (8, 45).
- Use of drug enriched normal plasma (i.e. plasma from healthy donor in which the drug is added in vitro) to assess reagent sensitivity to DOAC may not reflect the same reagent sensitivity as seen in patient samples (8).
- Correlation between *in vivo* DOACS plasma concentrations and PT prolongation depends on the reagent and is generally poor (38).

Clinical pearls with aPTT and DOACS

- Most aPTT reagents demonstrate a curvi-linear, drug dependent concentration effect with DOACS meaning the aPTT in seconds tends to plateau with increasing drug concentration (8, 25, 35–37, 39, 40).
- All aPTT reagents demonstrate variable degree of sensitivity to DOACS (8, 25, 35–37, 39, 40).
- Within a single manufacturer, aPTT reagents are generally more sensitive to dabigatran than anti-Xa DOACS (5).
- Use of commercial calibrators or controls to assess local sensitivity to DOAC may not be optimal for all reagent systems (8, 45).
- Use of drug enriched normal plasma to assess reagent sensitivity to DOAC may not reflect the same reagent sensitivity as seen in patient samples (8).
- Correlation between DOACS plasma concentrations in patients and aPTT prolongation in seconds depends on the reagent and is generally poor (46).

Clinical pearls: known or unknown DOAC exposure

PT or aPTT may not be sufficiently sensitive to rule out significant levels of drug (>30 ng/mL) (5, 44).

- Dabigatran affects the aPTT (for most reagents) more so than PT (for most reagents) (36, 37).
- STA®Neoplastine CI Plus is sensitive to rivaroxaban, but not apixaban (39–43).
- Dade Innovin® is insensitive to all anti-Xa DOACS (39, 40, 43).
- Apixaban impacts the PT at a given drug concentration to a lesser degree than rivaroxaban, edoxaban or betrixaban (42).
- *In vitro* studies may not reflect same degree of reagent sensitivity as samples from patients on DOAC therapy (8).
- Use of commercial calibrators and controls to locally assess DOAC sensitivity may be misleading to reagent sensitivity as compared to real patient samples (8, 45).

A prolonged PT and/or aPTT in a patient with known DOAC exposure should be considered to be secondary to the drug, until proven otherwise.

- Studies suggest mixing studies, factor assays in samples containing DOAC may gave factitious results (39, 47, 48).
- Antibiotic use may impact intestinal flora which may result in acute vitamin K factor deficiency, thereby affecting PT (and aPTT) (49).
- Lipoglycopeptide antibiotics may affect the PT and aPTT due to interference with phospholipids within PT/aPTT reagents (50).
- Lupus anticoagulants may result in prolonged aPTT (and with less frequency, the PT) (51).

Tab. 2 Effect of dabigatran on coagulation assays (5, 8, 13, 14, 20, 22, 26, 27, 32, 37, 41, 43, 45–47, 52, 53).

effect	clot based assays	chromogenic based assays	immunoAssays (e.g. LIA, ELISA)	clinical impact of reported test result
linear relationship between prolonged clotting time and increased reported result	• PT/INR[1,2] • aPTT[1,2] • thrombin time • ecarin based assays		none	• diagnosis and/or • management
relationship between dabigatran presence and factitiously decreased reported result	• fibrinogen[2,3] • factor activity[1] (II, V, VII, VIII, IX, X, XI, XII)		none	• (mis)diagnosis and/or • (mis)management
relationship between dabigatran presence and factitiously increased reported result	• inhibitor screen[1,2] • inhibitor assay[1,2] • lupus anticoagulant[1] • protein C activity [1,2] • protein S activity [1,2] • APCR [1,2]	• antithrombin[2] (thrombin substrate)	none	• (mis)diagnosis and/or • (mis)management
no effect	• reptilase time	• antithrombin (factor Xa substrate) • protein C activity • plasminogen activity • alpha-2-antiplasmin • factor XIII activity • factor VIII activity	• protein C antigen • total/free protein S antigen • VWF activity and antigen	none – desired testing, when clinically necessary or relevant

[1] reagent dependent
[2] concentration dependent
[3] For fibrinogen – if measured using the Clauss method, most reagents will not be affected. For PT-derived measurements, results are more likely to be factitiously increased.

- Compromised liver function or consumption coagulopathy may also result in prolonged PT and/or aPTT.

A prolonged PT and/or aPTT in a patient with unknown DOAC exposure would require further assessment, including, but not limited to:

- Mixing studies
  - Presence of inhibitor or factor deficiency
- Thrombin time (TT)
  - Markedly prolonged TT would suggest dabigatran presence especially if the TT does not normalize with heparinase and if the reptilase time is normal, but cannot completely exclude rare causes (e.g. dys-, hypo-, afibrinogenemia, inflammatory responses, to name a few) (52).
- Chromogenic anti-Xa test
  - Positive "signal" would suggest presence of anti-Xa class of drugs, including heparins or DOACS (41, 43, 46, 53, 54).
  - Cannot readily differentiate anti-Xa class of drugs, including heparins or DOACS, except for some tests, specifically designed to be insensitive to heparins (e.g. the Biophen Direct FXa inhibitors® assay).

> Consultation with appropriate laboratory personnel to interpret the test results in a patient with unknown or unclear medication history should be considered (8).

Clinical pearls: quantifying DOAC concentration

In order to properly interpret the results of such quantitative assays, it is essential to know the clinical situation for which the quantitative measurement is requested. Specific cut-offs have been established in perioperative setting to support the administration of reversal agents in high-risk procedures (i.e. 30 ng/mL) or in bleeding patients (i.e. 50 ng/mL) (19, 34). In any cases, it is mandatory to know the delay since the last dose was taken in order to know if the plasma concentration is increasing or not. In a random sample, comparison of the patient's plasma level with the results of on-therapy ranges observed in large phase-3 trials may be helpful (32).

If quantifying DOACS is required: Rapid methods calibrated using commercial or locally prepared DOAC plasma of known concentrations.

- Dabigatran – only commercially prepared calibration material would be acceptable as dabigatran is administered as a prodrug (dabigatran etexilate) that is converted into the active drug, dabigatran *in vivo*.
- Anti-FXa DOACS – commercially prepared or locally prepared calibration material using drug dissolved in Dimethyl sulfoxide (DMSO).

Rapid tests can be easily adapted and performed on most automated coagulation analyzers, and for clot based assays using

Tab. 3 Effect of anti-Xa DOACS on coagulation assays (5, 8, 13–15, 24–26, 28, 30, 32, 35, 38–42, 45, 48, 53, 56, 58).

effect	clot based assays	chromogenic based assays	immunoAssays (e.g. LIA, ELISA)	clinical impact of reported test result
relationship between prolonged clotting time and increased reported result	• PT/INR[1,2,3] • aPTT[1,2,3]		none	• diagnosis and/or • management
relationship between DOAC presence and factitiously decreased reported result	• factor activity[1,2,3] (II, V, VII, VIII, IX, X, XI, XII)	• factor VIII[2] • factor IX	none	• (mis)diagnosis and/or • (mis)management
relationship between DOAC presence and factitiously increased reported result	• inhibitor screen[1,2,3] • inhibitor assay[1,2,3] • lupus anticoagulant[1,2] • protein C activity [1,2] • protein S activity [1,2] • APCR [1,2]	• antithrombin[2] (factor Xa substrate) • unfractionated heparin, low molecular weight heparin, or • heparinoids/penta-saccharide (factor Xa substrate)	none	• (mis)diagnosis and/or • (mis)management
no effect	• fibrinogen • thrombin time • reptilase time • ecarin based assays	• antithrombin (thrombin substrate) • protein C activity • plasminogen activity • alpha-2-antiplasmin • factor XIII activity	• protein C antigen • total/free protein S antigen • VWF activity and antigen	none – desired testing, when clinically necessary or relevant

[1] reagent dependent
[2] concentration dependent
[3] Apixaban usually not affecting result

manual methods (e.g. tilt tube, fibrometer) as well:

- For dabigatran, drug calibrated assays to quantitate plasma drug concentration:
  - Dilute thrombin time (20, 37)
  - Ecarin clotting time (21, 37)
  - Ecarin chromogenic assay (22, 37)
  - Dilute Russell's Viper venom time (26, 27)
  - Chromogenic anti-IIa method (55)
- For anti-FXa DOACS drug calibrated assays to quantitate plasma drug concentration:
  - Chromogenic anti-Xa method (25, 28, 33, 38, 39, 41–43, 56)
  - Dilute Russell's Viper venom time (26, 27)

For non-emergent sample testing, in addition to methods described above, mass spectrometry, or equivalent, methods for quantifying DOACS could be considered, whether performed locally or at reputable reference laboratories (33, 57–59).

Clinical pearls: DOACS may affect a range of laboratory tests and caution must be used when interpreting these data

- Dabigatran presence may affect other coagulation assays: factitiously low or high results (▶Tab. 2).
- Anti-FXa DOACS may affect other coagulation assays: factitiously low or high results (▶Tab. 3).
- Test methods based on latex immunoassay (LIA), enzyme-linked immunoassay (ELISA), or chromogenic methods without thrombin or anti-Xa substrates or methodologies are not affected by DOACS presence.
- Coagulation tests affected by DOACS but are not required for assessing the anticoagulant effect of DOACS (e.g. used for diagnostic purposes) should be avoided.
- If diagnostic tests are required while on DOACS, a trough collection may minimize the DOAC effect, but a concomitant DOAC level would be useful in interpreting the test result.

Conclusion: DOAC anticoagulation and the clinical laboratory

It is essential for any clinician who treats, or may encounter, patients with DOAC exposure consults with the laboratory on any (normal or abnormal) coagulation test result(s) to discuss the merits and limitations of the test in that setting. Additionally, any coagulation assay that employs clot based principles, or methods that have thrombin or anti-Xa activation or substrates should be avoided or the results interpreted with caution. Lastly, it is essential that the clinical laboratory have an algorithmic testing plan for adequately assessing the presence of all DOACS that are approved for clinical

use, and provide the treating clinician with potential diagnostic strategies, especially in emergent situations, which may be necessary to provide optimal care and patient safety.

Comment

The authors recently published an overall laboratory guidance document: *International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. Thromb Haemost 2018; 118(03): 437–450.*
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Conflict of interest

RCG provides expert testimony for laboratory monitoring or rivaroxaban and dabigatran, advisory board for NovoNordisk, Roche Diagnostics; JD is the founder of QUALIblood s.a., reports personal fees from Stago, Daiichi-Sankyo, Roche Diagnostics and Roche, outside the submitted work; DA reports personal fees from Stago and Roche outside the submitted work, clinical advisor to Instrumentation Laboratory.

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