
Armando Tripodi\textsuperscript{1}, Giancarlo Di Iorio\textsuperscript{2}, Giuseppe Lippi\textsuperscript{3}, Sophie Testa\textsuperscript{4}, Cesare Manotti\textsuperscript{5}

\textsuperscript{1}Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, IRCCS Cà Granda Maggiore Hospital Foundation and Università degli Studi di Milano, Milano, Italy. \textsuperscript{2}Laboratory of Clinical Chemistry, Anticoagulation Service, General Hospital, Pescara, Italy. \textsuperscript{3}Academic Hospital, Laboratory of Clinical Chemistry and Hematology, Department of Pathology and Laboratory Medicine, Parma, Italy. \textsuperscript{4}Hemostasis and Thrombosis Center, General Hospital, Cremona, Italy. \textsuperscript{5}Anticoagulation Service, Fidenza, Italy.

Correspondence: A. Tripodi, Via Pace 9, 20122-Milano, Italy. Phone: +39 02 50320725; FAX: +39 02 50320723; email: armando.tripodi@unimi.it

Word count: 2,245

FCSA, Italian Federation of Thrombosis Services
SIMeL, Italian Society of Laboratory Medicine
SIBioC, Italian Society of Clinical Biochemistry
CISME\textsuperscript{L}, Italian committee for standardization of laboratory tests
ABSTRACT
At variance with vitamin K antagonists, the new oral anticoagulants (NOAs) can be prescribed at fixed dosage without adjustment by laboratory testing. However, this does not necessarily mean that the laboratory does not play a role for their management. This position paper represents the consensus document of three Italian scientific societies dealing with laboratory issues in thrombosis and hemostasis. It is aimed at reviewing (i) which test(s) should be used to evaluate the anticoagulant effect of each of the NOAs presently available (i.e., dabigatran, rivaroxaban and apixaban); (ii) the patients to be investigated and (iii) the timing of investigation.
INTRODUCTION

Currently, heparins and vitamin K antagonists are the drugs of choice for treatment and prevention of venous thromboembolism (1), prevention of systemic embolism and stroke in atrial fibrillation (2) and prophylaxis of patients with mechanical prosthetic heart valves (3). These drugs are effective and safe, but difficult to be managed because of the need for frequent dose-adjustment by laboratory testing (unfractionated heparin and vitamin K antagonists) and subcutaneous or intravenous administration (heparins). These pitfalls made research to develop new oral anticoagulants (NOAs) aimed at retaining the advantages of conventional drugs, but not requiring dose-adjustment by laboratory testing. NOAs that target such individual activated coagulation factors as X or thrombin have been developed and investigated in phase III clinical trials for efficacy and safety in the treatment and prevention of thromboembolism in many clinical conditions (see ref. n. 4 for review). Presently, three drugs are in an advanced process of release and will be available very soon in Italy and other countries. These include dabigatran (Pradaxa®, from Boehringer Ingelheim), a direct thrombin inhibitor; rivaroxaban (Xarelto®, from Bayer) and apixaban (Eliquis®, from Pfizer), which are instead direct factor Xa inhibitors. This position paper represents the present state of the art on laboratory testing of these drugs and summarizes the consensus of three scientific societies dealing with laboratory issues in thrombosis and haemostasis in Italy.

LABORATORY EVALUATION OF THE EFFECT OF NOAs

NOAs are not yet widely available for treatment and prevention of thrombosis and information on which test(s) should be used to evaluate their anticoagulant effect is scanty and is derived mainly from the experience stemming from phase III clinical trials of patients with venous thromboembolism or atrial fibrillation, or studies of pharmacodynamics and pharmacokinetics in healthy volunteers. Hence, the following recommendations should be taken as the present state of the art and can be subjected to modification as soon as more information will be available. Due to
the different mechanisms of action, each of the presently available drugs will be considered separately.

**Which test(s) for Dabigatran**

Dabigatran is a direct thrombin inhibitor (4) and is thereby able to affect all global coagulation tests that are based on thrombin generation and fibrin formation as endpoints such as the prothrombin and activated partial thromboplastin times (PT and APTT) and congeners (5). In principle dabigatran is also able to affect the anti-factor IIa activity, that can be assessed by the measurement of residual thrombin upon addition to the test plasma of an excess amount of this enzyme. While no information is presently available for the latter test, it is known that the PT and APTT are dose-dependently prolonged by increasing concentrations of dabigatran (5). However, the two tests show different patterns: PT prolongations are linearly related with dabigatran concentrations, but the responsiveness (i.e., the slope of the best-fit line relating the prolongation of the clotting times with the drug concentrations) is rather poor (5). Conversely, the APTT is not linear, but more responsive (5). It is anticipated that there will be a large variability of the clotting time prolongation obtained with the same method, but different reagents in response to the same quantity of dabigatran, thus making standardization of results across laboratories rather challenging. This unfavourable characteristic makes the generalization of procedures stemming from prolongation of clotting times in response to dabigatran virtually unfeasible. Results should therefore be interpreted according to the reagent used for testing. In this respect, calibration plasmas obtained by spiking pooled normal plasmas with known and increasing amounts of dabigatran are commercially available in lyophilized form. These plasmas may help translating clotting times obtained for patients with any given method/reagent into concentrations of the drug by means of local calibration curves. Although no accurate information can presently be given, it can be estimated that 150 mg dabigatran twice daily (i.e., the dose administered to patients with atrial fibrillation) corresponds roughly to a $C_{\text{trough}}$ (i.e., the plasma level measured just before the next dose) of 200 ng/mL.
circulating drug and that 220 mg once daily (i.e., the dose administered to patients undergoing major orthopaedic surgery) corresponds roughly to a $C_{\text{trough}}$ of 67 ng/ml circulating drug (6). Other tests such as the ecarin clotting time (ECT) or the thrombin clotting time (TCT) are suitable to measure dabigatran. While ECT possesses good linearity and responsiveness, TCT possesses good linearity but it is too much responsive: the TCT is prolonged nearly 15-times the basal value following a single dose of dabigatran (5). However, simple modifications of the TCT test have been developed that make it still linear and adequately responsive (7).

**Recommendation.** On the basis of the above observations it is recommended to use the modified TCT or ECT for dabigratran measurement. Results of the above tests should be expressed as ratio (patient-to-normal) clotting times or as dabigatran concentrations by interpolation of the patients clotting time from a calibration curve prepared by testing sets of dabigatran-calibration plasmas with the local method/reagent. It is important to realize that calibration curves depend on the method/reagent used for testing and cannot be generalized even for the same method performed in combination with a different coagulometer.

**Which Test(s) for Rivaroxaban**

Rivaroxaban is a direct factor Xa inhibitor (4) and as such it is able to affect many global coagulation tests. Rivaroxaban is also able to affect the anti-factor Xa activity that can be assessed by the measurement of residual factor Xa upon addition of excess amounts of this enzyme to the test plasma. A modification of the latter test has been reported to be adequately linear and responsive to rivaroxaban (8). The PT and APTT are dose-dependently prolonged by increasing concentrations of rivaroxaban. They are also adequately linear and responsive (9). Other tests such as the dilute Russell viper venom tests (dRVVT) and the HepTest® may be useful alternatives, but their dose-response is not linear (9). Although no accurate information can be given owing to the large inter-reagent variability it can be estimated that a single oral dose of 10 mg rivaroxaban corresponds roughly to 200 ng/mL circulating drug (9). Standardization of results across
method/reagent both for PT/APTT or anti-factor Xa activity poses the same problems as for dabigatran (9, 10). Results should therefore be interpreted according to the reagent used for testing. In this respect, calibration plasmas obtained by spiking pooled normal plasmas with known and increasing amounts of rivaroxaban are commercially available in lyophilized form as for dabigatran. These plasmas may help translating clotting times obtained with any given method/reagent into concentrations of the drug by means of local calibration curve. An alternative method of standardization for the PT when used to test for rivaroxaban has been proposed and warrants consideration and further investigation (11). This method is based on the calculation of sensitivity indexes for commercial thromboplastins specific for rivaroxaban and their use to convert PT-ratio into a standardized scale similar to that used for vitamin K antagonists (11).

**Recommendation.** On the basis of the above observations it is recommended to use the anti-factor Xa activity or the PT for rivaroxaban measurement. The first is not yet readily available in many clinical laboratories especially in emergency situations and/or stat testing. Results of the PT should be expressed as ratio (patient-to-normal) clotting times. Results reporting in terms of the regular international normalized ratio (INR) (i.e., the one valid for patients on vitamin K antagonists) is highly discouraged as it considerably magnifies the between-thromboplastin variability of results (11). Results for the anti-factor Xa activity or for the PT can also be expressed as rivaroxaban concentrations by interpolation of the patient plasma optical density or the clotting time from a calibration curve prepared by testing sets of rivaroxaban-calibration plasmas with the local method/reagent. It is important to realize that calibration curves depend on the method/reagent used for testing and cannot be generalized.

**Which Test(s) for Apixaban**

Apixaban like rivaroxaban is a direct factor Xa inhibitor (4). Precise information on the effect that this drug may have on coagulation tests is not presently available. It is anticipated that most of recommendations given above for rivaroxaban should also apply to apixaban (12). However, this is
not presently known and the reader should refer to the instructions given by the manufacturer of this drug.

**Patients to be Investigated**

If one gives for granted that NOAs do not require dose-adjustment based on laboratory testing, the following situations in which the laboratory may help should be taken into consideration.

(i) Patients presenting in emergency with thrombotic or hemorrhagic events and no clear indication on their treatment. In these circumstances physicians may benefit from knowing which type of drug is being taken by the patient and whether the concentration of the drug is within the expected therapeutic limits.

(ii) Immediate reversal of the drug anticoagulant effect. Although no antidote is yet available for NOAs, in patients with life-threatening hemorrhagic events the drug should be immediately stopped and appropriate therapeutic measures promptly established. The recent Evidence-based Clinical Practice Guidelines of the American College of Chest Physicians concluded that there is still insufficient clinical experience to guide management of major bleeding in patient taking NOAs. So that, the approach remains mostly serendipitous and relies largely on by-passing agents (1, 13). Prothrombin complex concentrates (PCCs) or recombinant factor VIIa are candidate therapeutic materials for immediate reversal and physicians may need to evaluate by simple laboratory methods whether or not the reversal has been achieved. In this respect, it has been reported that laboratory tests are able to show reversal of anticoagulation mediated by PCCs in healthy volunteers treated with rivaroxaban, but not in those treated with dabigatran (14).

(iii) Pre-surgical screening. Laboratory testing should be performed immediately before surgical procedures to ensure that the drug is no longer circulating.
(iv) Patients with impaired renal function require periodic laboratory testing as chronic treatment with NOAs may lead to drug accumulation.

(v) Laboratory testing may also be required in patients with chronic liver disease as some of these drugs are metabolized by the liver.

(vi) Drug-to-drug interactions. Although NOAs interfere with other drugs much less than vitamin k antagonists, there is still a certain degree of interaction and therefore suspicion or knowledge that this may occur should prompt laboratory measurement of the drug anticoagulant effect.

(vii) Finally, laboratory testing may be required in patients with low body weight or in those who are obese as the dose of the drug usually given to individuals with normal body weight might be inadequate for these patients.

**Recommendation.** *On the basis of the above observations it is recommended to test plasmas from patients on NOAs when they present in emergency with thrombotic or hemorrhagic events; if they require immediate reversal of the drug anticoagulant effect or surgical procedures; patients with suspected or established impaired renal function or chronic liver disease; whenever there is suspicion or knowledge of drug-to-drug interaction; finally, testing should be performed in patients with high or low body weight.*

**When Testing**

Although in some occasions testing is required in emergency (e.g., bleeding patients) and this may occur any time, it is important to realize that NOAs are subjected to variation in their concentrations depending on the time elapsed from drug intake to blood collection. Most of these drugs reach peak plasma levels (as assessed by coagulation tests) approximately after two hours from intake. The rate of disappearance from circulation is variable and depends mainly on the coagulation tests being used. It is therefore important to get acquainted about the pharmacodynamics of the relevant drug according to the method used for testing.
**Recommendation.** On the basis of the above observations it is recommended to consider the time elapsing from the drug intake to the time when blood has been collected, keeping in mind that peak values are reached approximately at two hours from intake and that the effect declines thereafter, but is dependent from the laboratory method being used. Whether it is more appropriate to measure the $C_{trough}$ (i.e., the plasma level measured just before the next dose) or the $C_{max}$ (i.e., the maximal concentration) of the drugs is still a matter of debate (6).

**INTERFERENCE OF NOAs WITH HEMOSTASIS PARAMETERS**

It is important to realize that the measurement of many common hemostatic parameters such as antithrombin, fibrinogen, activated protein C resistance, protein C/S and detection of lupus anticoagulants are heavily and variably affected by NOAs (15-18). For example, in patients on rivaroxaban or dabigatran, the antithrombin inhibitory activity will be considerably overestimated if it is measured against factor Xa or thrombin, respectively; in patients on dabigatran, fibrinogen might be underestimated if it is measured as coagulation activity by means of the Clauss method.

Hence, to avoid misclassifications or misdiagnoses it is recommended to test for the above parameters after discontinuation of the treatment (19). Whenever discontinuation is unfeasible, caution should be exerted in the interpretation of results. In this regard, laboratory workers are encouraged to report results for patients on NOAs with an interpretative comment (20) as many clinicians may not be aware of the complex interactions that NOAs may have with the most common laboratory tests.

**CONCLUSIONS**

Although NOAs (at variance with vitamin K antagonists) proved to be effective and safe when administered at a fixed dose, the laboratory is still required to manage treated patients in many circumstances (21-25). Clinical laboratories are therefore encouraged to set up and make available
simple tests able to measure the anticoagulant effect of these drugs. Physicians on the other hand are encouraged to rely on clinical laboratories for assistance while dealing with patients on NOAs.
REFERENCES


