Management of Patients Treated with Direct Oral Anticoagulants – the Clinical Laboratory Perspective

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LEARNING POINTS

- Direct Oral Anticoagulants (DOACs) do not require laboratory testing for dose adjustment *("monitoring")*. However, *"measuring"* the anticoagulant effect of DOACs is occasionally required.
- *Measuring is required:* (a) before starting treatment, (b) before invasive/surgical procedures, (c) during adverse (thrombotic/hemorrhagic) events, and (d) before initiating thrombolytic therapy.
- *Measuring is potentially useful:* (a) after reaching stable therapeutic levels of long-term anticoagulation, (b) before and after reversal of anticoagulation, (c) to assess potentially interfering drugs, and (d) to assess dosage in patients with extreme body weights, and in patients with compromised kidney function.
- *Tests of choice*: (a) dilute thrombin time or ecarin clotting (chromogenic) test for dabigatran, (b) anti-Factor Xa activity or the prothrombin time with responsive thromboplastins for rivaroxaban, and (c) the anti-Factor Xa activity for apixaban.
- *Caution* should be exercised when interpreting the results of the most common hemostatic parameters, such as antithrombin, and fibrinogen, as DOACs may considerably affect measurement.

INTRODUCTION

Direct oral anticoagulants (DOACs) targeting activated coagulation factors have been developed and compared to low molecular weight heparins (LMWH) in patients undergoing orthopaedic surgery, for their efficacy and safety, and with warfarin in patients with venous thromboembolism or atrial fibrillation. Cumulatively, clinical trials have demonstrated that DOACs are at least comparable to standard treatment with the considerable advantage of oral administration and no need for laboratory testing to individualize dose-adjustment. Currently, three such drugs have been approved in many countries and will be increasingly used within the next few years ***(Table 1)***. They include dabigatran (Pradaxa®, Boehringer Ingelheim) targeting thrombin, and rivaroxaban (Xarelto®, Bayer) and apixaban (Eliquis®, Pfizer) both targeting Factor Xa. An additional DOAC targeting Factor Xa, edoxaban (Lixiana®, Daiichi Sankyo), has now completed clinical trials for the prevention of stroke in atrial fibrillation and the treatment/prevention of venous thromboembolism (1,2).

Although these drugs do not require laboratory testing for dose-adjustment, such testing may be warranted in certain situations. The Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) has recently issued a consensus statement from an expert panel on DOACs and laboratory testing (4) which provides further information. The present article describes the author's personal opinion on when and how patients treated with DOACs should be investigated in the clinical laboratory.

Table 1 DOACs and their target coagulation factors

DOAC	Target factor
Dabigatran (Pradaxa®)	Thrombin
Rivaroxaban (Xarelto®)	Factor Xa
Apixaban (Eliquis®)	Factor Xa
Edoxaban (Lixiana®)	Factor Xa

PERFORMING TESTING

Creatinine Clearance

As DOACs are largely excreted through the kidney, (3) they tend to accumulate in the blood of patients with chronic renal impairment. As a consequence, assessment of the creatinine clearance before initiation, and subsequently at regular intervals during treatment, is mandatory to prevent the risk of bleeding following drug accumulation. This evaluation is particularly important with elderly patients in whom renal clearance may be compromised and may change over time.

Hemostasis Testing – When to Test

Hemostasis tests should be performed in the following situations:

1. **At baseline.** (before initiating anticoagulant therapy). Certain patients may have slightly abnormal baseline results. If these abnormalities go unnoticed, the interpretation of results during anticoagulation could be affected.
2. **During adverse events.** Patients should be tested when adverse events occur (hemorrhage or thrombosis). This information helps physicians determine if such events are caused by over- or under-dosage of the drug.
3. **Before surgical or invasive procedures.** Patients should be tested immediately before undergoing surgical or invasive procedures to determine whether residual drug is still circulating. Recent reports in the literature (5) indicate that drug elimination within a few days following discontinuation of therapy cannot be taken for granted. Furthermore, when timing of the last dose is not precisely known, laboratory testing is essential to rule out residual circulating drug, especially when emergency surgery is required.
4. **Before thrombolytic therapy.** Approximately 50% of patients taking oral anticoagulants worldwide are treated to prevent stroke and systemic embolism in patients with atrial fibrillation. Despite warfarin or DOAC therapy, some patients may still develop a stroke. The recommended treatment for such patients is thrombolytic therapy (6), which is however, associated with haemorrhage risk and may be life-threatening if added to circulating DOACs. Hence, laboratory testing is warrented to ensure the absence of residual circulating drug prior to initiation of thrombolytic agents.

There are other situations in which laboratory testing could be useful. ***(Table 2)***. They include:

1. **Reversal of anticoagulation.** Hemostasis testing may be beneficial when immediate neutralization of anticoagulation is needed during life-threatening hemorrhage. Although experience is limited, recent reports demonstrate that efficacy of the prothrombin complex concentrates (PCC) infusion could be evaluated with hemostasis testing (7). Indeed, laboratory tests were normalized upon infusion of PCC in healthy subjects treated with rivaroxaban. However, in those subjects treated with dabigatran, thrombin clotting time remained prolonged even after infusion of PCC (7). Therefore, caution should be used in the interpretation of results.

   An antidote for dabigatran has been developed and investigated in animal models (8). Antidotes specifically designed for other DOACs will presumably follow. If each DOAC requires a different antidote, physicians in emergency departments must be able to determine not only the level of anticoagulation, but also the type of drug used. Because such information may not be readily available, qualitative lab tests specifically designed for each DOAC may provide valuable insight. Urinalysis test cartridges to provide rapid identification are currently under development (9).
2. **Long-term anticoagulation.** Upon achievement of steady--state chronic anticoagulation (1-2 weeks after initiation), laboratory testing may be useful to assess the drug concentration in plasma. Dabigatran concentrations in treated patients have been reported to vary 117 – 275 ng/mL at peak and 61 – 143 ng/mL at trough levels (10) ***(Fig 1)***. Rivaroxaban concentrations in treated patients have been reported to vary 22 – 535 ng/mL at peak and 6 – 239 ng/mL at trough levels (11). Because of the considerable variability between subjects, it can be logically assumed that individual patients may present with different circulating drug concentrations despite being treated with the same dose. If this holds true, the interpretation of test results during adverse events cannot be generalized and, therefore, knowledge of the steady-state DOAC concentration in individual patients may be useful.

3. **During clinical visits.** During routine follow-up visits (perhaps annually), lab testing will generally be ordered for patients taking DOACs. Such test results may be useful to determine and assess variations in anticoagulant levels over time.
4. **Drug-to-drug interaction.** Unlike warfarin, the interaction of DOACs with other drugs appears to be negligible. However, there may be interactions that are still not well known. Hemostasis testing before and after (1-2 weeks) introduction of an additional drug may be useful to identify particular interactions.
5. **Body weight.** The "one-size-fits-all" approach might not be adequate for DOAC treatment in patients with extreme body weight. For such patients, measurement of DOAC blood levels may be not only useful, but necessary to determine the ideal dosage. This has been evaluated in the post-*hoc* analysis of data from clinical trials of dabigatran in atrial fibrillation (12). Laboratory assessment of the drug anticoagulant effect as well as assessment of adverse events in patients with extreme body weight may prove useful.

Table 2 Usefulness of DOAC laboratory testing

When testing is useful	When testing is potentially useful
• At baseline (before initiating DOAC therapy) • During adverse events (hemorrhage or thrombosis) • Before surgical or invasive procedures • Before thrombolytic therapy	• Reversal of anticoagulation • Chronic anticoagulation • During routine clinical visits • Drug-to-drug interaction • Extreme body weight

Which Test?

Most of the hemostasis tests that are based on thrombin generation and fibrin formation, as well as the inhibitory activity measured against the relevant Factor (i.e., thrombin or Factor Xa), are affected by DOACs. However, it should be recognized that such tests may behave differently based on the drug used. The following section discusses assays that are useful for particular DOACs ***(Table 3)***. More extensive discussion can be found in additional literature (13, 14).

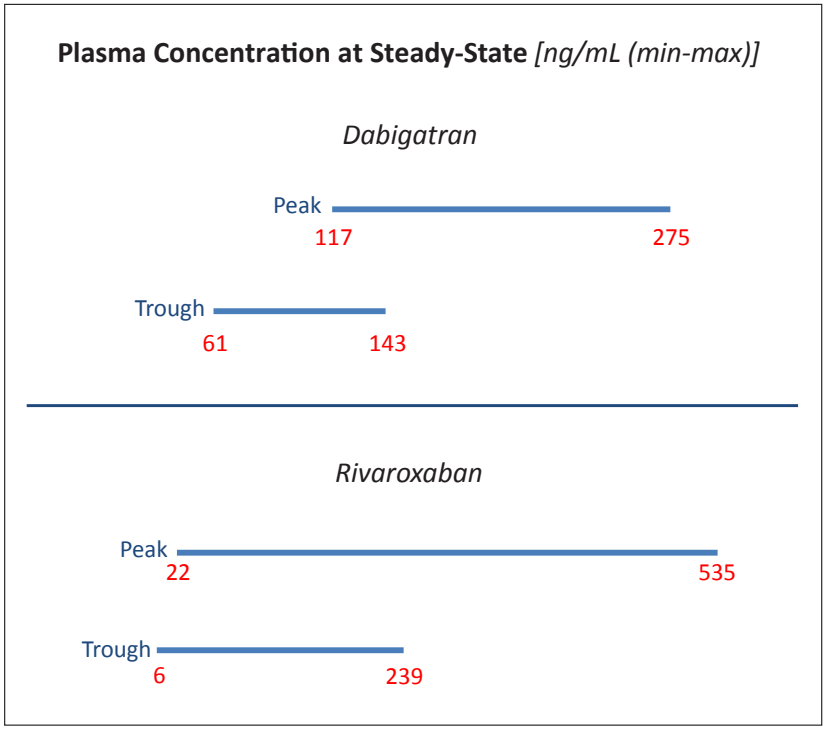

Figure 1
Patients treated with 150 mg dabigatran twice daily or 20 mg rivaroxaban once daily.

Dabigatran Prothrombin (PT) and activated partial thromboplastin time (APTT) are not useful tests for measuring dabigatran. Although they are variably prolonged following intake of this drug, PT is poorly responsive and APTT (though more responsive than the PT) shows a curvilinear dose-response. Furthermore, commercially available APTT formulations behave differently with the same plasma concentration of dabigatran (15). Thus, establishment of "universal" (i.e., valid for all APTT reagents) cut-off values alerting physicians to bleeding or thrombotic risk, would be very difficult and/or not possible. Alternatively, the conventional thrombin clotting time (TT) that assesses the conversion of fibrinogen to fibrin may be the test of choice. However, while it is extremely responsive to dabigatran, (16) and therefore cannot be used to measure plasma concentrations, a normal TT can be used to rule out dabigatran in plasma. In contrast, its diluted version (dTT) proved adequately responsive to dabigatran: 200 ng/mL dabigatran (i.e., the expected median dabigatran plasma level in patients taking 150 mg twice daily) prolonged the dTT approximately 2x the baseline value and can, therefore, be used to measure concentrations (17). The ecarin clotting (or chromogenic) test (ECT) is another test that can be used to measure dabigatran. ECT prolongations following dabigatran administration are linearly related to dosage: 200 ng/mL dabigatran concentrations can prolong the ECT 3x over baseline (16).

Rivaroxaban The effect of rivaroxaban can be assessed with the anti-Xa (18) or PT assays (19). While anti-Xa assays are not yet widely available, especially in emergency departments, PT assays can be considered a valid alternative, provided that a sensitive thromboplastin is used for testing. The PT prolongation in response to 200 ng/mL rivaroxaban is approximately 2x the baseline value, but is highly variable depending on the thromboplastin used for testing (19, 20). The responsiveness of the PT to rivaroxaban follows a different pattern from that observed for warfarin; thromboplastins that are very responsive to warfarin may be poorly responsive to rivaroxaban (19, 20). The variable responsiveness to rivaroxaban can (in principle) be minimized by correcting the PT ratio (patient-to-normal) with a sensitivity index valid for rivaroxaban. This can be determined for commercial thromboplastins relative to the World Health Organization (WHO) thromboplastin standard (20).

Apixaban PT is poorly responsive to apixaban and cannot be used for this drug. A valid alternative is the anti-Xa assay (21). However, limited experience in the laboratory is available. The same considerations will likely apply to edoxaban.

Table 3 DOACs and specific tests

DOAC	Test
Dabigatran	Dilute Thrombin Time Ecarin clotting (chromogenic) test
Rivaroxaban	Anti-Factor Xa Prothrombin time (with responsive thromboplastins)
Apixaban	Anti-Factor Xa

Expression of Test Results

The most convenient method to expressing PT, dTT or ECT results is the clotting time ratio (patient-to-normal) or drug concentration-equivalents interpolating the patient clotting time or optical density (OD) from a calibration curve. This is prepared by plotting clotting times (or OD) vs. drug concentrations for a normal pooled plasma added with increasing concentrations of the relevant drug (22).

Timing of Blood Drawing

Because of their relatively quick onset of action and short half-life, DOACs reach peak plasma concentrations approximately two hours after ingestion, and trough concentrations 24 or 12 hours after ingestion, depending on whether the drug is administered once daily or twice daily. Knowledge of the time from drug administration to blood draw is, therefore, essential to optimize interpretation of results.

Interference with the Most Common Hemostasis Parameters

Measurement of some of the most common hemostasis parameters may be influenced by DOACs ***(Table 4)***. *Fibrinogen* activity measured with the Clauss method may be underestimated in patients taking dabigatran. This effect is variable depending on the reagent used for testing (23). *Antithrombin* activity may be considerably overestimated in patients taking rivaroxaban, apixaban or dabigatran, depending on the target enzyme (e.g., FXa thrombin) used for testing (23, 24). *Activated Protein C resistance* when measured with APTT-based methods may be underestimated in patients on DOACs (25, 26). *Protein C or Protein S* anticoagulant activity measurement may be variably overestimated (26).

Individual coagulation factors have been reported to be underestimated (26), including *Factor XIII* activity when measured by functional assays in patients on dabigatran (27). Finally, detection of *lupus anticoagulants* may be affected (26). Therefore, measurements of the most common hemostasis parameters should be postponed until one week after discontinuation of DOAC administration or results should be interpreted with caution.

Table 4 DOAC interference with the most common hemostasis parameters

Parameter	Interference
Fibrinogen	Possible underestimation in patients taking dabigatran (variable effect based on reagent used for testing)
Antithrombin	Possible considerable overestimation in patients taking rivaroxaban, apixaban or dabigatran, based on target enzyme (e.g., FXa or thrombin) used for testing
Activated Protein C resistance	Possible underestimation when measured with APTT-based methods
Protein C or Protein S	Possible overestimation of anticoagulant activity
Individual coagulation factors	Possible underestimation of procoagulant activity
Detection of lupus anticoagulants	Possible interference, based on test used for detection
Factor XIII Activity	Possible underestimation of the activity in patients taking dabigatran

CONCLUSIONS

Although not strictly needed for dose-adjustment, lab testing is useful in the management of patients on DOACs. Relatively simple tests can be used to measure the effect of these drugs in special situations. Furthermore, results stemming from the measurement of some of the most common hemostasis parameters might be considerably influenced by DOACs.

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