

Laboratory Monitoring of Anticoagulation: Where Do We Stand?

Armando Tripodi, Ph.D.,¹ and Antonius van den Besselaar, Ph.D.²

ABSTRACT

The treatment of choice for acute venous thromboembolism is anticoagulant therapy with fast-acting drugs (unfractionated or low-molecular-weight heparin or fondaparinux) aimed at preventing thrombus extension, followed by extended prophylaxis with vitamin K antagonists aimed at preventing recurrence. Experience accumulated over the years has demonstrated that strict laboratory monitoring is required for unfractionated heparin and vitamin K antagonists, making use of these drugs problematic for patients and physicians and prompting researchers to develop new anticoagulants equally effective but without the requirement for laboratory monitoring. The results of clinical trials to date, albeit limited, suggest that these new drugs will probably keep their promise. However, the definitive answer will come subsequent to these clinical trials, when clinicians will start to use these drugs to treat patients in the real world. It is likely that some sort of laboratory monitoring will be required at least for selected categories of patients. Accordingly, clinical laboratories should still be prepared to monitor patients, although the numbers may hopefully decrease sharply in the next decade or so.

KEYWORDS: Heparin, low-molecular-weight heparin, vitamin K antagonists, fondaparinux, new anticoagulant drugs

Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and pulmonary embolism (PE), is a frequent disease with an estimated incidence of first event of ~2 per 1000 inhabitants per year in Western countries.¹ Although not fatal in most cases, DVT may progress in the potentially fatal PE and the debilitating postthrombotic syndrome.² As a consequence, VTE may be regarded as a major cause of morbidity and mortality in Western countries³ and probably in Asia⁴ where it was until recently considered to occur less frequently. Currently, the treatment of choice for acute VTE is anticoagulant therapy with fast-acting drugs aimed at preventing thrombus exten-

sion and its sequels, followed by extended prophylaxis aimed at preventing recurrence.⁵ The fast-acting drugs currently employed are unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH) and the synthetic pentasaccharide fondaparinux, which are all administered parenterally.⁵ Extended prophylaxis is achieved by oral anticoagulants such as vitamin K antagonists (VKAs).⁵ There are other drugs under development, which may become suitable alternatives to the above, both for treatment and prophylaxis.⁶ The question of whether patients treated with the above drugs should undergo some sort of laboratory monitoring aimed at adjusting appropriate dosage to prevent

¹Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, University Medical School and IRCCS Ospedale Maggiore Policlinico, Mangiagalli and Regina Elena Foundation, Milan, Italy; ²Department of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, The Netherlands.

Address for correspondence and reprint requests: Armando Tripodi, Ph.D., Via Pace 9, 20122 Milan, Italy (e-mail: armando.tripodi@unimi.it).

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hemorrhage or thrombotic recurrence has long been debated. For some of the oldest ones (i.e., UFH and VKAs), the experience accumulated over the years has demonstrated that strict laboratory monitoring is required, making use of these drugs challenging for patients and physicians and prompting researchers to develop new anticoagulants equally effective but not requiring laboratory monitoring. The results of clinical trials to date, albeit limited, suggest that these new drugs probably will keep their promise. However, the definitive answer will come only after these drugs begin to be used to treat patients outside the clinical trials. It is likely that some sort of laboratory monitoring will still be required, at least in selected categories of patients. This article aims to review the current situation with respect to the laboratory methods (and their potential drawbacks) that may be employed to monitor old and new drugs for treatment and prophylaxis of VTE.

VITAMIN K ANTAGONISTS

VKAs are oral anticoagulant drugs that are derivatives of 4-hydroxycoumarin (warfarin, phenprocoumon, and acenocoumarol [nicoumalone]) and that interfere with the biosynthesis of vitamin K-dependent coagulation factors. Although these drugs have different pharmacokinetic profiles, their pharmacodynamic mechanism of action overlaps.⁷ In the steady state of treatment, the relative concentrations of the vitamin K-dependent clotting factors II, VII, IX, and X are reduced to similar but not identical levels.⁸ For most indications, the optimal therapeutic interval is achieved when patients have an international normalized ratio (INR) from 2.0 to 3.0.⁵

Prothrombin Time and INR

Traditionally, laboratory monitoring of VKA is performed with the one-stage prothrombin time (PT) test using citrate plasma. The PT test was developed by Quick et al in 1935.⁹ With modifications, this test continues to be the principal method for monitoring VKA therapy. The PT is sensitive to variation in the activity of three vitamin K-dependent factors; that is, factor II (prothrombin), factor VII, and factor X. "Prothrombin time" therefore is a misnomer, and the correct scientific name has been established as "plasma-coagulation, tissue factor-induced; time."¹⁰ However, in daily practice, the expression PT remains in use.

Many modifications of Quick's original PT reagent and technique have been developed.¹¹ Also, many different coagulometers are used to determine the PT. As a consequence, the results obtained by different laboratories could not be compared directly. Standardization of the PT may be accomplished by

transformation of the PT values obtained with any reagent/instrument into standardized values. The INR has been recommended as the universal scale for reporting of the PT in VKA monitoring.¹² It should be emphasized that the INR has been defined only for patients on long-term anticoagulant therapy and not for screening of extrinsic pathway factors. The INR is based on two principles: (a) the establishment of an international standard for thromboplastin in conjunction with a well-described method for using this standard; and (b) the calibration of other PT systems against the international standard by testing fresh samples from both healthy individuals ("normals") and patients who have been on VKA for at least 6 weeks.¹³ The calibration includes the selection of patients with INR values in the range 1.5 to 4.5, the calculation of an orthogonal regression line for log-transformed PT, and the exclusion of outlying measurements.¹³ In the recommended calibration model, the international sensitivity index (ISI) of a PT system is defined as a quantitative measure of the system's responsiveness to the defect induced by VKA. The ISI is derived from the orthogonal regression line calculated for both normals' and patients' log(PT) measurements, assuming that the same line is valid for both normals and patients. In many cases, the assumption of a single straight line through normals and patients appears to be valid, but occasionally a deviation is observed. The logarithms of the PT values of the normals may lie systematically away from the regression line estimated using only patients' plasma samples. In case of marked deviation, the assignment of an ISI would not be meaningful. Consequently, application of an inappropriate ISI for calculation of the INR would result in a bias. A deviation from the ISI model can be handled in two ways: (a) a modified calibration model is used, e.g., as described by Tomenson¹⁴; (b) the magnitude of the bias at INR 2.0 and at INR 4.5 is assessed, and if the bias is not greater than 10%, it may be ignored.¹³ An example of the INR correction according to Tomenson has been shown in the case of the reagent Normotest, which demonstrated nonlinear calibration lines.¹⁵ Manufacturers of thromboplastin reagents do not always report small deviations from the ISI calibration model. With the advent of whole-blood point-of-care monitors for INR (see next section), any appropriate calibration model can be fixed in the microprocessor of the instrument, e.g., a polynomial regression model.¹⁶ In this way, any INR bias due to nonlinearity can be avoided.

Point-of-Care Whole-Blood Coagulation Monitors and INR

Point-of-care test (POCT) monitors are increasingly being used for monitoring of VKA treatment.¹⁷ Some types of POCT instruments can be used with citrate blood or citrate plasma.^{18,19} Other types are being used

for home PT testing with native capillary blood.^{20–22} In several cases, the POCT systems could be calibrated by adopting the ISI model.^{16,19,21} It can be concluded from the published studies that PT-INR self-testing may be considered as a suitable alternative to conventional laboratory testing.²³ For the PT-INR to be reliable, manufacturers of POCT monitors should calibrate their devices against international standards for thromboplastin with procedures similar to those recommended for conventional laboratory systems.^{24,25} Potential users of a new system or a new lot of test strips may wish to validate these before using the system in clinical practice. In The Netherlands, new test strip lots for POCT monitors are assessed by independent centers when they are introduced onto the market.²⁶ Small interlot differences were observed for the CoaguChek S (Roche Diagnostics, Indianapolis, IN), which were deemed not of clinical importance.²⁶

Training of patients and implementation of appropriate quality assessment schemes are also essential prerequisites for the success of PT-INR self-testing. External quality assessment (EQA) of POCT monitors may be performed with sets of lyophilized plasmas at different INR levels.²⁷ EQA is useful to assess imprecision and to reassure users about the comparability of their results with those obtained by other users of the same devices. Some authors advised caution in the interpretation of results obtained with lyophilized plasmas.²⁸ When POCT monitors are calibrated only for analysis of whole blood, results for plasma cannot be used to assess accuracy without supporting evidence derived from whole-blood analysis.²⁸ EQA should be an inherent component of patient self-testing of oral anticoagulation. In a recent study, various methods of EQA were evaluated.²⁹ It was concluded that patients are independently able to undertake a formal EQA scheme.

UNFRACTIONATED HEPARIN

Heparin is endowed with the remarkable ability to bind the plasma protein antithrombin (AT) and to convert this relatively slow inhibitor into a potent anticoagulant.³⁰ Intravenously administered UFH is a mainstay of inpatient anticoagulation therapy for a variety of clinical indications. Because the anticoagulant response to UFH varies widely among individuals, it is standard care to monitor UFH and make dose adjustments based on the results of coagulation testing. Based on the results of an observational clinical study,³¹ use of the activated partial thromboplastin time (APTT) to monitor UFH with a therapeutic range of 1.5 to 2.5 clotting time prolongation over the basal value became standard practice. However, use of the APTT is complicated by the variable response of different coagulometers and reagents to heparins. It has been recommended that

hospital laboratories calibrate a therapeutic range for their APTT reagent-coagulometer combination to provide heparin levels of 0.2 to 0.4 units/mL as measured by protamine sulfate titration or to an anti-factor (F) Xa level of 0.3 to 0.7 units/mL. In a recent study, the interlaboratory agreement of the anti-FXa–correlated APTT was compared with that of the traditional 1.5- to 2.5-times the midpoint of normal method.³² It was concluded that the anti-FXa–correlation method does not seem to enhance interlaboratory agreement in UFH monitoring compared with the traditional 1.5- to 2.5-times method. Similar conclusions were obtained in another study showing similar heparin dosage adjustment decisions using an empiric APTT therapeutic range versus a heparin concentration-derived APTT therapeutic range.³³

The anti-FXa assay specifically determines the anticoagulant activity of UFH by measuring the ability of heparin-bound AT to inhibit a single enzyme (i.e., FXa). The principle is very simple: test plasmas are added with excess FXa, which is inhibited by UFH (or LMWH), and the residual FXa is eventually measured using a specific chromogenic substrate. Optical density readings are then converted into anti-FXa activity by interpolation from a dose-response curve constructed by using increasing amounts of the brand of UFH (or LMWH) used for treatment. In recent years, the chromogenic anti-FXa assay has become automated, cost-effective, and more accessible to clinicians. Thus, in many institutions, UFH is monitored directly against the anti-FXa rather than indirectly via the APTT. However, differences up to 30% in mean UFH levels were demonstrated between separate anti-FXa assays.³⁴ Apart from binding to AT, UFH is involved in nonspecific binding to other plasma proteins and platelet-derived proteins. Some anti-FXa assays are performed in the presence of exogenous dextran sulfate, aiming at reducing the influence of heparin-binding proteins other than AT. Furthermore, the UFH anti-FXa assays can be performed with and without addition of exogenous AT. This needs to be considered in the context of the endogenous AT levels in different clinical settings/patient populations. In a recent study, the anti-FXa activity for a given dose of UFH was found to vary significantly based on the anti-FXa assay and the (pediatric) population being monitored.³⁵ It was suggested that an anti-FXa assay without addition of exogenous AT and dextran sulfate provides the best physiologic measure of the UFH effect in children.³⁵

Thrombin generation for the control of heparin treatment has been compared with the APTT.³⁶ Relative to the baseline value of the individual, the heparin effect was recognized by the APTT in 55% of the cases and by the thrombin generation method in 98%.

LOW-MOLECULAR-WEIGHT HEPARIN

LMWH represents a family of sulfated polysaccharides with molecular weight (2000 to 9000 Da) of approximately less than half that of the parent molecule UFH from which the individual LMWH is obtained by fractionation or depolymerization.³⁷ Because of the relatively low molecular weight, the anti-FIIa activity of LMWH is considerably reduced whereas the anti-FXa activity is preserved so that the anti-FXa/anti-FIIa ratio is greater than 1.³⁷ However, it should be acknowledged that there are great variations among commercial preparations of LMWH with respect to the molecular weight and consequently the anti-FXa/anti-FIIa activity ratio. The above characteristics do not allow monitoring patients by means of the APTT as per UFH (see earlier), because this test is both scarcely and variably prolonged as a consequence of treatment. However, the widely accepted notion that the LMWH does not prolong the APTT should be viewed with caution as some of the commercial brands do so, depending on their more favorable activity ratio toward FIIa. Numbers of studies performed over the years (reviewed in Ref. 37) have cumulatively shown that LMWH can be used for prophylaxis and treatment of VTE with considerable advantages over UFH. Among them, the most important from the practical standpoint are the route of administration (subcutaneous once or twice per day for LMWH versus intravenous for UFH) and no requirement for laboratory monitoring, both making LMWH an ideal drug for short-term anticoagulation of patients outside the hospital setting. The latter issue has been the focus of much attention and many debates for many years and is still probably unresolved. An early review by Boneu³⁸ concluded that in the majority of patients given LMWH for prophylaxis or treatment of VTE, laboratory monitoring is not required. This corroborated the results and conclusions stemming from clinical trials that were designed to assess the efficacy and safety of LMWH given at a fixed dose. However, the increased use of LMWH in daily practice for the treatment of acute VTE allowed accumulation of information that escaped the attention of those who designed the clinical trials where patients were highly selected. This information caused the American College of Pathologists³⁹ to recommend monitoring in selected groups of patients treated for VTE, including those who are over- or underweight; are pregnant; in those who have renal insufficiency; and in children. In all these specific conditions, there might be a risk of over- or under-treatment or the risk of accumulation (as occurs in patients with renal insufficiency given that the kidney is the main route of elimination of LMWH). Recently, the yes/no debate was reopened by Harenberg⁴⁰ who added further indications requiring monitoring, such as patients on extended therapy for various clinical

conditions. The other dualists, while reiterating that clinical trials did not show any association between major hemorrhage and activity measurement, acknowledged that for some groups of patients, monitoring may be appropriate.⁴¹ Altogether, there seems to be consensus among the experts that prophylaxis does not require monitoring, whereas therapy of VTE does, at least for some categories of patients; this brings one to the point of making a decision regarding which kind of test should be used. The most often used test to monitor LMWH has been and still is the anti-FXa activity assay, determined in plasmas supplemented, or not, with AT and using chromogenic substrates specific for FXa. Another widely used method is the Heptest, a clotting assay based on FXa inactivation.⁴² Despite their simplicity, anti-FXa assays are affected by various drawbacks.^{43,44} For instance, Kitchen et al⁴³ have shown results obtained for plasmas from patients treated with two different brands of LMWH by using different methods for anti-FXa activity and found mean values spanning from 0.28 to 0.69 U/mL for one preparation and from 0.43 to 0.69 U/mL for the other. Furthermore, results obtained with the Heptest were significantly lower than those obtained with chromogenic methods.⁴³ This between-method variability occurred notwithstanding the fact that the assays were standardized against the first international World Health Organization (WHO) standard for LMWH.⁴³ Probably, the differences recorded by these authors are somewhat underestimated if one considers that different laboratories use different instruments and different standards to perform their assays. As a matter of fact, results of the national U.K. National External Quality Assessment Scheme surveys on coagulation showed interlaboratory coefficient of variation (CV) values up to 20% (Kitchen, personal communication). The reasons for this relatively high between-method variability, although not precisely known, may rest on the variable influence of instruments, standards,⁴⁵ and the variable thrombin inhibition of LMWH.⁴⁶ Finally, it should be realized that the inhibitory effect of LMWH on FXa as it occurs for UFH is dependent on AT.⁴⁷ Some commercial reagents do involve the addition of exogenous AT to optimize its assay concentration, whereas others do not. It is therefore possible that the variable anti-FXa activity obtained with different methods may depend on the variable activity of AT present in the patient plasmas. The above considerations clearly demonstrate that there is high heterogeneity among different methods, which may influence management of patients. As a consequence, adoption of a universal therapeutic interval might be problematic, although some guidelines recommend target anti-FXa activity for VTE treatment spanning from 0.5 to 1.1 U/mL for sample collected 4 hours after subcutaneous injection regardless of the method used for testing.³⁹

FONDAPARINUX

As mentioned previously, the anticoagulant activity of heparin is dependent on its interaction with AT. The sequence that permits high-affinity binding contains an essential pentasaccharide. This relatively short chain makes AT able to efficiently inhibit FXa but not thrombin. Fondaparinux is the synthetic analogue of the pentasaccharide and therefore may be regarded as an indirect (AT-dependent) FXa-inhibitor.⁴⁸ Its development and use in the treatment of VTE was the logical consequence of the elucidation of the above mechanism. Besides being a selective FXa inhibitor, fondaparinux has some interesting properties: it is homogenous, can be given once daily subcutaneously, has high bioavailability, does not cross-react with heparin-induced thrombocytopenia (HIT) antibodies, and has a relatively long half-life. These properties, along with the successful clinical trials designed to assess safety and efficacy, probably made it the first selective FXa inhibitor to receive U.S. Food and Drug Administration (FDA) approval for prevention and treatment of VTE. Efficacy and safety of treatment have been evaluated in clinical trials (reviewed in Ref. 49) where the drug was administered at a fixed dosage without laboratory control. Therefore, it appears that this treatment does not require monitoring. However, as happened for LMWH, it might be that extensive use of fondaparinux may bring to our attention categories of patients for whom monitoring may be advisable. Furthermore, it should be realized that treatment with fondaparinux may occasionally need reversal, and therefore a test would be needed to assess the success or otherwise of the reversal (i.e., the persistence of anticoagulation). The current test of choice would be again based on anti-FXa activity. Additional tests have also been used such as the prothrombinase-induced clotting time assay^{50,51} or the thrombin generation test.⁵² The latter proved highly effective in detecting the fondaparinux effect and also its reversal.⁵²

Idraparinux is a further generation of the synthetic pentasaccharide that has been modified from fondaparinux by hypermethylation. This modification increases its affinity for AT to such an extent that its half-life is much more prolonged (17 versus 80 hours for fondaparinux and idraparinux, respectively). This property, coupled with its complete bioavailability and predictable anticoagulant response, permits treatment of VTE by subcutaneous injection once weekly not requiring laboratory monitoring.⁵³ Protamine sulfate, which is used to reverse anticoagulation after heparin or LMWH, does not bind fondaparinux or idraparinux. Therefore, a biotinylated version of idraparinux has been developed.⁵⁴ This drug (provisionally coded as SSR 126517) retains the anticoagulant properties of idraparinux but also permits rapid reversal in case of need using intravenous injection of avidin.

DIRECT FACTOR Xa OR THROMBIN INHIBITORS

As mentioned previously, heparin, LMWH, fondaparinux, and idraparinux mediate their anticoagulant activity through binding to AT. Efforts have been made over the past decade to develop drugs that may inhibit directly FXa or thrombin.

Rivaroxaban

Rivaroxaban is a potent and selective direct inhibitor of FXa regardless of whether the enzyme is free or bound to the prothrombinase complex.⁵⁵ It is absorbed from the gastrointestinal tract, its bioavailability is considerable, and the half-life is around 5 and 12 hours in the young and the elderly, respectively.⁵⁶ Clinical trials (reviewed in Ref. 49) have been performed with fixed doses, and the treatment does not apparently require laboratory monitoring. However, the drug prolongs both the APTT and PT (the latter being more responsive) in a reagent-dependent fashion. Furthermore, like all the other FXa inhibitors, rivaroxaban can also be monitored by anti-FXa assays. On the basis of the limited experience, this latter test should be considered the test of choice if monitoring is found to be required.

Apixaban

Apixaban is another direct FXa inhibitor with properties similar to those of rivaroxaban.⁵⁷ Phase II clinical trials for this drug have been performed at fixed dosage,⁵⁷ and again no laboratory monitoring was taken into consideration in those studies. The drug prolongs APTT and PT only slightly at therapeutic concentrations. Laboratory monitoring, if required, should be based on FXa inhibition.

Dabigatran

Dabigatran etexilate is a new, orally active direct thrombin inhibitor.⁵⁸ Clinical trials with fixed dosage are under way.⁶ Laboratory monitoring if needed for special categories of patients will become apparent at a later stage. Although there is not very much information available in the literature, this drug should prolong the conventional coagulation tests PT, APTT, and the ecarin clotting time.⁵⁹ Therefore, if some sort of laboratory evaluation will be needed, the above tests could be used.

Hirudin and Argatroban

Other direct thrombin inhibitors such as recombinant hirudin (lepirudin) and argatroban were developed years ago and are now licensed for patients who in the course of treatment with heparin develop HIT. Both are

reported to need laboratory monitoring to adjust the dosage,⁶⁰ especially in patients with impaired renal function, and the APTT seems the test of choice. Dosages aimed at prolongation by 1.5 to 2.5 from baseline (lepirudin) or 1.5 to 3.0 (argatroban) are required.⁶⁰ However, it should be realized that no between-assay standardization has been achieved to date, and this may be a problem if one considers that there is a different responsiveness of commercial APTT reagents to the anticoagulant action of these drugs.⁶¹ Finally, it should be realized that argatroban greatly increases the INR, and this may be a problem when argatroban is stopped and the patient is switched to VKA. It could in fact be very difficult to monitor VKA and reach an appropriate therapeutic interval when the two drugs are concomitantly effective. Because waiting to resume VKA therapy after the effect of argatroban vanishes may expose the patients to the risk of rethrombosis, the alternative could be monitoring VKA by means of a FX chromogenic assay.⁶⁰

CONCLUSION

Laboratory monitoring is needed when the variation in the individual response to a given drug is so large that a paradigm dose-standard effect is unattainable. Evidence accumulated over several decades has shown that this is certainly the case for UFH and VKA, which are used for treatment and extended prophylaxis of VTE, respectively. Whereas laboratory monitoring of VKA has been reasonably standardized, monitoring of UFH has been more complex and far reaching. However, one can anticipate that even if standardization of UFH therapy had been achieved, research toward developing new alternative drugs to replace UFH and VKA would have not been slowed. As a matter of fact, laboratory monitoring is a burden for clinicians and patients alike. LMWH has apparently alleviated one of the problems, and probably the new anticoagulants will resolve the rest. However, this does not necessarily mean that laboratory monitoring of antithrombotic drugs should be dismantled. As mentioned, there may be categories of patients treated with LMWH who still need monitoring, and this may probably apply also to some of those patients who will be treated with the new anticoagulants. For instance, one would argue that whereas LMWH is usually given for a relatively short period of time, the new drugs will be given for extended (sometimes lifelong) prophylaxis. Perhaps, without laboratory monitoring, the compliance for these drugs may prove more difficult than anticipated from the experience gained from clinical trials. As mentioned, rapid reversal of anticoagulation may occasionally be needed, and some laboratory method might be needed to assess whether and to what extent this has been achieved.

In conclusion, we believe that the clinical laboratory should still be prepared to continue monitoring patients, although the numbers may hopefully decrease sharply in the next decade or so. It is also important to invest time and effort in developing and validating new tests that more closely mimic what occurs in vivo. In this respect, thrombin generation tests would appear to be the most promising candidates as all the above-mentioned drugs ultimately share the same mechanism of action: they have been designed to directly or indirectly decrease thrombin activity.⁶²

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