Review Article

# Monitoring of Anticoagulant Therapy in Heart Disease: Considerations for the Current Assays

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## Abstract

*Clinicians should be aware of new developments to familiarize themselves with pharmacokinetic and pharmacodynamic characteristics of new anticoagulant agents to appropriately and safely use them. For the moment, cardiologists and other clinicians also require to master currently available drugs, realizing the mechanism of action, side effects, and laboratory monitoring to measure their anticoagulant effects. Warfarin and heparin have narrow therapeutic window with high inter- and intra-patient variability, thereby the use of either drug needs careful laboratory monitoring and dose adjustment to ensure proper antithrombotic protection while minimizing the bleeding risk. The prothrombin time (PT) and the activated partial thromboplastin time (aPTT) are laboratory tests commonly used to monitor warfarin and heparin, respectively. These two tests depend highly on the combination of reagent and instrument utilized. Results for a single specimen tested in different laboratories are variable; this is mostly attributable to the specific reagents and to a much lesser degree to the instrument used. The PT stands alone as the single coagulation test that has undergone the most extensive attempt at assay standardization. The international normalized ratio (INR) was introduced to "normalize" all PT reagents to a World Health Organization (WHO) reference thromboplastin preparation standard, such that a PT measured anywhere in the world would result in an INR value similar to that which would have been achieved had the WHO reference thromboplastin been utilized. However, INRs are reproducible between laboratories for only those patients who are stably anticoagulated with vitamin K antagonists (VKAs) (i.e., at least 6 weeks of VKA therapy), and are not reliable or reproducible between laboratories for patients for whom VKA therapy has recently been started or any other clinical conditions associated with a prolonged PT such as liver disease, disseminated intravascular coagulation, and congenital factor deficiencies. In contrast to marked progress in the standardization of PT reagents for INR reporting, no standardization system has been globally adopted for standardization of PTT reagents. Recently College of American Pathologists recommend that individual laboratories establish their own therapeutic range by using aPTT values calibrated against accepted therapeutic unfractionated heparin (UFH) levels calibrated against accepted therapeutic UFH levels performing anti-Xa test (which is the most accurate assay for monitoring UFH therapy).*

*Herein, we review recent data on the monitoring of conventional anticoagulant agents. Marked interlaboratory variability still exists for PT, INR, and PTT tests. Further research should be focused on improving the standardization and calibration of these assays.*

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## Introduction

The final effect of the coagulation cascade is to produce thrombin, which acts on fibrinogen to generate the fibrin clot. Thrombin, factor IIa (FIIa), is produced from prothrombin by the action of activated factor X (FXa). Anticoagulants are frequently prescribed for patients with a variety of cardiovascular diseases to prevent thrombosis, to treat present thrombosis, or to reduce the recurrence of thromboembolic events after a first episode of thrombosis. For decades, two main classes of anticoagulants have been widely used by cardiologists: orally administered vitamin K antagonists (VKAs), in particular warfarin, or parenterally administered unfractionated heparin (UFH). Since their introduction into clinical practice in the late 1980s, low-molecular-weight heparins (LMWHs) have replaced UFH for many indications; however, UHF remains the drug of choice in selected patient groups due to its short half-life, its higher safety among patients with renal failure, and the fact that its anticoagulant effects are typically reversible with protamine sulfate.[1]

Currently available anticoagulants have several drawbacks. UFH and LMWHs, for example, must be given parenterally, which limits their usage in the outpatient setting and causes a rare but potentially fatal complication of heparin-induced thrombocytopenia.[2] Warfarin offers the convenience of oral administration but its antithrombotic effect takes hold only during the following three to five days and is subject to interaction with a host of foods and other drugs,[3] often making anticoagulant control hard to obtain. Finally, warfarin and heparin have narrow therapeutic window with high inter- and intra-patient variability; thus, the use of either drug needs careful laboratory monitoring and dose adjustment to ensure proper antithrombotic protection while minimizing the bleeding risk. Therefore, there has been a desire for simple, safer, fixed dosing new oral anticoagulation drugs with high bioavailability and predictable pharmacokinetics without need for monitoring. There are now several new oral agents targeting multiple points in the coagulation cascade that have the potential to alter the nature of anticoagulant therapy.[4] Clinicians should be aware of new developments to familiarize themselves with the pharmacokinetic and pharmacodynamic characteristics of these agents to appropriately and safely use them. For the moment, cardiologists and other clinicians also require to master currently available drugs, realizing the mechanism of action, side effects, and laboratory monitoring to measure their anticoagulant effects.

More importantly, for achieving best quality, laboratories require to concentrate not only on analytical steps but also on other steps in laboratory testing i.e. pre-analytic and post-analytic ones. Analyzing the causes of laboratory errors, Plebani and Carraro ascribed 68% of laboratory errors to pre-analytic phase.[5] Several other studies have shown similar results.[5, 6] In vitro coagulation test results are more sensitive to pre-analytic variables related to the quality of patients' citrated plasma samples than most other laboratory assays. Pre-analytic variables have significant impacts on the coagulation protein-enzymatic reactions occurring in prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays. Pre-analytic variables may be due to phlebotomy (time for tourniquet application optimally < 1 minute), collection tubes (standardized tubes and ensure the appropriate blood-to anticoagulant agent ratio), specimen transportation (as soon as possible), centrifugation conditions (time and speed to ensure obtaining platelet poor plasma < 10,000/ul), and processing.[1, 7, 8] Since the accuracy of coagulation results is directly associated with the sample quality influenced by these pre-analytic variables, it is essential to recognize applicable pre-analytic variables that could have occurred before actual coagulation testing beginning at the time of phlebotomy.

## Warfarin therapy

Warfarin is the most frequently used VKA and the only oral anticoagulant available in Iran. VKAs act indirectly and inhibit the gamma carboxylation of the vitamin K-dependent coagulation factors II (prothrombin), VII, IX, and X.[9] The mechanism of the action of warfarin justifies its delayed onset and offset of action; synthesized coagulation factors prior to warfarin intake would continue to function until degraded and replaced by inadequately carboxylated molecules, leading to a slow onset of action whereas the long half-life of warfarin explains the slow offset of action.[9] As a result, in most circumstances, warfarin must be initiated in conjunction with a rapidly acting anticoagulant such as heparin; it also must be discontinued several days before surgeries to reduce the likelihood of excessive bleeding.

### Laboratory control

The complex pharmacokinetics and pharmacodynamics of warfarin necessitate the inconvenience of frequent coagulation monitoring and dose adjustment. The PT has been applied for monitoring warfarin since the introduction of this drug in 1941. First emerged in the early 1900s, PT is a commonly used screening test which assesses the global function of the extrinsic clotting pathway, and specific cause of abnormal PT needs more specific and specialized coagulation tests. The PT is performed by adding a reagent known as thromboplastin to citrated plasma. Thromboplastin is a mixture of tissue factor, phospholipid, and calcium ions, and is used to initiate clotting as measured in the PT assay. Initially, the tests were performed by the manual clotting techniques (e.g., tilt-tube method) and reagents prepared by individual laboratories, causing a wide variability of results.

In the late 1960s, commercially prepared reagents and instrumentation for detecting the end point of the PT became popular and decreased the degree of variability up to a point. However, manufacturers' reagents differed from one another, and the same lot numbers from an individual manufacturer revealed similar variability.[10] The PT stands alone as the single coagulation assay which has undergone major efforts for standardization. The desire to standardize PTs began in the mid-1960s and the process was developed methodically during the 1970s and 1980s.[11-15] One must note that the impetus for PT reagent standardization was to improve therapy for one particular group of patients chronically anticoagulated with VKA therapy. This PT standardization was required due to the narrow therapeutic index of warfarin and the high risk of clotting or bleeding because of underdosage and overdosage, respectively. An influential study demonstrated that reduced warfarin dosing and as a result low incidence of bleeding in countries using sensitive thromboplastin reagents dramatically accelerated the international interest to standardize PT results and underscored the impetus for the rapid clinical adoption of the international normalized ratio (INR).[16] Poller and Taberner[17] also brought the importance of both the degree and the nature of this variability into attention in an epidemiologic study which showed that both the rate of hemorrhagic complications and the dosage of warfarin differed in various regions of the world. This difference was attributed to the varied sensitivities of the thromboplastin reagents used to perform the PT.

### *International Normalized Ratio*

Thromboplastin reagent can be produced by a variety of methods, including tissue extraction, tissue culture, and molecular biological (genetic) technologies. Thromboplastins from various sources and methods of manufacture contain various concentrations and mixtures of components, and this result in the fact that thromboplastins vary in responsiveness to a decrease in the vitamin K-dependent coagulation factors. An unresponsive (insensitive) thromboplastin causes less prolongation of the PT for a given decrease in vitamin K-dependent clotting factors than a responsive (sensitive) one. In 1983, the world health organization (WHO) produced a 'gold standard' in an attempt to offset variation in thromboplastin reagent responsiveness and enhance standardization of PT reporting.[18, 19] The INR was rapidly accepted of in Europe and other regions of the world to such an extent that it was nearly universally used by the end of the 1980s; however, the acceptance in the United States was slower and it came almost a decade later. At the time the INR was developed, PT reagents in the United States (originated typically from rabbit) were relatively insensitive to factor deficiencies as compared to those consumed in Europe or Canada (originated typically from bovine or ovine). The INR was intended to ''normalize'' all PT reagents to a WHO reference thromboplastin preparation standard, such that a PT measured anywhere in the world would result in an INR value similar to that which would have been achieved had the WHO reference thromboplastin been utilized. Therefore, INRs from a given laboratory would be comparable to that from any other laboratory across the world.[19]

In order to account for PT reagent responsiveness, an International Sensitivity Index (ISI) is assigned to each commercial lot number of thromboplastin reagent. The ISI is a calibration parameter that defines the sensitivity of the reagent as compared to a WHO International Reference Preparation (IRP) which, by definition, has an ISI of 1.0. The more sensitive the reagent, the lower the ISI value.[20] Highly sensitive thromboplastins (ISI, approximately 1.0) produced by recombinant technology are now available. It was speculated that the use of low-ISI reagent could improve the laboratory monitoring of oral anticoagulant therapy.[21] Unfortunately; however, low-ISI reagent, either tissue- or recombinantly-originated, would not improve INR precision or reproducibility between laboratories.[22] The INR is a mathematical conversion of the PT measured as follows:

$$\mathrm{INR} = \left(\frac{\text{Patient PT}}{\text{Mean Normal PT}}\right)^{\mathrm{ISI}}$$

Where Patient PT = measured prothrombin time, Mean Normal PT = geometric mean PT of the reference range, ISI = International Sensitivity Index, specific to each reagent-instrument combination. To determine an INR or establishing a local ISI, geometric, not arithmetic, mean PT is recommended based on the fact that PT values in a ''normal'' population are distributed log-normally.[23] The mean normal PT is the geometric mean of the PT of almost 20 healthy individuals obtained via the blood-collection system in use locally and tested with the same make and lot of thromboplastin as that of the ISI in use. Even though the INR system has improved PT reporting, it is still related to unexpectedly high degrees of inconsistency in values between laboratories and even within one laboratory between two different instruments.

An important source of variation in the INR system is the manufacturer-assigned ISI value as compared to the "actual" ISI value according to the local calibration of the thromboplastin against an IRP. The INR system of PT standardization was originally on the basis of manual PT determination, and a single ISI value for each batch of thromboplastin reagent was assigned[18, 24].Although many laboratories in Iran still detect PT manually, in all accredited centers worldwide and in ours, the manual PT has been replaced by fully automated coagulometers , and many studies have revealed that the ISIs of thromboplastin reagents vary based on the type of instrument used.[25-28] Some manufacturers have developed 'instrument-specific' ISIs; nevertheless, this does not solve the problem completely

due to many possible instrument/reagent combinations and because ISIs are often different even among individual types of instruments with the same thromboplastin. Causes for why the ISI value of the thromboplastin reagent in a particular laboratory on a particular instrument may be different from the "actual" ISI value (variation in local ISI) include, but are not restricted to, imprecision in the assignment of ISI by the manufacturer, incorrect ISI value used by the laboratory in the INR calculation, and the local effect of the coagulation instrument on the ISI.

For various reasons, including the need for manual PT testing with a WHO reference standard thromboplastin, and that WHO standard thromboplastin is not readily available routinely, ISI calibration by using the WHO-recommended procedure is not always possible in routine hospital laboratories. Moreover, the WHO procedure needs a sample of 60 fresh plasmas from stabilized orally anticoagulated patients and 20 fresh plasmas from normal individuals. Thus, thromboplastin reagents that are used in the laboratory are not calibrated directly against the IRP; they are instead calibrated against secondary standards held by the manufacturers. In fact, these reagents may be three or more calibration steps away from the IRP. A definite amount of imprecision in the ISI value occurs and is allowed with each calibration. If a thromboplastin reagent is three steps away from the IRP, up to 15% variation in the ISI value in comparison with that determined against the IRP may be observed. This imprecision in the ISI of the thromboplastin used locally is merely one reason for the difference in the INR system.

Another reason for variation is that different coagulation instruments can have significant effects on the ISI of thromboplastins. ISI values are, thus, instrument- and reagent-specific. Variation in ISI values between apparently identical coagulation instruments using the same thromboplastin may occur. ISI values are considered "generic" if the ISI determined for a thromboplastin is provided for a group of instruments that use the same general method for end-point detection, such as manual, photo-optical, or mechanical methods. This general scheme of assigning an ISI is problematic because not all instruments within a group (i.e., not all optical systems) function in the same way. Whenever possible, laboratories should use thromboplastin reagents with instrument-specific ISI values, as this improves INR accuracy.

For a more detailed review of variations in local ISI, the reader is referred to CLSI document H54 Procedures for Validation of INR and Local Calibration of PT/INR Systems.[23]

Notably, INR results between instruments or between laboratories show increased variation in values, even as high as 20%, when the therapeutic range of INR (i.e., INR 2-4.5) is exceeded.[29] This variability occurs due to the fact that above this value INR, results become very sensitive to changes in PT as determined in seconds. It is unknown how reproducible INRs > 4.5 are between laboratories, and no certified or calibrator material exists to detect the accuracy of INRs more than this value. Also, to our knowledge, there is no study on the variability of INRs > 4.5 in association with using different thromboplastin reagents or instruments. On the other hand, laboratories should report INRs more than 4.5 because international guidelines recommend different reversal VKA treatments to be undertaken when the INR is > 5 vs. > 9.[9] Although the accuracy of INRs reported in this range is not known, clinicians are relying on these values for particular therapies for the different ranges.

Furthermore, theoretically, determination of the highest INR a laboratory could report is dependent on the upper limit of the PT measurable range. Using INR equation simply converts the upper limit of the PT measurable range into an INR. For instance, if the PT limit is 125 seconds, the geometric mean PT is 12.5 seconds and the reagent ISI is 1, with the highest INR the laboratory could report being $(125/12.5)^{1.0} = 10$. If the same measured PT and geometric mean PT are applicable, but the reagent ISI is 1.4, the highest INR the laboratory could report is $(125/12.5)^{1.4} = 15.9$. The practice of reporting INRs greater than 10 is questioned and some laboratories simply report their INR at ‘‘greater than or equal to 10’’.

Therefore, it seems vital for any individual laboratory to verify the ISI assignment for its own unique local PT system, i.e., thromboplastin/coagulometer combination.[30,31] Although such plasmas are not yet available in Iran, certified plasmas are well-characterized plasmas that have INR values assigned to them in order to verify that the ISI used locally is correct. If the INR values of the certified plasmas determined locally vary from the assigned INR values of these plasmas, this suggests that local INR calibration is necessary or perhaps a different thromboplastin reagent should be applied.

To overcome the aforementioned restrictions and to provide guidance to both suppliers (manufacturers or reference laboratories) and laboratories as consumers of the certified plasmas, a working group of the International Society of Thrombosis and Haemostasis, Subcommittee on Control of Anticoagulation, has recently produced guidelines on ISI calibration and INR determination.[32] The reader is referred to this guideline for a detailed discussion on preparation, certification ,and use of certified plasmas for INR detection and internal quality control for INR performances .External quality control for INR performances is also available with a number of national and international schemes ,including that from the WHO.

### *Is INR reliable in all clinical situations?*

One must keep in mind that INRs are reproducible between laboratories for only those patients who are stably anticoagulated (i.e., at least 6 weeks of VKA therapy), and are not reliable or reproducible between laboratories for

patients for whom VKA therapy has recently been started or any other clinical conditions associated with a prolonged PT such as liver disease, disseminated intravascular coagulation, congenital factor deficiencies.[31, 33-35] It is specifically suggested that PT numbers, in seconds, be used instead of the INR for reporting PT results for patients with liver disease.[36]

### *PT/INR Point-of-care testing*

Considering the large number of patients on chronic anticoagulant therapy, there has been an increasing interest to determine the INR at the point of care. The Point of Care Testing (POCT) is defined as: "Diagnostic testing performed near to or at the site of the patient care which results in possible change in the care of the patient". As compared to testing performed in the clinical laboratory, it would be more convenient for patients to perform testing at home or at a local community clinic. Such a system would also generally provide savings of time and transportation costs. Moreover, a growing body of evidence has revealed that PT/INR self-testing is a trusted method for monitoring long-term oral anticoagulation therapy, and it improves patient outcomes.[37]

As the safety and effectiveness of VKAs therapy is dependent on having patients within the target INR ranges (thromboembolic events rise at INR < 2.0 and bleeding risk at INR > 4.5, disproportionately), the POCT must yield reliable INR values. The INR variability between two methods, POCT vs. clinical laboratory, is not significantly different from previously documented interlaboratory INR variability, and POCT testing does not impose additional variability to INR results.[9] However, optimal calibration and quality control systems as well as reference with expert centers are required to achieve and maintain an acceptable quality standard.

### *What clinicians need to know about PT/INR laboratory monitoring*

1. Despite the fact that the PT coagulation test has undergone the most extensive attempt for assay standardization, progress in INR standardization of thromboplastins and calibration of coagulometers have not completely eliminated the variability in reagent/instrument within and between laboratories. A national quality control program and accreditation of laboratories by the Iranian Society of Pathology may solve the problem in the interpretation of the PT results.

2. Factors such as dietary vitamin K intake, dietary factors other than vitamin K, alcohol use, concomitantly taken drugs, herbal medicines, or supplements influence warfarin metabolism and INR results and are of clinical importance for warfarin-treated patients (Table 1).[3, 38-41] Therefore, in the case of remarkable dietary and lifestyle changes and at commencement and discontinuation of concomitant drug therapy, close monitoring of anticoagulation is recommended.[42]

3. Although biologic variables such as age, gender, and genetic differences influence warfarin metabolism and INR results, these factors are not of clinical importance and are only responsible for a total approximate 10% INR coefficient of variability (CV).[43]

4. Clinicians should be aware of pre-analytic variables, including the system of blood drawing, the tubes and citrate concentrations in use, the amount of blood collected, the tendency to reject unsuitable specimens, the storage temperatures, and the times between collection and analysis, as potentially important factors for having a direct influence on the quality of results and on their clinical reliability.

5. In contrast to the concept that the PT reagents are only sensitive to VKAs, the well-known interference of heparin with INR has been reported.[44, 45] When PT reagents sensitive to heparin are used in a patient treated concurrently with heparin and warfarin, the INR values obtained are falsely elevated. Nonetheless, fortunately, most of the current PT reagents are not affected by therapeutic levels of heparin (concentrations of heparin as high as 1 U/ml).

6. According to the data from the leading manufacturers and suppliers of reagents-coagulometers in Iran, only a small number of laboratories provide routinely performing and evaluating daily quality control; this may result in variety in PT results in different laboratories.

7. One must keep in mind that INRs are reproducible between laboratories for only those patients who are stably anticoagulated (i.e., at least 6 weeks of VKA therapy) and are not reliable or reproducible between laboratories for patients for whom VKA therapy has recently been started or any other clinical conditions associated with a prolonged PT such as liver disease, disseminated intravascular coagulation, and congenital factor deficiencies.[31, 33-35] It is specifically suggested that PT numbers, in seconds, be used instead of the INR for reporting PT results for patients with liver disease.[36]

8. INR is the only important and reliable parameter for the monitoring of patients who are stably anticoagulated (i.e., at least 6 weeks of VKA therapy).

## *Antithrombin (AT) therapy: UFH and LMWH*

Heparin products are a heterogeneous mixture of highly negatively charged and sulfated polysaccharide units. The prototype of all heparin derivatives, UFH, has been used as anticoagulant in a wide variety of clinical setting, especially in patients with hematologic and cardiovascular disorders, for almost a century; the reader is referred to an excellent review on this subject for more detail.[1] LMWHs came into popular use in the late 1980s because of their relative superiority to

Table1. Overview of interactions of the selected food and drugs with warfarin (Coumadin)

| Food, drug or drug class | Effect | Mechanism of action | Recommendations |
|---|---|---|---|
| Antibiotics | | | |
| Most agents, but specially trimethoprim-sulfamethoxazole, metronidazole, ciprofloxacin, clarithromycin , erythromycin | ↑ INR | Reduction in synthesis of vitamin K by intestinal flora | Select alternative antimicrobial therapy for patients who are taking warfarin |
| Rifampin | ↓ INR | Induction of cytochrome P450 isoenzyme 2C9 | Select alternative antibiotics |
| Antifungals | | | |
| Fluconazole, miconazole | ↑ INR | Inhibition of cytochrome P450 isoenzyme 2C9 | Select alternative antifungals |
| Acetaminophen | ↑ INR | Direct interference with vitamin K cycle | Prescribe lowest possible dosage of acetaminophen and monitor INR |
| Antiplatelet agents | | | |
| Acetylsalicylic acid (ASA), clopidogrel, ticlopidine | ↑ INR | Altering platelet function | Limit the dosage (e.g., ASA<100 mg/day) and monitor INR |
| NSAIDs | ↑ INR | Direct mucosal injury, Altering platelet function ? | If concomitant use is necessary, use a cyclooxygenase-2 inhibitor and monitor INR. |
| Antidepressants | | | |
| SSRIs | ↑ INR | Interference with primary hemostasis; some such as fluoxetine also inhibit cytochrome P450 isoenzyme 2C9 | Select alternative antidepressants |
| Levothyroxine | ↑ INR | Increasing the catabolism of clotting factors | Prescribe lowest possible dosage of levothyroxine and monitor INR |
| Alternative remedies | | | |
| Gingko biloba, dong quai, fenugreek, chamomile | ↑ INR | Unknown | Avoid concomitant use |
| St. John's wort | ↓ INR | Unknown | Avoid concomitant use |
| Vitamin K containing food/supplements | | | |
| Leafy vegetables, certain legumes, cauliflower, and some vegetable oils (e.g., rapeseed and soyabean ) | ↓ INR | Main dietary source of phylloquinone (vitamin $K_1$) | Control dietary vitamin K content |
| Liver of animals and some fermented foods including cheese | ↓ INR | Main dietary source of menaquinone (vitamin $K_2$) | Control dietary vitamin K content |
| Foods with antiplatelet effects | | | |
| Garlic, and onion | ↑ INR | Antiplatelet effects? | Control dietary intake |
| Grapefruit | ↑ INR | Inhibition of cytochrome P450 isoenzyme 1A2 and 3A4 | |
| Cranberry | ↑ INR | Unknown | - |
| Alcohol intake | | | |
| Acute binges (>3 drinks daily) | ↑ INR | Induction of the hepatic metabolism of anticoagulants? | Preferably avoid alcohol consumption or limit intake to 1-2 drinks per day. |
| Chronic alcohol ingestion | ↓ INR | potential to increase the clearance of warfarin | Preferably avoid alcohol consumption or limit intake to 1-2 drinks per day. |
| Coenzyme Q10 (an herbal supplement) | ↓ INR | Having chemical structure similar to vitamin K | Either be avoided or used consistently while on warfarin therapy |
| Caffeinated beverages | | | |
| Cola, coffee, tea, hot chocolate, chocolate milk | ↑ INR | Unknown | These foods should be avoided or limited |

NSAIDS, nonsteroidal anti-inflammatory drugs; SSRIs, selective serotonin reuptake inhibitors

UFH. They are synthetically derived from standard heparin by either filtration or controlled depolymerization, which yields chains with lower mean molecular weights. Standard heparins (UFH preparations) have molecular weights of 5,000 to 30,000 daltons, while LMWHs weigh ranging from 1,000 to 10,000 daltons.

The longer chain lengths and the dense negative charge surrounding the molecules result in considerable nonselective binding of UFH to cells and proteins, which reduces its anticoagulant effect. UFH is also limited by inter-patient variability, unstable pharmacokinetics, and potential side effects such as hemorrhage and heparin-induced thrombocytopenia.[46] LMWH interacts less readily with platelet factor 4, decreasing the risk of heparin-induced thrombocytopenia.[47]

Based on these limitations, UFH therapy is usually restricted to the hospital setting, where it can be laboratory monitored to guide its dosage properly. As compared to UFH, the products of LMWH bind significantly less to plasma proteins, have enhanced bioavailability, interact less with platelets, and yield a predictable pharmacokinetics. LWMH is administered typically by subcutaneous abdominal injections without need for laboratory monitoring; it can, therefore, be administered either in-hospital or out of the hospital. However, these drugs have their own shortcomings, such that the anticoagulant effects of LMWHs cannot be sufficiently neutralized[48] and that it is currently impossible to monitor their serum levels by point-of-care clinical methods. Given the lack of monitoring and blood level control, LMWHs are unsuitable for patients in emergency situation such as those with acute coronary syndrome taking LMWHs who are at high risk of bleeding complications after the occurrence of an urgent surgical intervention.[49]

In contrast to direct thrombin inhibitors such as hirudin which bind directly to thrombin and are able to inactivate clot-bound thrombin,[50] both UFH and LMWH inhibit thrombin formation through binding to AT, a naturally occurring plasma protein with anticoagulant properties. However, the heparin/AT complex does not effect on clot-bound thrombin; instead, by inducing conformational changes in the AT molecule, heparin accelerates AT ability to inactivate thrombin and factor Xa and dramatically increase the anticoagulant activity of AT. In other words, coagulation is suppressed by AT-dependent inactivation of serine proteases involved in the coagulation cascade-particularly thrombin (FIIa) and activated factor X (FXa). The ability of heparin to bind to FXa and FIIa is directly associated with the size of the heparin. Any size heparin will bind to AT to inactivate Xa, but in order to inactivate thrombin the heparin molecules with at least 18 polysaccharide units are only large enough to bridge and bind both AT and thrombin simultaneously. It is believed that merely one third of the administered dosage of UFH binds all three factors to exert an anticoagulant effect.[1] LMWHs with a polysaccharide chain of shorter than 18 monosaccharides are large enough to bind both FXa and AT, but too short to bridge to and bind thrombin, and their activity is predominantly directed at the inactivation of FXa.[1] Therefore, UFH has roughly equivalent AT and anti-Xa effect, whereas the AT activity of each individual commercially available LMWH products is dependent on the relative proportion of molecules containing 18 or more monosaccharides.

### *Laboratory monitoring of UFH*

The aPTT is laboratory test commonly used to monitor UFH anticoagulant effect. Nearly four decades ago, Basu et al. at McMaster University[51] in a retrospective analysis of patient data suggested that an aPTT equal to 1.5 to 2.5 times the mean control could reduce the risk of recurrent thromboembolism. A subsequent experimental study by the same group (McMaster group) on a rabbit model, using the same aPTT reagents of thrombus extension, supported the 1.5 to 2.5 therapeutic range.[52] Based on these studies, an aPTT ratio (measured by dividing the reported therapeutic range of aPTT by the control value for the reagent) of 1.5 to 2.5 was widely adopted as the UFH therapeutic range. Still, the correlation of this therapeutic PTT values with the clinical outcome is uncertain because it has not been validated in prospective studies and due to the fact that over the years the new aPTT reagents and instruments have been available.

### *The aPTT and clinical outcome*

Early clinical studies supported a relationship between an aPTT ratio< 1.5 within the 24 or 48 hours of starting UFH and recurrent thrombosis.[53-55] Despite the fact that the data were less secure, the relationship between UFH concentrations more than 0.7 or 0.8 anti-Xa IU/ml with bleeding was also reported.[56, 57] Consequently, it seemed that the aPTT had a well-defined association with clinical outcome (recurrent thrombosis and bleeding).

However, in later prospective studies comparing UFH with LMWH in the treatment of thrombotic disease, it became clear that UFH was effective for the treatment of venous thromboembolism, only if the therapy was commenced with an proper dosage (initiated as a bolus of at least 5,000 IU, followed by a continuous intravenous infusion of at least 30,000 IU/24 h).[58-60] In addition, as various aPTT reagents were used to monitor the UFH dose, the anticoagulant effects related to a target aPTT ratio of 1.5 to 2.5 would have varied markedly among studies.[61] Accordingly, to re-examine the relationship between the risk of recurrent venous thromboembolism and the aPTT response to adequate dose of UFH, a meta-analysis was performed on five studies that provided data in this regard that showed the total recurrence rate was 6.3% in patients whose aPTT ratios were < 1.5 within the first 24 to 48 hours as compared to 7% in patients

whose aPTT ratios were higher than the lower limit of the therapeutic range; thus, this result brought into question the findings of early studies.

It is not surprising for the aPTT to be of limited value for predicting clinical outcome in patients receiving UFH because it has been estimated that below 50% of the variation in the UFH serum concentration is reflected by the aPTT,[7, 62, 63] with the remaining variability explained by the variables that are independent of the anticoagulant effect of UFH, including pre-analytic variables such as the sample collection methods and processing; analytic variables, in particular the combination of the reagent and instrument used for calculating the aPTT; and biologic variables, which include clotting factor levels and variables that influence the pharmacokinetics of UFH and the dose-response of the aPTT to UFH.[1, 7] Thus, the dosage of UFH seems to be more reliable than the aPTT in predicting clinical efficacy.

***The aPTT accuracy and standardization for reagents among laboratories***

In 1953, the aPTT test was first introduced as a two-stage assay to differentiate hemophilic from normal plasma. The test was modified to a one-stage assay in 1958 and was further modified, as the test which is used today, in 1961. To perform the test, a surface activator and diluted phospholipid are mixed into citrated plasma, after which calcium is added and the clotting time is measured. The aPTT is primarily a measure of the function of the intrinsic and common pathways of coagulation. The test is regularly used for the monitoring of treatment with UFH.

The McMaster group suggested a PTT ratio of 1.5 to 2.5 by using their aPTT reagent matched to a heparin level of 0.2 to 0.4 IU/ml as measured by a protamine sulfate titration assay.[64] At the time of this study, the wide variability in different aPTT reagents and test methods was not considered. Be that as it may, with the availability of further aPTT reagents (and coagulometers), it became clear that each reagents demonstrated different sensitivities for the PTT to heparin; and as was mentioned before, the use of aPTT is complicated by the variable response of various methods and commercially available reagents to heparin (analytic variables).[65] In other words, PTT therapeutic ranges derived from heparin levels of 0.2 to 0.4 IU/ml via the protamine sulfate assay are reagent specific.

Once it was proven that the aPTT failed to accurately reflect the plasma heparin level, efforts focused on improving the assay precision by creating reagent-specific therapeutic ranges. The use of therapeutic ratios was widely supplanted by PTT therapeutic ranges calibrated using anti-Xa heparin measurements. On account of the fact that the data from the McMaster group studies revealing a heparin level of 0.2 to 0.4 IU/ml via the protamine assay were equivalent to a level of 0.35 to 0.70 IU/ml by a factor Xa heparin assay,[64] this association formed the basis for the development of guidelines by both the College of American Pathologists (CAP)[7] and the American College of Chest Physicians,[1] which recommended a 0.3 to 0.7 IU/ml therapeutic range for UFH using an anti-Xa assay. However, by contrast to the marked progress in the standardization of PT reagents for INR reporting, prolonged efforts by the medical community to establish a method of standardization for PTT reagents has achieved little success and no standardization system has been globally adopted thus far.[1, 66-69]

In an attempt to improve the precision of the assay, the most recent CAP recommendations for the laboratory monitoring of UFH using the aPTT are that individual laboratories establish their own therapeutic range using aPTT values calibrated against accepted therapeutic UFH levels using the anti-Xa test (which is the most accurate assay for monitoring UFH therapy).

To produce a therapeutic range for the first time, the CAP recommends firstly a collection of plasma samples from patients receiving IV heparin therapy (ex vivo samples) and secondly analysis via the aPTT and heparin assay.[70] A therapeutic aPTT range can be measured by determining the PTT values corresponding to anti-Xa levels of 0.3 and 0.7 IU/ml. Changes in reagent lots and/or instrumentation should be accompanied by a revalidation of the therapeutic range. Laboratories may consider repeating the same validation process or analyzing and comparing the results with the original PTT reagent lot (or method) versus the new PTT lot on the samples from patients administered IV heparin therapy to determine clinically equivalent response. The mean difference between the two lots must not be more than 7 seconds. Because each subsequent reagent lot is compared against the preceding one, laboratories must monitor the total of differences from the reagent lot used in the original validation to be certain that the cumulative mean PTT difference is not over 7 seconds.[7]

If this type of standardization cannot be feasible, the use of an aPTT ratio ranging from 2.0 to 3.0 or 3.5 may be preferable with most modern aPTT reagents and instruments in use than an aPTT ratio of 1.5 to 2.5, which frequently demonstrates inadequate UFH concentrations.[61, 71]

Direct measures of UFH concentration with enzymatic assays such as the anti-Xa assay are of interest because these assays are not influenced by most pre-analytic variables (e.g. under-filled collection tube which is a common problem) and biologic variables that interfere with the aPTT and may be suitable for automation but are nonetheless complex, expensive, and reagent-intense. Furthermore, there are limited published data on the safety and effectiveness of anti-Xa assays for a routine monitoring and managing of UFH therapy. One recent study demonstrated that there were patients on intravenous UFH therapy in a medical intensive care unit in whom no measurable heparin levels by 3 different anti-Xa assays were identified.[72]

### *Monitoring LMWH: When and how?*

LMWHs have better bioavailability, substantially longer half-life, dose-independent clearance, and decreased protein-binding than UFH and these are factors that render their anticoagulant response more predictable. These characteristics obviate the need for laboratory monitoring for the vast majority of the cases on LMWH therapy. However, in special groups for whom it is desirable to measure the circulating level of LMWH, including pregnant patients (whose weight is constantly changing); those with renal failure; and neonates or other low-weight patients for whom weight-based dosing may not be accurate, anti-Xa activity assays are recommended.[73] The aPTT is not practical for monitoring patients receiving LMWH, because LMWHs specifically inhibit factor Xa, and to a lesser degree thrombin as compared to standard heparin[74, 75] and as a result have only little if any effect on the aPTT. Moreover, the anti-thrombin activity of LMWH is much less than its anti-factor Xa activity and the aPTT prolongation largely depends on low thrombin activity.[76] Hence, only anti-Xa activity assays can be used to monitor LMWH.

Anti-Xa activity assays detect the amount of anti-Xa activity in a sample. The functional activity of heparin (any UFH, LMWH or fondaparinux) is evaluated through adding enough antithrombin (AT) to bind all the available heparin and then the ability of the AT-heparin complex to neutralize activated clotting factors, FXa ,or FIIa is tested in a chromogenic or clotting assay.[51] Between two major methodologies, clot-based versus chromogenic substrate assays, chromogenic anti-Xa activity assays are the methodology of choice and recommended for LMWH monitoring.[73] The cost of the anti-factor Xa assay is about three times the cost of aPTT, but it is available for monitoring LMWH and UFH and is much less costly and easier to perform than protamine sulfate titration.

There are other clinical situations in which the determination of plasma anti-factor Xa activity may be more appropriate as the monitoring test; for instance, the actual concentration of heparin is hard to measure in patients on heparin therapy in whom the aPTT is raised above 180 s - often related to underlying liver disease and not related to heparin therapy. Among patients with an aPTT > 180 s, patients with an anti-Xa activity < 1 IU/ml in comparison with those with an anti-factor Xa activity > 1 IU/ml have a significantly lower bleeding risk (23% versus 57%).[77] The second example is in heparin resistance once high doses of UFH fail to elevate the aPTT into the therapeutic range. In this situation, the anti-factor Xa assay is a safe and effective method for tracking the patient, rather than further increasing the dosage in response to unchanged aPTT value, which adds to the risk of bleedings.[64]

There are many different commercially available anti-Xa activity assays which unfortunately are not standardized, and considerable interassay result variability exists among them.[78-80] In addition, the results of LMWH in a single anti-Xa assay vary, including marked variability from various LMWH lots from an individual manufacturer.[81] Considering the composition of the varying commercial LMWHs and related variable performance in laboratory assays, laboratories need to calibrate the chromogenic anti-Xa assays against an international standard when using the a new dispensed LMWH.[73] There is limited evidence that a single LMWH calibration curve can be used for a variety of different LMWHs.[82]

Finally, it is notable that there is no readily available bedside assay such as the application of the Activated Clotting Time (ACT) with UFH to evaluate the anticoagulant effect of LMWHs. This makes the safety and efficacy of the utility of LMWHs problematic, particularly in the catheterization laboratory.

### *Role of Activated Clotting Time (ACT) in UFH monitoring*

The ACT was first introduced in 1966 and has been demonstrated to be insensitive to lower UFH concentrations ever since.[83] The ACT is essentially a POCT of coagulation that is used to monitor the anticoagulant effect of UFH on-site in patients when higher-intensity anticoagulation is needed. The test has several limitations, including the fact that it is not as accurate as other assays and that clotting times achieved by the various activated clotting time devices cannot be used interchangeably.[7, 84] The fact that the ACT, alongside UFH, is prolonged by antiplatelet agents including abciximab has supported the limited specificity of this test.[85] In spite of these limitations, the ACT is common in clinical practice, particularly when evaluating anticoagulation related to interventional cardiology procedures and cardiopulmonary bypass surgery. Errors in ACT measurements can also occur due to technical causes; thus, standardization or calibration of the ACT is required.[86] To improve test results and concomitantly physicians' reliance on the ACT, quality control systems are required to be carefully constructed and tracked. The control program must be easy to use and precise and be utilized in conjunction with very reliable, stable, and reproducible control material; if the control is not within acceptable range, patient testing must not be performed.

### *Summary of what clinicians need to know about aPTT laboratory monitoring*

1. Like PT, aPTT results are highly dependent on the combination of reagent and instrumentation and type of heparin brand used. Thus, depending on the reagent/instrument and commercial preparations of heparin used, each laboratory needs to standardize and calibrate its unique therapeutic dose for heparin therapy.

2. It is noteworthy that according to the leading importing firms and suppliers of reagents-coagulometers in Iran, almost none of the laboratories in the country requests control reagents of the ACT test, indicating that there is a lack of adequate control program for this test.

3. There are limited outcomes data with regard to anti-Xa heparin monitoring; however, as the tariff for this test is not too high in Iran, on the basis of the present outcomes and data currently available it is recommended that laboratories switch to anti-Xa heparin monitoring on most occasions.

4. Warfarin may increase the aPTT; thereby collecting samples from patients taking warfarin and heparin simultaneously should be used only if the INR is < 1.3.[87]

## Conclusions

Anticoagulants are a mainstay of cardiovascular therapy; however, currently available anticoagulants have several shortcomings, including the need for the frequent monitoring and adjusting of dosages and poor patients' acceptance.

Except for the use of INR for monitoring of the patients on chronic warfarin therapy, other coagulation tests applied for the monitoring of anticoagulants did not enjoy of favorable standardization.

The INR was introduced to be a reliable and precise measure of VKA anticoagulation. Although the INR system has improved PT reporting, it is still associated with unexpectedly high degrees of inconsistency in values between laboratories and even within one laboratory between two different instruments.

The aPTT is an inaccurate measure of the anticoagulant intensity of UFH. Less than 50% of the variability in plasma UFH concentrations is explained by the aPTT, with the remaining variability justifiable by pre-analytic, analytic, and biological factors that influence the dose response of the aPTT to heparin. Despite known serious limitations, the aPTT continues to be the most widely test to monitor IV heparin therapy in clinical practice. The reliance on the aPTT is expected to continue due to its availability and familiarity of clinicians with this test.

Standardization of the aPTT used to monitor unfractionated heparin may be acquired by following the recommendation that individual laboratories develop their own therapeutic range using aPTT values that correspond to accepted therapeutic unfractionated heparin levels (0.2 to 0.4 IU/ml by protamine titration or 0.3 to 0.7 IU/ml by anti-Xa assay).

Supplanting UFH with LMWH and other new anticoagulants that do not require routine laboratory monitoring will increase the challenges laboratories encounter in validating their PTT therapeutic ranges. Therefore, many laboratories may fail to comply with current accreditation recommendations. In an effort to obtain enough samples, laboratories might decide to either draw more than 2 samples from each patient or perform calibration with fewer than 30 samples. Either of these approaches would have the effect of raising the imprecision of the estimated therapeutic range. Anti-Xa assays represent an attractive surrogate for the PTT in order to track UFH; limited outcomes and high cost, however, restrict the utilization.

Clinicians should bear in mind that a well-known interference of heparin with INR has been reported. On the other hand, warfarin may increase the aPTT. Consequently, either of these tests may overestimate the effect of continuing therapy after the discontinuation of the other medication.

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