The Laboratory and the New Oral Anticoagulants

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BACKGROUND: The new oral anticoagulants (NOAs) dabigatran, rivaroxaban, and apixaban have proved effective and safe when used in clinical trials, without a need to adjust the dose in response to laboratory testing. This demonstrated efficacy does not necessarily mean that the laboratory, considered the mainstay for the management of the old anticoagulants, will no longer play a role in treatment with NOAs.

CONTENT: Laboratories are involved in the management of anticoagulants in 2 ways. The first, monitoring, implies laboratory testing to assess the drug’s effect and to adjust the dosage to maintain anticoagulation within the therapeutic interval. This consideration applies to the old drugs. The second way, measurement, implies laboratory evaluations of drug effect to determine whether patients are under- or over-anticoagulated, information that can be useful for decision-making in special circumstances. The latter applies to NOAs.

SUMMARY: Measurements of the effect of NOAs are indicated in several situations: (a) patients with adverse events (i.e., thrombotic/hemorrhagic), particularly those who present with overdosage owing to excessive drug intake or decreased clearance; (b) patients undergoing surgical procedures for ensuring that no residual drug remains in the circulation; (c) patients requiring anticoagulation reversal because of life-threatening hemorrhage; (d) patients with renal insufficiency, who are likely to accumulate the drug in the circulation; (e) patients with liver failure, because NOAs are metabolized by the liver; (f) patients taking other drugs that might increase/decrease the effects of NOAs via drug-drug interactions. The choice of tests is based on such characteristics as availability, linearity of the dose-response curve, standardization, and responsiveness to increasing drug dosage. Practitioners need to be aware that NOAs can interfere with the measurement of common hemostasis parameters.

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Deep vein thrombosis and pulmonary embolism, collectively known as “venous thromboembolism,” are frequent diseases, with an incidence of nearly 2 individuals per 1000 inhabitants per year (1). Acute venous thromboembolism requires prompt treatment with fast-acting drugs such as heparins and vitamin K antagonists (VKAs)3 to limit thrombus extension. Heparins are a family of sulfated mucopolysaccharides that include unfractionated heparin (UFH), which has a relatively high molecular weight, and low molecular weight heparin (LMWH), which is obtained from UFH by depolymerization (2). Both drugs exert their antithrombotic activity by binding to and potentiating the anticoagulant activity of the naturally occurring anticoagulant antithrombin (2). The discovery that binding of antithrombin to UFH or LMWH requires a unique sulfated oligosaccharide sequence (i.e., the pentasaccharide) led to the development of fondaparinux, a synthetic antithrombotic drug that mimics the essential pentasaccharide sequence (3). Although effective and relatively safe, UFH, LMWH, and fondaparinux also have pitfalls: UFH is usually administered by continuous infusion and requires laboratory monitoring to adjust the dosage (4), whereas LMWH and fondaparinux do not require frequent laboratory monitoring but are administered subcutaneously. VKAs are coumarin-like drugs that interfere with the carboxylation of vitamin K–dependent coagulation factors, thus limiting their activity (5). Approximately 2% of the general population in Western countries is currently on VKAs, and the most frequent indications are to prevent stroke and systemic embolism caused by atrial fibrillation (about 50% of patients) (6), patients with mechanical prosthetic heart valves (7), and treatment/

3 Nonstandard abbreviations: VKA, vitamin K antagonist; UFH, unfractionated heparin; LMWH, low molecular weight heparin; NOA, new oral anticoagulant; Fxa, activated factor X; INR, international normalized ratio; rFVIIa, recombinant activated FVII; PT, prothrombin time; APTT, activated partial thromboplastin time; dRVVT, dilute Russell viper venom test; TCT, thrombin clotting time; ECT, ecarin clotting time; Cmax, peak NOA concentration in plasma; Ctrough, nadir NOA concentration in plasma; ISIVKA, International sensitivity index for VKA; APC, activated protein C; APC-R, APC resistance; LA, lupus anticoagulant.
prevention of venous thromboembolism (8). Notwithstanding their recognized safety/efficacy, VKAs have several drawbacks that have the potential to deny lifesaving prophylaxis to many patients worldwide. Because of their relatively narrow therapeutic window and their interaction with the diet and other drugs, VKAs require tight clinical and laboratory control, not only to maintain patients within the therapeutic interval for preventing thrombotic recurrence but also to provide adequate hemostatic potential. The percentage of time spent within the therapeutic interval is inversely related to the risk of adverse events (9) and must be maximized by the combined action of skilled clinical and laboratory personnel, which can be best achieved through dedicated anticoagulation clinics. Such clinics are not widely available worldwide, however.

Owing to these limitations, demand has been increasing to develop new oral anticoagulants (NOAs). Most efforts have been directed at targeting and inhibiting specific coagulation factors without the intermediation of antithrombin or carboxylation, as occurs with heparins/fondaparinux or with VKAs, respectively. Presently, NOAs have been used successfully in targeting activated factor X (FXa) (rivaroxaban or apixaban) and thrombin (dabigatran) (Fig. 1). These drugs, which have undergone phase III clinical trials to assess their safety/efficacy, are no less effective or safe than VKAs when used at fixed dosages [see (10) for a review]. Hence, they are now recommended for therapeutic use without the need for regular laboratory monitoring. The aim of this article is to review the current situation and discuss how and when the laboratory can help clinicians manage patients treated with NOAs.

New Oral Anticoagulants

Three NOAs have already been (or are being) licensed for use in patients with cardiovascular diseases. Rivaroxaban (Xarelto®; Bayer) and apixaban (Eliquis®; Pfizer) are direct FXa inhibitors, and dabigatran (Pradaxa®; Boehringer Ingelheim) is a direct thrombin inhibitor (10). These NOAs have relatively short half-lives (8–15 h), which translates into a relatively short onset or offset of anticoagulant action, characteristics that make them more easily managed than VKAs in special situations, such as the initiation or discontinuation of the treatment. Furthermore, these drugs possess a greater bioavailability than VKAs and have little or no interaction with other drugs or the diet. All of these characteristics make the anticoagulant action of NOAs more predictable than for VKAs; hence, dose adjustment guided by laboratory testing is not needed. Concluding that the management of NOAs no longer requires the laboratory, however, is an oversimplification that should be tempered.

Monitoring vs Measurement

The laboratory may be involved in the management of antithrombotic drugs in 2 ways. The first (here referred to as “monitoring”) implies the use of specific laboratory tests to assess the drug’s anticoagulant effect and to adjust the dosage to maintain anticoagulation within a prespecified therapeutic interval. This concept applies to VKAs and UFH. The second way (here referred to as “measurement”) implies laboratory evaluation of the drug’s anticoagulant effect, not necessarily to use the results to adjust the dosage but simply to see whether the patient is under- or over-anticoagulated, information that may be extremely useful for making decisions in special circumstances. This concept applies to NOAs, and the following sections discuss the most important situations that require measurement of a drug’s effect.

Who Should Be Tested?

As mentioned above, NOAs are not yet widely available, and therefore the still scanty information on how they affect laboratory tests of hemostasis derives mostly from measurements of plasma samples from patients participating in clinical trials, plasma samples from healthy individuals participating in pharmacodynamics/pharmacokinetics studies, or pooled plasma samples from healthy individuals spiked in vitro with increasing amounts of the relevant drug. This lack of data notwithstanding, information has started to accumulate, and it is reviewed below. It is important to realize, however, that the range of drug concentrations and
their effects on laboratory testing in nonbleeding and bleeding patients are not yet known and should be established by appropriate postmarketing, long-term clinical trials for each test and drug.

CHECKING COMPLIANCE
Adherence to the prescribed therapy is of paramount importance for any antithrombotic drug. One can assume that the efficacy and safety of VKAs rest mainly on the fact that patient compliance can be rigorously checked with regular laboratory testing. VKAs have a long-lasting action, and an observed reduction in the international normalized ratio (INR) can pick up a missed single dose during the steady state, even a few days before the laboratory check. This advantage is not applicable to NOAs, which have relatively short half-lives, so that missing a dose a few days before laboratory testing and resuming treatment soon before testing would probably escape the attention of the laboratory.

ADVERSE EVENTS
Patients presenting in an emergency department with adverse events (thrombotic/hemorrhagic) should be investigated with laboratory tests to evaluate whether they are under- or over-anticoagulated. This consideration is particularly important in cases of overdosage, in which the risk of bleeding may be increased because of excessive intake or decreased clearance of the drug (see below).

SURGICAL PROCEDURES
Patients undergoing surgical or invasive procedures must be checked immediately before the procedure to ensure that no residual drug remains in the circulation, as that may cause serious bleeding events. Owing to the short half-lives of NOAs, common practice is to discontinue the treatment at least 2 days before the procedure. In patients with chronic renal failure (see below), however, discontinuation of dabigatran at least 4 days before surgery has been reported to be required to allow complete clearance of the drug from the circulation (11).

REVERSAL OF ANTICOAGULATION
No antidotes are presently available to reverse the anticoagulant effect of NOAs. Although reversal is seldom required because of the relatively short half-lives of the drugs, there are instances of life-threatening hemorrhage (i.e., intracranial) when anticoagulation must be promptly neutralized. There would be clinical benefit to know whether reversal has been achieved. Our experience is still limited, but prothrombin complex concentrates or recombinant activated FVII (rFVIIa) may be more useful and effective than plasma. Eerenberg et al. (12) have recently reported on a study of healthy individuals who received dabigatran or rivaroxaban for 2.5 days and then underwent reversal of anticoagulation via infusion of prothrombin complex concentrates or placebo. The effect of reversal was monitored with a panel of coagulation tests that showed peculiar results: Reversal was effective for rivaroxaban but not for dabigatran. If the pattern obtained with the 2 drugs and laboratory testing truly represents what occurs in vivo, the physician in charge of anticoagulant reversal might need to know which drug the patient is taking. That might be problematic for patients who are unconscious, but the laboratory could help by performing coagulation tests specific for rivaroxaban or dabigatran (see below).

RENAI INSUFFICIENCY
The kidneys excrete NOAs, especially dabigatran (approximately 80%) but also rivaroxaban (approximately 30%) and apixaban (approximately 25%) (10). Therefore, patients with renal insufficiency tend to accumulate the drug in the circulation, thereby increasing the risk of bleeding. This problem may be addressed by checking via appropriate laboratory testing before and during treatment, not only for creatinine clearance but also for the anticoagulant effect of the drug. It is important to realize that renal function, although normal or near normal at the beginning of treatment, can deteriorate during treatment. Therefore, patients (especially the elderly) should be checked regularly for signs of drug accumulation.

LIVER IMPAIRMENT
The liver metabolizes NOAs, especially apixaban (approximately 75%) but also dabigatran (approximately 20%) and rivaroxaban (approximately 30%) (10). Little information is available on the effect of these drugs in patients with liver impairment because they have been excluded from phase III clinical trials. Caution should therefore be exerted, and laboratory measurements should be made for such patients.

DRUG–DRUG INTERACTION
Although few interactions between NOAs and other drugs have been reported, one should realize that the patients who are candidates for chronic anticoagulant treatment are very often elderly and therefore may present with comorbidities that make them more prone to take additional drugs that might increase/decrease the anticoagulant effects of NOAs. Hence, if one suspects or knows that the patient is taking additional drugs that may interact with NOAs, the level of anticoagulation should be checked with appropriate laboratory testing.
Which Test(s)?

NOAs are able to limit the activity of the specific coagulation factor they are targeting. Accordingly, measuring anti-FIIa (i.e., thrombin) activity in plasma could be a reliable and specific means of evaluating the dabigatran effect. Similarly, the measurement of anti-FXa activity could be suitable for rivaroxaban and apixaban. In addition, the times for such global tests as the prothrombin time (PT), the activated partial thromboplastin time (APTT), the dilute Russell viper venom test (dRVVT), and the thrombin clotting time (TCT) are prolonged after NOA administration and could therefore be suitable alternatives for measuring the effect of rivaroxaban, apixaban, or dabigatran. The choice of the most appropriate test should be based on the characteristics of the test and on the drug being used. Given the limited experience accumulated thus far, the following test characteristics should be considered (Tables 1 and 2).

### Table 1. Main characteristics of tests to assess the anticoagulant effect of dabigatran.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Tests</th>
<th>Anti-FIIa</th>
<th>APTT</th>
<th>PT</th>
<th>ECT</th>
<th>TCT</th>
</tr>
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<tr>
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<td>Yes</td>
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<td>Linearity of dose–response curve</td>
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<td>Yes</td>
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<td>No</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Responsiveness to drug concentration</td>
<td></td>
<td>?</td>
<td>Yes</td>
<td>No</td>
<td>?</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* a The regular TCT test is overly responsive to dabigatran; however, the modified test uses diluted plasma and is adequately responsive.

### Table 2. Main characteristics of tests for assessing the anticoagulant effect of rivaroxaban.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Tests</th>
<th>Anti-FXa</th>
<th>APTT</th>
<th>PT</th>
<th>Heptest</th>
<th>dRVVT</th>
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<tbody>
<tr>
<td>Availability</td>
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<td>Yes</td>
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<tr>
<td>Linearity of dose–response curve</td>
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<td>Yes</td>
<td>Yes*</td>
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<tr>
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<td>Responsiveness to drug concentration</td>
<td></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* a The regular TCT test is overly responsive to dabigatran; however, the modified test uses diluted plasma and is adequately responsive.

### AVAILABILITY

Once NOAs have been introduced into worldwide use, we presumably will be dealing with millions of patients. These patients may bleed anywhere and anytime; therefore, physicians will need simple and readily available laboratory tests to help them manage emergency situations, such as reversal of anticoagulation due to excessive drug intake or accumulation. There is no point in setting up sophisticated tests if these tests cannot be run in every hospital, with simple equipment, and by relatively unskilled personnel.

### LINEARITY IN THE DOSE–RESPONSE CURVE

The test response (i.e., the prolongation of the clotting time or the absorbance) should be linearly related to the drug dose.

### RESPONSIVENESS

The slope of the best-fit line relating test response to the plasma concentration of the drug should be sufficiently high to make the test adequately responsive for distinguishing similar but not identical concentrations. The drug concentration needed to double the test response can be taken as a simple and reliable index of test responsiveness (the smaller the concentration, the better the responsiveness).

### STANDARDIZATION

Results for any given coagulation analyte are known to vary considerably when measured with the same method but with different reagents. This lack of standardization across reagents is one of the critical concerns in managing patients on VKAs and has been addressed by the development and use of the INR. This scale makes PT results relatively independent of the thromboplastin used for testing, thus allowing the adoption of “universal” therapeutic intervals for treated patients (13). Standardization is not needed for NOAs because these drugs do not require dose adjustment by laboratory testing. One could argue, however, that some sort of standardization could be of value even for NOAs. For example, if a cutoff value (beyond which the risk of bleeding is deemed high) were established for the PT with one of the commercial thromboplastins, this information could be easily generalized to all of the other thromboplastins, providing that a system of standardization were available.

The following sections discuss the most appropriate tests to be used for each of the drugs that are presently available. These tests are based on the considerations discussed above (see Tables 1 and 2) and on the limited data available in the literature thus far.
Dabigatran

ANTI-FIIa ACTIVITY
Since FIIa (thrombin) is the target of dabigatran, the anti-FIIa assay should be the test of choice for this drug. This test is based on the measurement of residual thrombin by a synthetic chromogenic peptide upon addition of excess thrombin to a patient’s plasma sample. In principle, this method should be relatively easy to run in an ordinary coagulometer. It is not presently available in most hospitals, and standardization of results across reagents is a matter of concern. No information is presently available on the test characteristics, such as linearity for and responsiveness in patients on dabigatran (Table 1).

PROTHROMBIN TIME
The PT also would be an excellent test, because it is readily available in clinical laboratories and is easily performed by personnel with little expertise. Prolongation of the PT is related linearly and dose-dependently to the plasma dabigatran concentration, but the responsiveness is not very high. Reportedly, 200 μg/L (i.e., the plasma concentration that should be expected in plasma after a dosage of 150 mg dabigatran twice daily) prolongs the PT about 1.2 times the basal value (14). Although the issue has not yet been investigated thoroughly, standardization of results across thromboplastins is a matter of concern (Table 1).

ACTIVATED PARTIAL THROMBOPLASTIN TIME
This test is readily available and easy to run. Its prolongation is related dose-dependently but not linearly to the dabigatran concentration (14). The responsiveness is adequate, because a plasma dabigatran concentration of 200 μg/L prolongs the APTT by about 2.5 times the basal value (14). Standardization across reagents will be an issue (Table 1).

ECARIN CLOTTING TIME
The ecarin clotting time (ECT) is a global test that reflects the activity of the coagulation cascade downstream from FII (15). Clot formation is obtained by a venom extract (ecarin) from the snake Echis carinatus. Ecarin converts FII into meizothrombin, which is then measured with a specific synthetic chromogenic substrate. Although not yet widely used in clinical laboratories, ecarin is commercially available, and the test could be easily run in an ordinary coagulometer. ECT prolongations above the basal value are related linearly and dose-dependently to the dabigatran concentration; responsiveness is also adequate (i.e., 200 μg/L dabigatran prolongs the ECT by about 3 times the basal value) (14). All of these characteristics make the ECT a candidate for dabigatran measurement (Table 1).

THROMBIN CLOTTING TIME
The TCT exploits the final step in the coagulation cascade (i.e., the conversion of fibrinogen to fibrin). This test is performed by adding an optimal amount of purified thrombin to the plasma sample to be tested. The TCT is prolonged by a low fibrinogen concentration or by the presence of thrombin inhibitors, such as heparin or dabigatran. The TCT test is readily available in clinical laboratories and is relatively easy to run. TCT prolongations are related linearly and dose-dependently to the dabigatran concentration, but the responsiveness to the drug is excessive: A 200-μg/L dabigatran concentration may prolong the plasma TCT to nearly 15 times the basal value (14). Modifications to the test that involve diluting plasma samples before testing make the TCT test adequately responsive (i.e., the TCT is prolonged 3 times the basal value by 200 μg/L dabigatran) (16, 17). Because of these characteristics, the TCT is a candidate for dabigatran measurement (Table 1).

CHOICE
Given the characteristics of the tests described above, the modified TCT and the ECT are the best tests for measuring the anticoagulant effect of dabigatran.

Rivaroxaban

ANTI-FXa ASSAY
Given that FXa is the target of rivaroxaban, the anti-FXa assay should be the test of choice for this drug. This test is based on measuring residual FXa with a synthetic chromogenic peptide upon addition of excess FXa to the patient’s plasma sample (18). In principle, this test should be relatively easy to run in an ordinary coagulometer, but it is not readily available in most hospitals on a 24-h basis. The standardization of results across reagents is also a matter of concern (19) (Table 2).

PROTHROMBIN TIME
Because it is readily available in clinical laboratories and easy to run with little expertise, the PT also would appear to be an excellent test. The prolongation of the PT is related linearly and dose-dependently to the rivaroxaban concentration, and the responsiveness is adequate. A concentration of 200 μg/L (i.e., the plasma concentration that should be expected in plasma after a once-daily 10-mg dose) prolongs the PT 1.5 times the basal time (20). Standardization of results across thromboplastins is a matter of concern, however. Commercial thromboplastins vary widely in their responsiveness to rivaroxaban, the most responsive being RecombiPlasTin® (Instrumentation Laboratory) and Neoplastin Plus® (Diagnostica Stago) and the least responsive being Innovin® (Siemens Healthcare Diagnostics) (Table 3) (20, 21).
ACTIVATED PARTIAL THROMBOPLASTIN TIME

The APTT test is readily available and is easy to run. Although contrasting results have been reported, its prolongation is related dose-dependently and linearly to the rivaroxaban concentration (20). The responsiveness is adequate, as 200 μg/L prolongs the APTT by about 1.5 times the basal value (20), but standardization across reagents is anticipated to be an issue (Table 2). There are important variations among the commercial reagents, with Actin® FS (Siemens Healthcare Diagnostics) and PTT-A® (Diagnostica Stago) being the most responsive and the least responsive, respectively, to increasing rivaroxaban concentrations (20) (Table 3).

THE Heptest AND THE dRVVT

In principle, these tests would be readily available in most clinical laboratories and be relatively easy to run. Responsiveness (but not linearity) to increasing dose is adequate: A rivaroxaban concentration of 200 μg/L prolongs the basal value by about 3 times and 2.5 times when measured with the Heptest and the dRVVT, respectively (20). The superiority of these tests over the others needs to be demonstrated, however, and there is no information on standardization (Table 2).

CHOICE

According to the experience accumulated thus far, the anti-FXa assay and the PT are the best tests for measuring the anticoagulant effect of rivaroxaban in plasma.

Table 3. Responsiveness of commercial reagents for the PT test or the APTT test to increasing rivaroxaban concentrations.

<table>
<thead>
<tr>
<th>PT</th>
<th>APTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>RecombiPlasTin® (Instrumentation Laboratory)</td>
<td>Actin® FS (Siemens Healthcare Diagnostics)</td>
</tr>
<tr>
<td>Neoplatin Plus® (Diagnostica Stago)</td>
<td>STA® C.K. Prest® (Diagnostica Stago)</td>
</tr>
<tr>
<td>Neoplatin® (Diagnostica Stago)</td>
<td>PTT-LA® (Diagnostica Stago)</td>
</tr>
<tr>
<td>TriniCLOT® (Trinity Biotech)</td>
<td>STA® PTT-A® (Diagnostica Stago)</td>
</tr>
<tr>
<td>Thromborel® S (Siemens Healthcare Diagnostics)</td>
<td>Innovin® (Siemens Healthcare Diagnostics)</td>
</tr>
</tbody>
</table>

* Commercial reagents are listed in order of decreasing responsiveness for the PT test [Samama et al. (20); Tripodi et al. (21)] or the APTT test [Samama et al. (20)]. RecombiPlasTin and Neoplatin Plus were similar in their responsiveness.

Apixaban

Limited data are available for apixaban and laboratory coagulation tests for this drug. Contrasting results have been reported on the correlation between the prolongation of the PT or the APTT and the apixaban concentration (22, 23). The anti-FXa assay has been evaluated in a substudy of patients treated with apixaban (24), and it proved to be highly correlated and adequately responsive to the apixaban concentration in plasma. The problems of availability and standardization of this assay that have already been discussed for rivaroxaban also apply to apixaban.

When to Test?

Timing of testing is essential, because the onset/offset actions of NOAs are relatively fast. Therefore, on the assumption that the in vivo anticoagulant effects of NOAs are truly represented by the ex vivo coagulation tests, the time interval between drug intake and blood sampling should be carefully considered when laboratory results are interpreted. In general, NOAs reach their peak value in plasma (Cmax) 2 h after intake; values start to decline thereafter and reach their nadir value (Ctrough) approximately 12 h after intake. According to the literature, there might be differences between drugs, especially for the Ctrough: Rivaroxaban is barely or not detectable by the PT at 12 h after an intake of 10 mg (25), whereas the anticoagulant effect of a 200-mg dabigatran dose is still detectable (approximately 50% of the peak activity) 12 h after drug intake (14). Still debated is whether the Cmax or Ctrough value should be sought when it is feasible to choose the timing for blood draw (26). This consideration applies, for instance, to patients on chronic treatment who require drug measurement to check for drug accumulation, compliance, drug–drug interaction, and so forth. One should realize, however, that in most instances there is little choice in terms of the timing of blood draw and drug measurement, because patients require evaluation when they present with adverse events (bleeding or thrombosis). Together, the concepts discussed above reinforce the caution that should be exerted when interpreting laboratory tests.

Standardization in the Expression of Results

In contrast to VKAs, standardization in the expression of results is not mandatory for NOA tests, because results are not used for dose adjustment. Nevertheless, some sort of between-test, between-reagent harmonization of results would be welcome, because that would permit generalization of patient-management information across anticoagulation centers that use differ-
ent methods and reagents. Such standardization may be achieved through the use of calibration plasmas prepared by adding increasing amounts of the relevant drug to a pooled normal plasma. These calibration plasmas can then be tested locally with the relevant test, and the response (i.e., clotting time prolongation or absorbance reading) can be used to construct calibration curves specific for each method/reagent and drug. The drug concentration in the patient’s plasma sample obtained with the same method can be derived by interpolating the test response from the calibration curve (16, 19, 27). Assuming that the test response is linearly related to the drug concentration, one would expect this system of standardization to yield the same result for the patient’s plasma sample regardless of the method/reagent used for testing, provided that method- and reagent-specific calibration curves are available. The total response for any given test will depend on both the response induced by the drug concentration and the response of the pooled normal plasma (i.e., its baseline coagulant activity). Normal plasmas from different healthy individuals (and even pooled normal plasmas prepared from different sets of individuals) will not be equal with respect to their baseline coagulant activity. Hence, to assume that calibration curves obtained with different normal plasmas spiked with the relevant drug will give the same slope for the relationship between test response and drug concentration might be an oversimplification. Therefore, standardization by means of this procedure might not be as easily achieved as currently thought. Furthermore, this system of standardization is demanding, requiring preparation of sets of calibration plasmas specific for each NOA and method.

Another suitable system for results standardization, at least for the coagulation assays, could be to express results as the ratio of the patient’s clotting time to the normal clotting time. On the assumption that the clotting time for the normal plasma is proportional to that of the patient’s plasma treated with the relevant NOA, the ratio between the 2 values might be a reliable system of standardization. Although this system is theoretically sound and might improve the comparability of results, it does not resolve the problem entirely, however (see below). Such an approach could be improved by exploiting the principle of INR calibration. The INR was devised as an effective system of standardization to express PT results for patients on VKAs (13). Manufacturers provide the international sensitivity index (ISI) values for their thromboplastins, which are calculated as the slope of the orthogonal regression line describing the relationship between log-transformed PT values (in seconds) obtained by using a standard thromboplastin and the working thromboplastin to test plasmas from healthy individuals and patients on VKAs. The ISI, is then used to convert the PT to INR, according to the equation:

\[ \text{INR} = \left( \frac{\text{PT}_{\text{patient}}}{\text{PT}_{\text{normal}}} \right)^{\text{ISI}_{\text{VKA}}} \]

If all thromboplastins are calibrated against the standard available from the WHO, the INR value obtained for any given patient is similar, regardless of the thromboplastin used for testing (13).

This system of standardization has the potential to be adapted, with appropriate modifications, to the standardization of the PT for the measurement of rivaroxaban (21). In the modified system, PT results for patients on VKAs were replaced with PT values for a normal plasma spiked with increasing amounts of rivaroxaban. The paired data (standard vs working thromboplastin) were then used to estimate an ISI valid for rivaroxaban (13). This work demonstrated that this system of standardization is feasible, and it would be able to minimize the between-thromboplastin variation in PT results for patients on rivaroxaban (21). The system was validated by testing plasmas spiked with rivaroxaban with 6 commercial thromboplastins that had previously been calibrated against a common standard to determine the ISI value (21). The results for these plasmas were expressed as:

\[ \text{PT ratio} = \left( \frac{\text{PT}_{\text{patient}}}{\text{PT}_{\text{normal}}} \right)^{\text{ISI}_{\text{rivaroxaban}}} \]

\[ \text{INR}_{\text{VKA}} = \left( \frac{\text{PT}_{\text{patient}}}{\text{PT}_{\text{normal}}} \right)^{\text{ISI}_{\text{VKA}}} \]

\[ \text{INR}_{\text{rivaroxaban}} = \left( \frac{\text{PT}_{\text{patient}}}{\text{PT}_{\text{normal}}} \right)^{\text{ISI}_{\text{rivaroxaban}}} \]

by using the appropriate calibrations. The between-thromboplastin imprecision (assessed as the CV) was 14%, 29%, and 2% when results were expressed as the PT ratio, the INR, and the INR, respectively (21). These results show that the system of standardization is feasible and effective in reducing the between-thromboplastin imprecision. They also show that results expressed as INR for patients on rivaroxaban should not be used, because results expressed in this way dramatically magnify the between-thromboplastin imprecision. Whether this system of standardization can also be applied to other coagulation tests and NOAs requires further investigation.

**Interference Effects of NOAs on Common Hemostatic Parameters**

Patients on NOAs may be tested occasionally for such common hemostatic parameters as antithrombin, protein C, protein S, activated protein C (APC) resistance (APC-R), fibrinogen, lupus anticoagulant (LA) detection, or others. Physicians and laboratory workers must be cognizant that these parameters might be affected heavily and variably by the drug being used, de-
pending on the method used for testing (Table 4). A few examples follow.

**ANTITHROMBIN**

Antithrombin activity will be overestimated considerably when rivaroxaban or dabigatran is used and the antithrombin inhibitory activity is measured against FXa or thrombin, respectively (25, 28-30). These effects occur because the antithrombin inhibitory activity is usually assessed by measuring residual FXa or thrombin activity upon addition of an excess amount of the appropriate enzyme to the test plasma. Accordingly, FXa or thrombin will be inhibited in these assays, not only by antithrombin but also by rivaroxaban or dabigatran, thus explaining the overestimation of the antithrombin inhibitory activity. Overestimation can be avoided with methods that use FXa or thrombin as the target enzyme when the patient is being treated with dabigatran or rivaroxaban, respectively (Table 4).

**FIBRINOGEN**

Fibrinogen might be underestimated considerably if it is measured as clotting activity with the Clauss method and the drug being used is dabigatran. That is not surprising, because the Clauss method is based on the conversion of fibrinogen to fibrin by exogenous thrombin. Hence, the inhibition of exogenous thrombin by dabigatran leads to an underestimate of the fibrinogen concentration (28, 30). The extent of underestimation depends on the method used for testing (28), suggesting that the concentration (30) and/or the type of thrombin used for testing may play an important role (Table 4).

**APC RESISTANCE**

APC-R is assessed as the ratio of the clotting times for test plasmas obtained in the presence and absence of exogenous APC in an APTT-based method (31). By definition, a smaller ratio yields a larger APC-R value (31). Increased ratios were observed in plasma samples from FV wild-type individuals or FV Leiden carriers supplemented with increasing concentrations of dabigatran (28) or rivaroxaban (29). Methods based on the activation of coagulation at the prothrombinase level are unaffected (Table 4).

**LUPUS ANTICOAGULANTS**

Although the experience for the interference of NOAs on lupus anticoagulant testing is still very limited, clotting times for dRVVT- and APTT-based tests, which are recommended for LA detection (32) are dose-dependently prolonged by dabigatran or rivaroxaban, making LA detection problematic in patients on such treatment (Table 4).

In general, NOAs are likely to influence measurements of all hemostatic parameters that are based on coagulation assays, including protein C and protein S functional assays. Hence, testing is recommended for such parameters after (1 week) discontinuation of the treatment. If discontinuation is not feasible, results should be interpreted with caution, and the physician should be informed about the possible interference to avoid misdiagnoses or misclassifications.

**Concluding Remarks**

A statement that laboratory monitoring is not needed for patients on NOAs may be seen at the beginning of a review article or heard at a conference on NOAs. Although true, this concept has been emphasized to such an extent that it is misleading and potentially dangerous, because clinicians might be falsely reassured that laboratory testing is never needed when dealing with NOAs. On the contrary, the accumulating evidence indicates that this statement is an oversimplification. An interesting and paradigmatic case report published re-
ently (11) is illustrative of this situation. A 79-year-old man with chronic renal insufficiency and taking 150 mg dabigatran twice daily needed cardiac surgery. Accordingly, dabigatran treatment was discontinued 2 days before surgery, and he was given tranexamic acid and standard heparin anticoagulation preoperatively, followed by postoperative heparin reversal with protamine sulfate. The patient developed severe bleeding postoperatively despite receiving additional tranexamic acid, protamine, cryoprecipitate, and infusion of plasma and platelets. The patient was then given 3 “cardiac doses” of rFVIIa, which failed to control the bleeding. The TCT was measured at this point, and it was 129 s (reference interval, <30 s). Two additional “hemophilic doses” of rFVIIa resolved the bleeding. From this case report we learn that the patient developed massive postoperative bleeding while he still had therapeutic dabigatran concentrations (as demonstrated by the prolonged TCT), despite having been “off” dabigatran for 2 days before his surgery. Owing to the patient’s renal insufficiency, dabigatran was still present in his circulation; presurgical measurement of plasma and platelets. The patient was then given tranexamic acid, protamine, cryoprecipitate, and infusion of plasma and platelets. The patient was then given 3 “cardiac doses” of rFVIIa, which failed to control the bleeding. The TCT was measured at this point, and it was 129 s (reference interval, <30 s). Two additional “hemophilic doses” of rFVIIa resolved the bleeding. From this case report we learn that the patient developed massive postoperative bleeding while he still had therapeutic dabigatran concentrations (as demonstrated by the prolonged TCT), despite having been “off” dabigatran for 2 days before his surgery. Owing to the patient’s renal insufficiency, dabigatran was still present in his circulation; presurgical measurement of the TCT would have revealed this fact. Another example of the danger stemming from this oversimplification comes from a recent report by Harper et al. (33) on the use of dabigatran. A considerable proportion of the study patients who were treated with dabigatran presented with bleeding. A review of their history identified risk factors for bleeding (i.e., errors of prescription, renal insufficiency, and so on), but these patients apparently did not undergo laboratory testing during their bleeding episodes. Perhaps performing such simple tests as the TCT or the ECT would have been helpful to determine whether gross abnormalities of these tests had occurred in bleeding diatheses. One might suggest that laboratory testing be performed for all patients once the treatment’s steady state is reached. This basal value might be compared with the value obtained at the time of bleeding, thus giving an opportunity to assess whether laboratory testing could help identify groups of patients whose risk factors for bleeding were not apparent at the beginning of the treatment.

In conclusion, the statement that laboratory testing is not needed for patients on NOAs is an oversimplification, and although dose adjustment (based on laboratory testing) may not appear to be necessary, some sort of assessment of the anticoagulant effect may be useful in many circumstances.

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