Challenges in Variation and Responsiveness of Unfractionated Heparin

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Unfractionated heparin (UFH) has been in clinical use for more than half a century. Despite its undoubted contribution to the treatment and prevention of thrombosis, heparin is significantly limited by its variable biochemical composition and unpredictable pharmacokinetics. The situation is compounded by the fact that methods for monitoring heparin do not necessarily reflect its therapeutic effect. The activated partial thromboplastin time (aPTT) is a method for monitoring heparin therapy that is simple, cheap, and readily available. However, it is also poorly standardized and is affected by numerous factors—both analytic and preanalytic—that are unrelated to the heparin effect. Establishing an appropriate therapeutic range for the aPTT is challenging for smaller clinical laboratories, and the antifactor Xa method of measuring heparin levels is not widely available. The College of American Pathologists published consensus guidelines in an effort to improve the laboratory monitoring of UFH therapy. However, it seems unlikely that the laboratory problems associated with monitoring UFH will be resolved. Unfractionated heparin is highly antigenic and carries a significant risk of heparin-induced thrombocytopenia (HIT). Even in the absence of thrombocytopenia or thrombosis, the presence of heparin-associated antibodies may predict adverse clinical outcomes and strengthen the rationale for the ultimate replacement of UFH. Fortunately, alternatives to UFH, such as low-molecular-weight heparins, direct thrombin inhibitors, and more specific factor Xa inhibitors, are becoming available for clinical use. The pharmacokinetics of these agents are more predictable and rely much less on laboratory monitoring. Nonheparin agents also eliminate the risk of HIT. The emergence of these newer anticoagulants makes the continued use of UFH increasingly difficult to justify.

Key Words: unfractionated heparin, activated partial thromboplastin time, antifactor Xa assays, thrombocytopenia, low-molecular-weight heparins, direct thrombin inhibitors, pentasaccharides.

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Unfractionated heparin (UFH) is widely used in clinical medicine. Indeed, it is one of the most commonly administered parenteral drugs and is given to millions of patients annually. Like all anticoagulant therapies, the objective of heparin treatment is to provide a level of anticoagulation at which the patient is protected from thrombosis while remaining at minimal risk of bleeding. A significant increase has been reported in thromboembolic events when adequate heparinization is not achieved within the first 24–48 hours of therapy.[1]

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Unfortunately, achieving an ideal heparin effect is dependent on many factors, and close laboratory monitoring of therapy is needed. A number of laboratory methods to monitor the effects of heparin are in clinical use. Barriers to effective use of these methods are preanalytic variables (collection and preparation of the sample), analytic variables (laboratory reagents and instruments), biologic variables (clotting factor abnormalities), and therapeutic range issues.

Methods for Monitoring

The College of American Pathologists recommends monitoring of therapeutic heparin using a laboratory method with a defined therapeutic range.[2] When a UFH infusion is started, or after a dosage adjustment is made, monitoring should be performed at 6-hour intervals until a stable therapeutic response is achieved. At that point, monitoring may be performed daily, preferably at the same time each day. To avoid erroneous laboratory results, blood samples should be collected from an extremity other than that used for the UFH infusion. In addition, clinicians should be informed of the method used to monitor heparin therapy to assist in the interpretation of laboratory results.

Heparin dosages prescribed for treatment in patients with thrombosis are usually monitored by the activated partial thromboplastin time (aPTT) or a method based on the antifactor Xa or antifactor IIa activity of heparin. Some laboratories may use methods based on thrombin time or protamine sulfate neutralization. High heparin doses, such as those administered during percutaneous coronary intervention or open-heart surgery, typically are monitored using the activated clotting time.

Focus on the aPTT Assay

The initial concept of separate intrinsic and extrinsic pathways of blood coagulation is now outmoded (Figure 1). Nevertheless, this distinction still helps in understanding the factors that are measured by the prothrombin time and aPTT (Figure 2). The aPTT is widely used in clinical laboratories as a general screening test of the intrinsic coagulation system and to monitor heparin.

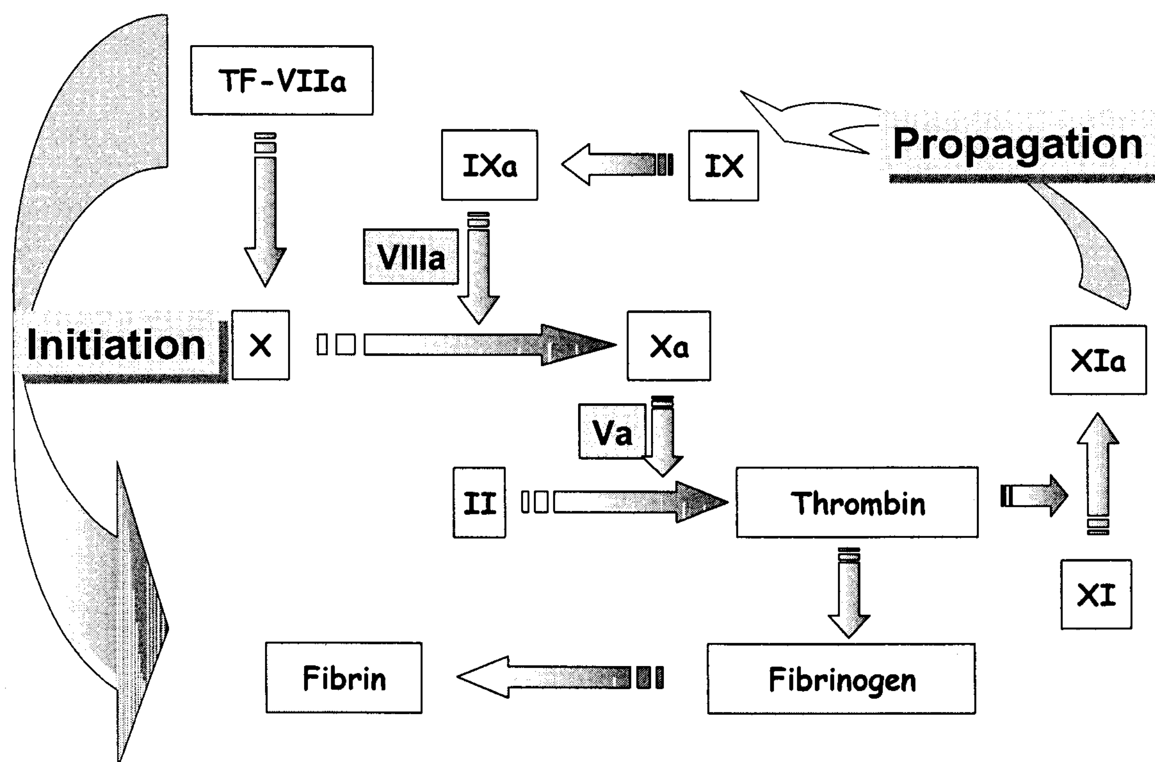

Figure 1. Current concept of the blood coagulation system. Clotting is started when flowing blood, containing factor VII, comes in contact with tissue factor (TF). The TF–factor VIIa complex rapidly activates factor X, leading to the generation of trace amounts of thrombin. The TF pathway is rapidly shut down by the TF pathway inhibitor (not shown). Coagulation activation then continues by means of a propagation pathway that is dependent on factors VIII, IX, and XI.

This test is performed by collecting the patient's blood into an evacuated tube containing sodium citrate. The addition of citrate prevents initial clotting by chelating the calcium contained in the sample. The blood then is spun in a centrifuge to separate the plasma from the cells. The citrated plasma is mixed with the aPTT reagent, which consists of an intrinsic pathway activator (typically celite) and a source of phospholipid (a required cofactor in the coagulation cascade).

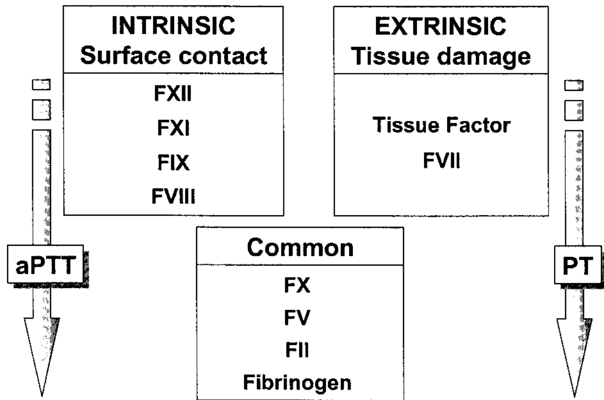

Figure 2. Coagulation factors measured by the prothrombin time (PT) and the activated partial thromboplastin time (aPTT).

After the plasma is recalcified to overcome the added citrate, the clotting time usually is recorded by an automated coagulation analyzer. The aPTT is prolonged by low (< 40%) levels of fibrinogen, prothrombin, and factors V, VIII, IX, X, XI, and XII, and by pharmacologic inhibitors (e.g., UFH) of these factors (Figure 3).[2]

The major advantage of the aPTT is that physicians and medical technologists have a great deal of familiarity with the assay. It has been used for many years and is immediately available at even the smallest hospitals. The aPTT is readily automated and relatively inexpensive. In contrast, a disadvantage of the aPTT assay is that it is a poorly standardized test that may be

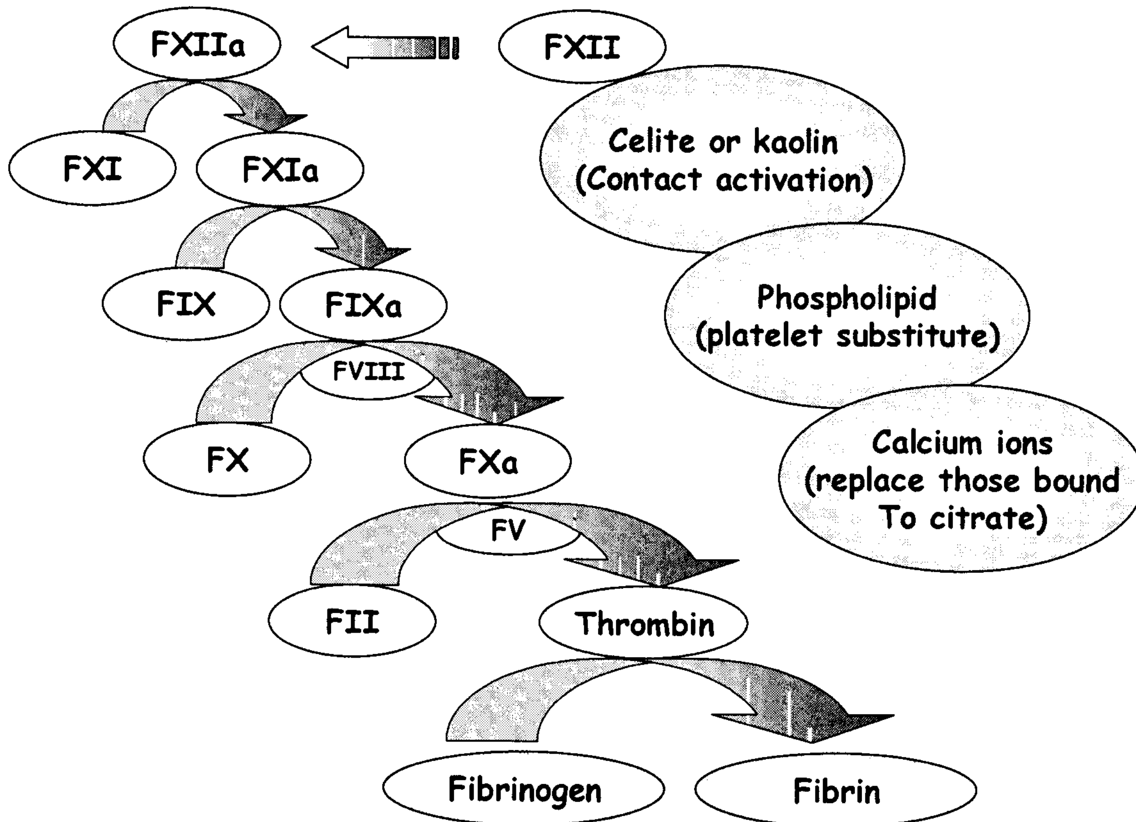

Figure 3. Principle of the activated partial thromboplastin time (aPTT). Plasma to be tested is mixed with the aPTT reagent, which is a mixture of phospholipids and a particulate contact activator (typically celite or kaolin). The plasma then is recalcified. The activator ensures maximal activation of factor XII, which is necessary to begin coagulation therapy in the absence of tissue factor (intrinsic pathway). Clotting time is proportional to the combined activity of factors II, V, VIII, IX, X, XI, and XII, and inhibitors of these reactions (e.g., heparin).

Table 1. Factors Affecting the Use of aPTT for Heparin Monitoring[2]

Preanalytic Variables	Analytic Variables	Biologic Variables
Time of blood sampling	aPTT reagent	Altered heparin pharmacokinetics or bioavailability (obesity, aging, hepatic or renal disease, altered production of heparin-binding proteins, general heparin resistance)
Site of blood sampling	Laboratory instruments	Altered aPTT dose response to heparin (factor VII, fibrinogen, antithrombin, or mild reduction of numerous coagulation factors)
Citrate concentration		Prolonged aPTT at baseline (lupus anticoagulants, factor XII, prekallikrein, high-molecular-weight kallikrein)
Centrifugation		
Sample storage		

aPTT = activated partial thromboplastin time.

affected by numerous factors other than the heparin concentration.

For example, the aPTT can be profoundly altered by preanalytic variables. However, even when these variables are well controlled, analytic variables such as the aPTT reagent-instrument combination can influence the assay's sensitivity to heparin. For this reason alone, each laboratory must determine its own therapeutic range. In addition, the aPTT is a global test influenced by a number of clotting factors. Patient-specific reductions or elevations of these factors can greatly affect the assay, causing overestimation or underestimation of the heparin response.

Heparin Monitoring: Preanalytic Variables

As a number of preanalytic variables may affect the utility of the aPTT for monitoring UFH therapy (Table 1), the College of American Pathologists provides recommendations regarding the management of these variables.[2]

Appropriate timing of blood sampling for UFH monitoring is critical. Samples should be collected every 6 hours until the patient's aPTT is within the therapeutic range.[2] Thereafter, samples should be drawn and tested daily. The daily sampling time should be standardized, because diurnal variations in clotting factors and other plasma protein levels may affect the test results despite a constant UFH infusion rate. A consistent time before 10 A.M. each day is preferred for sampling.

Blood samples should be drawn from the extremity opposite the site of the heparin infusion.[2] Care should be taken when drawing blood through indwelling lines because UFH may have been used as a flushing agent. The practice of obtaining a sample through this route should be discontinued whenever possible. Blood samples must not be drawn into a heparin anticoagulant tube; this error is difficult to recognize in the laboratory after the plasma has been separated.

The National Committee on Clinical Laboratory Standards recommends that all samples for coagulation testing, such as the aPTT, be collected into 3.2% sodium citrate.[3] The citrate concentration will affect assay results, particularly at higher heparin concentrations. This issue does not generally concern testing in an institution that uses a single citrate concentration. However, larger laboratories that service many hospitals may receive samples collected into both 3.2% and 3.8% sodium citrate. Furthermore, failing to draw the correct amount of blood (i.e., underfilling or overfilling the tubes) for the specified volume of citrate anticoagulant also will affect the results.[4]

The aPTT is dependent on the presence of phospholipids contained within the chosen reagent. However, additional phospholipids in the form of platelets may be present in the test plasma if the time or speed of centrifugation is inadequate. This may shorten the aPTT, and thus the heparin effect is underestimated. In addition, platelet factor 4 released from residual platelets can bind and inactivate heparin, thereby reducing its anticoagulant effects within the sample. Ideally, the platelet count in the plasma to be tested should be less than 10,000/µl.

It is recommended that blood for aPTT testing be centrifuged within 1 hour of phlebotomy to avoid these issues. Failure to do this will result in a significant shortening of the aPTT.[5]

The aPTT is a measure of the combined activities of numerous coagulation factors (fibrinogen, II, V, VIII, IX, X, XI, XII). If testing is delayed and the sample has not been kept cool, plasma levels of some of the more labile factors, such as factors V and VIII, may be reduced. This may spuriously prolong the aPTT. Once

separated through centrifugation, plasma samples are stable for up to 4 hours, whether refrigerated or maintained at room temperature.[5] When frozen plasma samples that contain significant numbers of platelets are thawed, the available amount of phospholipids increases, as does platelet factor 4 released from disrupted platelets. This causes shortening of the aPTT. Ideally, the aPTT should be performed on fresh, not frozen, samples.

Heparin Monitoring: Analytic Variables

The major source of analytic variability in monitoring UFH with the aPTT is the aPTT reagent itself. Different reagents may exhibit remarkable variation in their sensitivity to heparin.[6–8] Heparin sensitivity appears to be at least partly related to the phospholipid composition of the reagent, which may vary from reagent to reagent.[9] In addition, differences may exist between various lots of the same reagent; thus, the therapeutic range should be validated for each reagent lot change.[10]

The instrument used for clot detection in the aPTT assay also can be a source of variation, the effects of which may differ depending on the choice of reagent. Therefore, the therapeutic range must be established for each reagent-instrument combination used.

Heparin Monitoring: Biologic Variables

A number of biologic variables may alter the results of the aPTT assay (Table 1), such as conditions that influence the pharmacokinetics or bioavailability of heparin, alter the aPTT sensitivity to heparin, or cause an abnormal baseline aPTT.[6]

Relative deficiencies of clotting factors, whether congenital or acquired, will prolong the aPTT. For example, congenital deficiency of factor XII is often clinically silent but is associated with a markedly prolonged aPTT. Acquired clotting factor deficiencies, such as those produced by concomitant warfarin therapy, can have the same result. Whether congenital or acquired, lower levels of the procoagulant clotting factors may have the effect of increasing the observed sensitivity to UFH and overestimating its anticoagulant effect. In addition, the presence of autoantibodies to one coagulation factor or more (e.g., a lupus anticoagulant) can markedly increase the observed sensitivity to UFH and accentuate the prolongation of the aPTT.

Conversely, elevated levels of clotting factors increase thrombin generation and will shorten the aPTT. Due to the reduced heparin sensitivity, the effect of UFH anticoagulation is underestimated. For example, pregnancy is associated with a physiologic increase in coagulation factors and UFH binding proteins. Both can affect the aPTT response to UFH, thereby precluding the use of the therapeutic range referenced for nonpregnant women.[11]

Partly for these reasons, a pretreatment aPTT value should be obtained. Unusually short or prolonged aPTT results then can be recognized and an appropriate adjustment in monitoring strategy initiated. For some patients, a therapeutic range based on the ratio to baseline aPTT or a target concentration method (e.g., antifactor Xa assay) may be indicated. Alternatively, treatment may be switched to a low-molecular-weight heparin (LMWH), which does not usually require laboratory monitoring.

Heparin Resistance

Heparin resistance occurs when a patient requires relatively high doses of UFH (e.g., > 35,000 U/day) to achieve an adequate aPTT response.[12] There may be numerous reasons for apparent heparin resistance, such as antithrombin deficiency; increased heparin clearance; elevation in heparin-binding proteins; and increases in plasma levels of factor VIII, fibrinogen, and platelet factor 4. Patients demonstrating apparent heparin resistance should be tested with an antifactor Xa assay, and the results should be compared with the aPTT determinations. Adjusting the heparin dosage according to antifactor Xa levels has resulted in good clinical outcomes despite subtherapeutic aPTT.[13]

If the results of the aPTT and antifactor Xa are in relative agreement, resistance probably is due to decreased heparin bioavailability, an inadequate heparin dose, neutralization of heparin in vitro, or antithrombin deficiency when the antifactor Xa assay does not contain exogenous antithrombin. If the results of the aPTT and antifactor Xa assay are discordant, resistance probably is due to increased levels of factor VIII or an antithrombin deficiency when the sample is tested with an antifactor Xa assay that uses exogenous antithrombin. Antithrombin deficiency is relatively uncommon. When moderate decreases are observed in a patient receiving heparin therapy, it is more often

secondary to the heparin itself than to an actual clinical deficiency.

Therapeutic Range: aPTT Issues

Attempts have been made to identify a method to standardize the aPTT in a manner analogous to the international normalize ratio for monitoring oral anticoagulants. However, even when studies involved few aPTT reagents, efforts at standardization were only partially successful. As a result, aPTT standardization is unlikely to occur in the near future.[2] Because many variables affect the aPTT and its sensitivity to UFH, each laboratory should determine its own therapeutic range.[14] Ideally, this is accomplished by measuring the response to ex vivo heparin.

During this process, the aPTT and heparin concentration (e.g., antifactor Xa) are determined simultaneously for each plasma sample taken from 30–50 patients receiving therapeutic heparin. The aPTT is measured using the laboratory aPTT reagent-instrument system. A specific antifactor Xa assay is used to determine the heparin concentration. The study patients should span the complete heparin dosage range and should have a normal prothrombin time (e.g., no concomitant warfarin therapy). Linear regression analysis is performed on the plotted data, and the aPTT range that corresponds to a heparin concentration of 0.3–0.7 international units (IU)/ml (by antifactor Xa assay) then is determined as therapeutic (Figure 4).

This exercise should be repeated whenever a change occurs in the aPTT reagent lot or laboratory instrument. An alternative method for determining the therapeutic aPTT range is measuring the response to in vitro heparin. This process involves adding various concentrations of heparin to pooled normal plasma in vitro. Once the aPTTs are determined, a log-linear dose-response curve is produced. Although technically easier, this process is not recommended since it can be unreliable and does not produce equivalent results when compared with the ex vivo method.

Although comparing aPTT and antifactor Xa assay results for ex vivo plasma samples is the preferred method, it sometimes may yield relatively poor correlations (coefficient of determination < 0.5). Indeed, less than 50% of variation in the aPTT of heparinized plasma can be explained by the heparin concentration alone. Because the aPTT assay is a global test that is affected by many factors, it potentiates this discordance. Review of available options indicates better correlation with comparisons between aPTT and antifactor Xa assay than with comparisons between aPTT and protamine sulfate titration methods.[7]

Establishing the therapeutic aPTT range with the recommended ex vivo method can be a significant logistic problem for smaller facilities, such as most United States laboratories. Many laboratories in small hospitals perform only one or two aPTT assays/day. The College of American Pathologists has called for reagent manufacturers to provide calibrator plasma containing heparin at 0.2 and 0.4 IU/ml to assist in standardizing or calibrating the assay system for heparin monitoring.[2] Thus, smaller laboratories would need only to validate the manufacturer's data.[2]

The College of American Pathologists has published consensus recommendations for monitoring UFH therapy with the aPTT assay.[2] Before heparin therapy is started, a pretreatment aPTT assay and platelet count should be performed. The aPTT therapeutic range should be established for each reagent-instrument system and with each change of reagent lot or instrument. Preferably, this is accomplished through the comparison of ex vivo specimens with an appropriately validated heparin assay.

Clinical Application of the aPTT Assay

As discussed, in addition to the preanalytic and

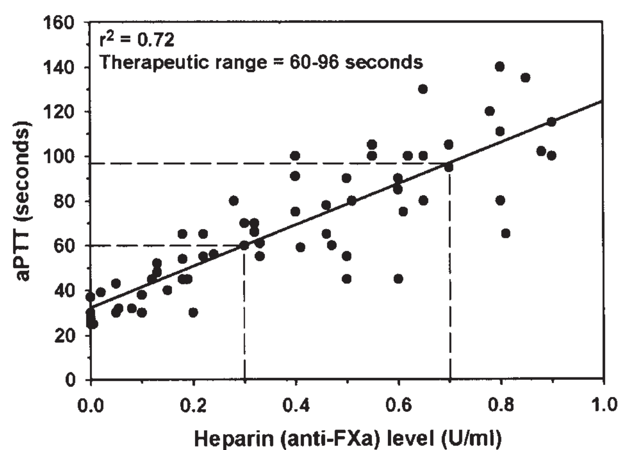

Figure 4. Plot of activated partial thromboplastin time (aPTT) versus heparin antifactor Xa levels in 65 patients receiving heparin for treatment of thrombosis. Regression analysis yielded a coefficient of determination of 0.72. Extrapolation of the line of best fit at points corresponding to 0.3 and 0.7 U/ml of heparin (by antifactor Xa assay) yielded an aPTT therapeutic range of 60–96 seconds.

analytic factors affecting aPTT responsiveness, hereditary or acquired causes of aPTT prolongation may be involved. These limitations have increasingly made the use of aPTT assays and interpretation of evaluable results fraught with error.

The reliability of the aPTT as a surrogate marker affecting the dosage of UFH was first challenged in a rather elegant pharmacokinetic model.[15] The patients involved had venous thromboembolism and were treated with a continuous intravenous UFH infusion. The daily dose of UFH was adjusted to maintain the aPTT, sampled at 8:00 A.M., at 1.5–2.5 times the control level. Once that level was achieved, the dose was kept constant. The aPTT then was measured every 4 hours for 48 hours.

The aPTT responsiveness demonstrated a circadian variation in all patients. Maximum values were achieved at night and minimum values in the morning. These circadian variations were reproduced for 2 consecutive days. Differences between night and morning values reached almost 50% with the aPTT assay. This circadian variation resulted from two rhythms—a circadian rhythm lasting 24 hours and an ultradian rhythm lasting 12 hours. They were detected by cosinor analysis ($p<0.01$). A circadian rhythm was detected individually in most of the patients with the aPTT assay.

All patients had a nocturnal peak in aPTT responsiveness on both days. In 60% of patients, this peak exceeded the upper desired limit of the aPTT. The clinical implications for this observation are borne out daily. Practitioners often respond to aPTT values drawn during this interval, when aPTT responsiveness peaks, reflecting what is thought to be a supra-therapeutic aPTT. If the heparin dosage is reduced based on this determination, the confirming 6-hour aPTT value is then obtained when sensitivity of the aPTT assay is minimal, most likely in the morning.

Logically, this often results in subtherapeutic aPTT values. Thus, circadian variance in aPTT responsiveness further complicates the management and monitoring of heparin therapy. The authors recommended consideration of alternatives to intravenous UFH therapy that produce a more predictable response and have less potential for adverse effects.

In a 6-month retrospective study at Massachusetts General Hospital and Harvard Medical School, patients were treated with intravenous heparin and warfarin for acute thrombosis or thromboembolism.[16] Of 311 eligible study patients, 134 were diagnosed with venous thromboembolism, 122 had cerebral arterial thrombosis, and 55 had confirmed peripheral arterial disease. Analysis of the data demonstrated that the dose response to UFH was highly variable. Even when a therapeutic aPTT target range was achieved, it was maintained through the next two consecutive measurements in only 29% of patients. This finding is important, especially in treatment of venous thromboembolism, since the recurrence rate has increased when aPTT values were not maintained in the therapeutic range during the first 24 hours of treatment.[17, 18]

In a similar manner, another study examined the relationship between aPTT and recurrent cardiovascular events and bleeding.[19] The study involved 5058 patients with acute coronary syndrome who received intravenous UFH. When subtherapeutic aPTT (< 60 sec) persisted for more than 48 hours, the increase in relative risk of a recurrent cardiovascular event was 1.84 (95% confidence interval [CI] 1.25–2.70) compared with patients whose aPTT values were 60 seconds or greater. However, higher aPTT values were associated with bleeding; for every 10-second increase in aPTT, the probability of major bleeding was increased by 7% (95% CI 3–11%, $p=0.0004$).

Another study used the Six Sigma approach to improve the safety and efficacy of acute anticoagulation with intravenous UFH.[20] Six Sigma is a new quality management philosophy that seeks a nonexistent error rate or outcome for the process to which it is applied. Results of a quality analysis demonstrated that fewer than half (47%) of all aPTT values determined were in the therapeutic range for 731 patients receiving weight-based therapeutic intravenous heparin. Of the remaining aPTT results, 18% were subtherapeutic, whereas 35% were deemed supratherapeutic.

In summary, even with the advent of weight-based heparin dosing nomograms as an effort to overcome complexities of the aPTT as a surrogate marker of anticoagulation, too many patients still have inappropriate aPTT values.

Focus on Antifactor Xa Assays

Therapy with UFH may be monitored by a target concentration method using an assay that specifically measures the heparin level. Two such methods are in use. One is the protamine sulfate

titration assay, which measures heparin by calculating the amount of protamine required to neutralize plasma heparin and normalize clotting time. The other is the antifactor Xa method, more commonly used, which measures heparin by quantifying the amount of residual factor Xa in the sample. The two assays do not produce similar results; this is reflected in their therapeutic ranges (0.2–0.4 IU/ml by protamine titration, 0.3–0.7 IU/ml by antifactor Xa chromogenic assay).

Antifactor Xa assays determine anticoagulant activity by measuring the ability of heparin-accelerated antithrombin to inhibit factor Xa. These assays are more specific than the aPTT since they measure inhibition of a single enzyme. Two different methods are used to conduct these assays: the clotting method and the chromogenic method. The chromogenic method may be preferred since it minimizes the influence of various antithrombin concentrations.

During the initial stage of a chromogenic antifactor Xa assay, a known excess of purified factor Xa is added to the patient's citrated plasma in the presence of enough antithrombin (endogenous or exogenous) to complex all of the available heparin. The antithrombin-heparin complex then rapidly inactivates the factor Xa. In the second stage of the assay, the amount of residual factor Xa is monitored using a chromogenic substrate that is relatively specific for this enzyme. In this test system, the residual factor Xa concentration is inversely proportional to the plasma heparin level (Figure 5).[2]

The major advantage of the antifactor Xa assay is the relative lack of factors (e.g., preanalytic, analytic, and biologic conditions) that affect its outcome. For example, it exhibits minimal interference from biologic factors, such as the presence of platelet-derived phospholipids, lupus anticoagulants, and elevated factor VIII activity, all of which can profoundly interfere with the

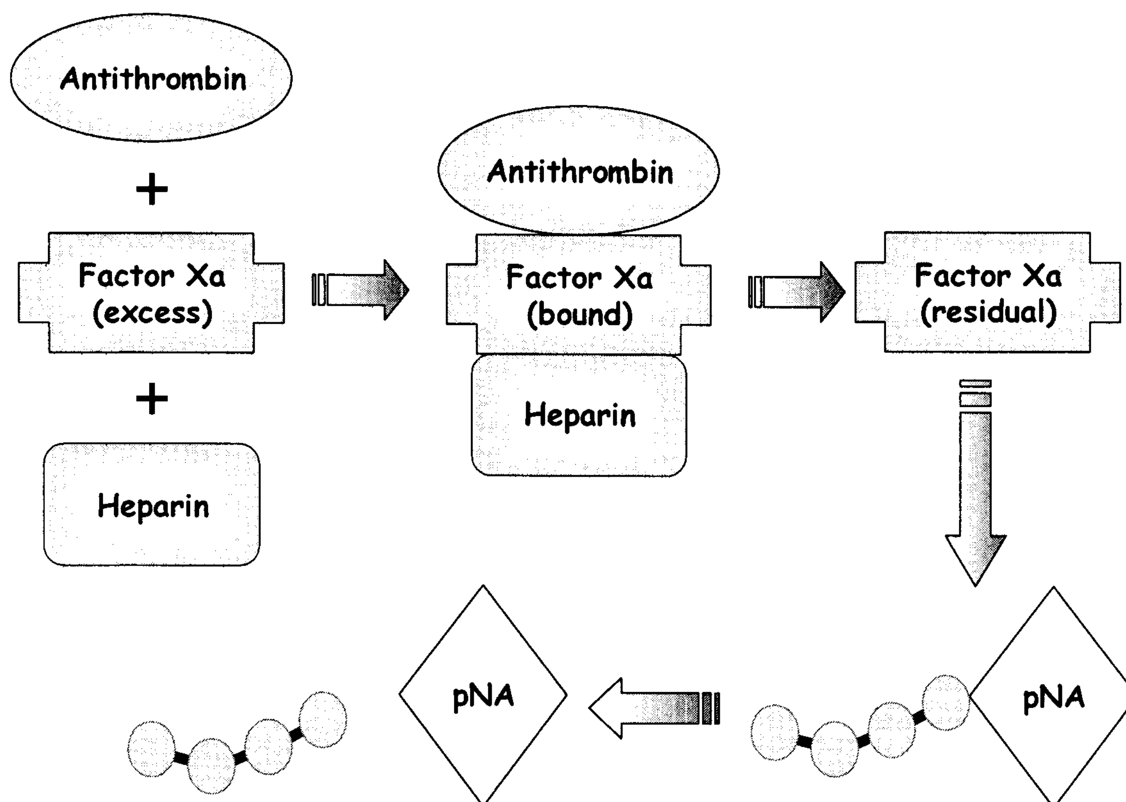

Figure 5. Principle of the antifactor Xa method of measuring heparin activity. The patient's plasma (containing heparin) is mixed with antithrombin and an excess amount of purified factor Xa. The antithrombin binds to factor Xa in a reaction catalyzed by the heparin. The amount of residual factor Xa, which is proportional to the heparin level, is measured using a specific chromogenic substrate. The substrate is initially colorless, but after cleavage by factor Xa, the free chromophore has a strong yellow color. The rate of cleavage of the substrate is measured spectrophotometrically. pNA = para-nitroanilide. (Adapted from reference 2.)

aPTT. In addition, concomitant administration of oral anticoagulants does not affect the assay outcome. Unlike the aPTT, different concentrations of sodium citrate (3.2% vs 3.8%) produce equivalent results.[21] The antifactor Xa assay can be performed on frozen plasma, an advantage for batch testing or for patients who live relatively far from the laboratory. Similar to the aPTT, the antifactor Xa assay can be automated and made available when results are required immediately.

Disadvantages of the antifactor Xa assay are its relative expense and limited, or lack of, availability in many laboratories. However, some evidence suggests that the antifactor Xa assay may reduce the number of anticoagulation monitoring tests and anticoagulant dosage changes required during therapy.[22] This may negate the additional cost of the assay compared with the aPTT. Another disadvantage is that smaller coagulation laboratories may lack the equipment needed to run chromogenic antifactor Xa assays. Testing then is referred to reference laboratories, with the inevitable increased turnaround times. In addition, the assay still requires significant attention to standardization, particularly with respect to calibrators. Another disadvantage is that different calibrators are required for different agents, such as UFH, LMWHs, or pentasaccharides.

Calibration and Standardization Issues

Little or no standardization exists among antifactor Xa assays. Most laboratories use commercially available kits, although some use reagents prepared in-house. The major difference in commercial kit formulation is that some kits provide purified exogenous antithrombin, which is added to the test plasmas, whereas others rely on the patient's own endogenous antithrombin activity. It is assumed that these assays will give comparable results if calibrated in the same way.

However, a group of investigators assessed the relationship between protamine titration and eight commercially available antifactor Xa assays.[23] Mean heparin level by protamine titration was 0.31 IU/ml, whereas mean antifactor Xa activity was 0.40–0.42 IU/ml for three clotting-based assays and 0.32–0.40 IU/ml for five chromogenic assays. Thus, the results of different antifactor Xa assays varied by up to 30%.

Assay calibration is perhaps the biggest single source of variation among antifactor Xa assays. For accurate results, the assay system must be calibrated with the same type and, preferably, the same lot of heparin used to treat the patient. If the assay is used to measure the antifactor Xa activity of a LMWH or pentasaccharide, the test system must be calibrated with the same agent.

The source of heparin used for calibration may vary. Some laboratories use heparin from the hospital's pharmacy department to ensure that the instrument is calibrated with the same heparin lot used for infusion preparations. However, in practice, this may be difficult because many lots are often in use in large institutions, and there may be significant variation from the stated potency between lots. Also, commercial heparin products are of relatively high potency (1000–10,000 U/ml). The need to significantly dilute these solutions before calibration is associated with potential opportunities for technical error.

As far as standardization is concerned, commercially available preparations for calibration that require little or no predilution may be preferable. Although the same heparin used for patient treatment is not used as a calibrator, the potential for technical error or interassay or interlaboratory variation is reduced.

Clinical Applications of Antifactor Xa Assays: Heparin and Beyond

Heparin concentration measurements may provide a target plasma therapeutic range, especially in unusual coagulation situations, such as pregnancy, morbid obesity, and renal impairment. In pregnant patients, aPTT results shorter than expected in relation to heparin concentration measurements may be indicative of increased circulating levels of factor VIII and increased fibrinogen levels.[24] Although pregnant patients may have therapeutic heparin concentrations measured by whole blood protamine sulfate titration or by the plasma antifactor Xa heparin assay, their aPTTs may not be significantly prolonged above baseline.[13] Many of these patients have very short pretreatment aPTT determinations.

The lack of correlation between aPTT and heparin levels was further investigated in 27 patients receiving intravenous heparin for either cardiovascular disease or deep vein thrombosis.[25] All patients had at least five paired sets of data (aPTT and antifactor Xa assay). Overall, the discordance between assays was 53%. For example, findings revealed that 68% of subtherapeutic aPTT values had corresponding

therapeutic heparin levels. The authors concluded that the aPTT does not appear to be a useful surrogate for heparin levels. Furthermore, they suggested that the safety and efficacy of heparin could be enhanced with expanded use of heparin levels.

Patients are identified as pseudo–heparin resistant when intravenous heparin dosages greater than 35,000 U every 24 hours produce a poor aPTT response and an adequate heparin concentration (> 0.3 U/ml by plasma antifactor Xa assay).[12] It is recommended that patients with subtherapeutic aPTTs have heparin concentrations monitored with a heparin assay. This may prevent unnecessary dosage escalations without compromising efficacy.[12, 13]

Heparin alone has minimal anticoagulant effects. When combined with antithrombin, the heparin-antithrombin complex catalyzes the inactivation of certain coagulation enzymes, thus inhibiting thrombus propagation. The normal plasma range for antithrombin is 70–145%. Anticoagulation may be difficult in antithrombin-deficient patients, defined as those with less than 50% of the amount expected in normal plasma. In addition, the aPTT assay is of limited value for these patients. This shortcoming of the aPTT assay is not seen with antifactor Xa heparin level determinations when used as the surrogate marker.[26]

Heparin concentrations also are used, when necessary, to monitor the efficacy and safety of LMWHs. Therapy with LMWHs is not routinely monitored with prothrombin time and aPTT assays since neither clotting time is significantly prolonged by these agents. Due to the predictive pharmacokinetic and pharmacodynamic properties of LMWHs, routine monitoring is usually not required.[24] However, heparin levels can be used to assess the safety and efficacy of LMWH prophylaxis and treatment dosages, particularly in patients who may require a dosage adjustment. These might be elderly patients or those with severe renal insufficiency or bleeding complications.

A therapeutically effective heparin plasma concentration range for venous thromboembolism prophylaxis is 0.2–0.4 IU/ml by antifactor Xa assay.[27] When antifactor Xa levels are used to assess therapeutic or treatment dosages of a LMWH, a therapeutically effective range for twice-daily dosing is 0.5–1.1 IU/ml when drawn 4 hours after a subcutaneous injection. For once-daily subcutaneous LMWH dosing, the therapeutic range is less certain. However, a range of 1.0–2.0 antifactor Xa IU/ml has been suggested.[28]

Data presented at the 2003 American Heart Association scientific sessions evaluated antifactor Xa activity as it related to outcomes in patients with acute coronary syndromes treated with the LMWH, enoxaparin.[29] A total of 803 consecutive patients with acute coronary syndromes were treated with the recommended dosage of enoxaparin except when a dosage adjustment was required. Antifactor Xa heparin levels were determined after patients had received at least two subcutaneous injections of enoxaparin. Rates of cardiac events and bleeding complications were assessed at 30 days. Antifactor Xa levels were correlated to the enoxaparin dose; levels were less than 0.5 IU/ml in 8.1% of the population. These levels were a direct consequence of underdosing and were associated with a significantly increased rate of death or myocardial infarction at 30 days. Bleeding event rates were low and did not correlate with antifactor Xa levels.

Conclusion

Unfractionated heparin has been the mainstay of intravenous anticoagulant therapy for many years. However, the drug has some significant disadvantages that limit its use. Heparin is a heterogeneous and relatively poorly standardized material. Coupled with its unpredictable pharmacokinetics, this results in significant challenges for clinicians in dosing and monitoring. Unfractionated heparin also carries a significant risk of heparin-induced thrombocytopenia.

Clinical use of heparin is further complicated by the fact that the available assays for monitoring heparin may not reflect the therapeutic effect of the drug. The aPTT assay, despite its simplicity and widespread availability, is poorly standardized for monitoring heparin therapy. In addition, it is beset by several preanalytic and analytic variables that confound its use. Many laboratories have difficulty establishing an appropriate therapeutic range for the aPTT, and methods of measuring heparin levels directly (e.g., the antifactor Xa assay) are not available in many institutions.

Unfortunately, it seems unlikely that any of these problems will be resolved in the near future. The College of American Pathologists has published consensus guidelines to assist in laboratory monitoring of heparin therapy. These recommendations highlight each phase of heparin monitoring in an attempt to minimize or

control, when possible, factors that may influence assay results. Recommendations for assisting in the laboratory monitoring of UFH, as adapted from the College of American Pathologists, are provided by the Heparin Consensus Group in Appendix 1.

Today's alternatives to heparin are LMWHs, direct thrombin inhibitors, and pentasaccharides. Under normal conditions, newer anticoagulation agents have more predictable dose responsiveness than heparin, thus negating reliance on routine laboratory monitoring. These newer agents also demonstrate reduced potential for heparin-induced thrombocytopenia and major bleeding relative to heparin. The efficacy, safety, and convenience associated with the newer anti-coagulants make the continued use of heparin increasingly difficult to justify.

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Appendix 1. Recommendations for Assisting in Laboratory Monitoring of Unfractionated Heparin Therapy

1. The therapeutic range for aPTT should be based on antifactor Xa levels using samples from at least 30 patients who are receiving heparin therapy and span the clinically relevant range.
2. The therapeutic range for aPTT should be determined by each laboratory and revalidated for each change in reagent, reagent lot, and reagent-instrument combination.
3. Commercially available plasmas and controls, calibrated to a common international standard, would facilitate the establishment and validation of therapeutic ranges for aPTT tests and measurement of antifactor Xa levels.
4. Blood samples for aPTT determination should be drawn into 3.2% sodium citrate, and tubes should be filled accurately to ensure the correct citrate:blood ratio.
5. A baseline aPTT and platelet count should be determined, with monitoring at 6-hour intervals after the start of heparin therapy, after each dosage adjustment, and daily thereafter.
6. Patients stabilized with heparin therapy may be monitored daily, preferably at the same time of day and before 10:00 A.M.
7. Plasma for aPTT tests should be separated within 1 hour after the blood is drawn, and should be centrifuged to ensure a platelet count below 10,000/µl. Samples for aPTT should not be frozen before testing.
8. Newer anticoagulants circumvent many of the UFH monitoring issues and may be the preferred agents of choice.