College of American Pathologists Conference XXXI on Laboratory Monitoring of Anticoagulant Therapy

Laboratory Monitoring of Unfractionated Heparin Therapy

John D. Olson, MD, PhD; Charles F. Arkin, MD; John T. Brandt, MD; Mark T. Cunningham, MD; Alan Giles, MD; John A. Koepke, MD; David L. Witte, MD, PhD

• ***Objective.*—To review the state of the art as reflected in the medical literature and the consensus opinion of recognized experts in the field regarding the laboratory monitoring of unfractionated heparin therapy.**

***Data Sources, Extraction and Synthesis.*—The authors made an extensive review of the literature. The draft manuscript was circulated to every participant in the consensus conference prior to the convening of the conference. Extensive discussion concerning all of the issues addressed in the manuscript as well as the resulting recommendations occurred. This information was then used to revise the manuscript into its final form.**

***Conclusions.*—The resulting manuscript has 23 specific recommendations regarding preanalytic, analytic, and postanalytic phases of monitoring and testing for complications related to unfractionated heparin therapy. This report contains detailed discussion of these recommendations and includes literature citations that support them. A number of issues for which consensus could not be reached are also discussed. A method is provided to assist laboratories, particularly small laboratories, in providing clinicians with an appropriate therapeutic range for the activated partial thromboplastin time, the most commonly used test in monitoring heparin therapy.**

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Unfractionated heparin is an anticoagulant that is used frequently in a wide variety of clinical settings. The drug is used in doses to achieve plasma concentrations from 0.1 U/mL or less (low-dose heparin) up to concentrations of 5.0 U/mL or more for extracorporeal circulation. Heparin is highly effective at inhibiting coagulation in vivo and in vitro, but has recognized complications, including an increased risk of thromboembolic disease if the therapeutic response is not achieved, an increased risk of bleeding if the therapeutic range is exceeded, a risk for the development of heparin-induced thrombocytopenia, and a risk for the development of osteoporosis with long-term administration.[1] These potential complications and the variable response to heparin led to the development of more than a dozen different laboratory methods to monitor the response of individual patients. The availability of such a variety of methods is evidence that no single approach has yet been found to be ideal.

In general, therapeutic monitoring goals, listed in order of preference, may be classified as follows: *primary,* titration to a clear, measurable, desired physiologic response; *secondary,* titration to a secondary physiologic response that correlates with the primary response; and *tertiary,* titration to a given concentration of the drug, called the target concentration strategy.[2] The secondary approach to monitoring unfractioned heparin, using the activated partial thromboplastin time (aPTT) or the activated clotting time, has been used for many years. Recently, the target concentration strategy is gaining support. Ten tenets have been proposed regarding the pharmacokinetics of a drug prior to using the target concentration strategy. These tenets have been previously reviewed for unfractionated heparin.[3] Although many are fulfilled, the wide variability of heparin preparations and patient response present some difficulties in fulfilling the recommended requirements.

Anticoagulant therapy is most commonly assessed by measuring the effect of the drug on a global clotting assay, such as the prothrombin time or aPTT. A long-standing hypothesis is that this type of measurement conforms to the secondary type of monitoring goal, that is, titration to a secondary goal (elongation of the clotting time) that corresponds to the primary physiologic response (anticoagulation with reduced thrombosis). This hypothesis has been one of the reasons for preferring the aPTT over more specific concentration assays, which would correspond to the tertiary goal of a target concentration strategy. There are now several reasons to question this hypothesis regarding the aPTT and heparin effect, including the following:

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From the Department of Pathology, The University of Iowa College of Medicine, Iowa City (Dr Olson); the Department of Laboratory Medicine, Boston (Mass) University Medical Center (Dr Arkin); the Department of Pathology, The Ohio State University, Columbus (Dr Brandt); the Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, and Department of Veterans Affairs Medicine Center, Kansas City, Mo (Dr Cunningham); the Department of Pathology, Queens University Kingston, Ontario, Canada (Dr Giles); the Department of Pathology, Duke University Medical Center, Durham, NC (Dr Koepke); and Laboratory Control Ltd, Ottumwa, Iowa (Dr Witte).

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Reprints: John D. Olson, MD, PhD, Department of Pathology, University of Iowa, 145 B MRC, Iowa City, IA 52242-1182.

Table 1. Levels of Evidence for Consensus Recommendations

Level 1	The recommendation is based on well-designed prospective studies, preferably more than 1.
Level 2	The recommendation is based on retrospective studies or multiple anecdotal studies that reach consensus.
Level 3	The recommendation is based on isolated anecdotal studies or the consensus of expert practitioners.

1. Prolongation of the aPTT by itself does not necessarily mean the blood is effectively anticoagulated. For example, patients with lupus anticoagulants may have a significant prolongation of the aPTT and still be at increased risk of thrombosis. Patients with deficiency of factor XII, prekallikrein, or high-molecular-weight kininogen typically have very prolonged aPTTs but are neither at risk of bleeding nor protected from venous thromboembolic disease (VTE).

2. The degree of prolongation of the aPTT in response to a clinically effective concentration of drug varies among different aPTT methods.[1,4–7] Thus, one cannot assume that a given degree of prolongation of the aPTT during heparin therapy translates into a specific level of anticoagulation.

3. Hirudin, like heparin, is a potent thrombin inhibitor. However, the degree of antithrombotic effect is quite different for heparin and hirudin at the same degree of prolongation of the aPTT. Some have suggested that this lack of concordance between equivalent antithrombotic effect and equivalent effect on the aPTT has led to the excessive rate of bleeding noted in some early clinical trials of hirudin.[8–11] It was assumed that because the aPTT was a global assay of coagulation, equivalent prolongation would correspond to equivalent antithrombotic effect for both agents. Clinical experience has shown that this conclusion is no longer viable.

4. Based on the observation that the response to heparin varies among aPTT methods, several groups have recommended that the therapeutic range for each reagent be determined relative to the plasma concentration. This recommendation may indicate that the aPTT is actually functioning as a surrogate assay for heparin concentration, rather than as a measurement of global effect on coagulation. Alternatively, the heparin assay may be used to document the sensitivity of the aPTT reagent while clinicians continue to use it, not as a surrogate heparin assay, but as a physiologic reflection of heparin effect.

The aPTT has maintained preeminence over the years for a variety of reasons, including the logical appeal of a physiologic measurement, a general level of clinical satisfaction with its use, the low cost, the overall technical reliability, ease and speed of performance, the widespread availability of the test, and the absence of a suitable alternative. Despite this, doubts about the efficacy of the aPTT for monitoring heparin therapy have persisted for decades and continue to accumulate. Replacing the aPTT with a direct assay of heparin or heparin activity has been an appealing option, one that has been made more attractive by recent advances in technology.

It is the purpose of this report to review the available techniques for monitoring heparin therapy for treatment of thromboembolic disease and maintenance of extracorporeal circulation. Recommendations of the consensus conference on laboratory monitoring of anticoagulation for the monitoring of unfractionated heparin therapy are reported and reflect the current understanding and consensus of those participating in this conference (Table 1).

ADMINISTRATION AND DOSING OF UNFRACTIONATED HEPARIN FOR THROMBOEMBOLIC DISEASE

Intravenous Heparinization

For many years, a standard bolus and initial infusion have been used to treat all patients, regardless of patient weight or clinical condition. In recent years, several groups have documented the advantage of using weight-based protocols for the administration of heparin.[12–15] Use of such protocols is associated with more rapid achievement of the target therapeutic response and a potentially lower rate of recurrent thromboembolic complications. An initial bolus of 70 to 80 U/kg is given intravenously followed by a constant infusion of 16 to 18 U/kg/hr. The response to heparin (aPTT or other assay) is checked after 6 hours, and the dose is adjusted according to the response. The response to heparin is again checked every 6 hours until the patient is in the target range; the response is then monitored on a daily basis.

Subcutaneous Heparin

For some time, subcutaneous administration of unfractionated heparin has been used effectively for prophylactic heparinization in high-risk settings, such as abdominal or orthopedic surgery. Laboratory monitoring of the response to heparin is usually not performed in this setting because the lower doses used have only a minimal or unmeasurable effect on the aPTT, and achievement of a target response has not been linked to clinical outcome. The efficacy of subcutaneous heparin in the therapy of VTE is controversial; however, one cross-over study suggests that it may be less expensive and preferred by patients.[16–18] In contrast, subcutaneous heparin may be the only therapeutic alternative for long-term anticoagulation, pregnancy being a case in point. Subcutaneous heparin therapy should be monitored in situations that require therapeutic levels of heparin. Such therapy usually requires less frequent monitoring than intravenous heparin administration.

Target Therapeutic Response for Treatment of Thromboembolic Disease

The target therapeutic response is framed by 2 objectives: (1) achieving a sufficiently high concentration of drug to minimize the risk of recurrence or extension of the thromboembolism and (2) maintaining the concentration of heparin at a level low enough to minimize the risk of bleeding. Regardless of the method used for monitoring, the range of heparin concentrations that correlates with these goals may be referred to as the therapeutic range. Surprisingly, no objective dose-finding studies have been performed in humans to define such a therapeutic range for heparin therapy of thromboembolic disease. However, animal studies and clinical trials of heparin have provided data that permit reasonable approximation.

The first clinical trial demonstrating a relationship between the response to heparin and the risk of recurrent

Table 2. Consensus Recommendations: General

1. Fixed, low-dose heparin does not require anticoagulant monitoring. (Level 3) (By Consensus)
2. Adjusted dose and therapeutic heparin require anticoagulant monitoring using a method with a defined therapeutic range.[4,19,20,25,36] (Level 1)
3. Upon initiation of therapy or after altering the dose of the continuous infusion of heparin, anticoagulant monitoring should occur at 6-hour intervals until a stable therapeutic response is reached.[14] (Level 1)
4. Monitoring heparin therapy given by continuous infusion that has reached a stable therapeutic response should be monitored daily, at the same time of day, preferably prior to 10 AM.[45] (Level 1)
5. Specimens used for monitoring heparin therapy should be collected from a different extremity than the one used for heparin infusion.[47] (Level 2)
6. Clinicians should be informed of the method used by the laboratory for monitoring heparin therapy and its recommended therapeutic range.[4,14] (Level 1)

thromboembolic disease was reported by Basu et al[19] in 1972. They found that patients with an aPTT less than 1.5 times control was associated with an increased risk of recurrent thrombosis. They used the classic Bell and Alton aPTT reagent, using kaolin as the activator and manual detection of the clot end point. A protamine titration assay indicated that this degree of prolongation corresponded to a heparin concentration of at least 0.2 U/mL. Subsequent clinical trials have also shown a relationship between a low heparin concentration or aPTT less than 1.5 times the control and recurrent thrombosis.[20–22] A study of a rabbit model of thrombosis was also supportive of this dose for effective anticoagulation.[23]

The upper limit of the therapeutic range, regardless of the method, has been more difficult to define, in part because bleeding in association with heparin therapy is often multifactorial in origin. In a prospective study of 280 patients treated for venous thromboembolism, bleeding was associated with an increased heparin concentration. The median daily heparin concentration among patients with bleeding complications in this study, as measured by a factor Xa inhibition assay, was 0.74 to 0.83 U/mL, compared with 0.24 to 0.36 U/mL among patients without bleeding complications.[24] A heparin concentration greater than 0.7 U/mL was associated with an increased risk of hemorrhage in patients receiving heparin following a course of thrombolytic therapy.[25] In an analysis of variables associated with bleeding during heparin therapy, a concentration greater than 0.8 U/mL by a factor Xa inhibition assay was associated with an increased risk of bleeding.[26] Thus, it appears that increased bleeding may occur when the heparin concentration exceeds 0.7 to 0.8 U/mL, as measured by a factor Xa inhibition assay. It should be noted that the rate of clearance of heparin molecules varies depending on molecular size; low-molecular-weight fragments, which preferentially catalyze the inhibition of factor Xa, are cleared more slowly. Thus, it is common to find a relatively higher concentration of heparin activity when a factor Xa–based assay in used than when a thrombin-based protamine neutralization assay is used.[7] For this reason, the upper limit of the therapeutic range for thrombin-based assays may be closer to 0.4 U/mL.[7]

The general recommendations for monitoring unfractionated heparin therapy are presented in Table 2.

MONITORING UNFRACTIONATED HEPARIN USING THE aPTT

aPTT Methods

According to data obtained from College of American Pathologists interlaboratory proficiency surveys, many aPTT systems are used in American laboratories. As early as 1973, there were at least 35 aPTT systems being used[27]; by 1997, results were reported for over 300 different reagent-instrument combinations.[28] When various concentrations of heparin are added to plasma in vitro and an aPTT is determined, a log-linear dose-response curve is obtained. This response suggests that the aPTT may function very well as a surrogate assay for heparin. In vitro heparinized and lyophilized plasmas have been included regularly on the College of American Pathologists proficiency surveys. The performance of the various aPTT systems has been reviewed and published periodically,[4,5,29] demonstrating differences in the dose-response characteristics among reagent-instrument combinations. These findings have been confirmed by several studies from individual laboratories as well.[6,7,30–34] These observations suggest the need for a method-specific therapeutic range for each aPTT method.

Additional studies have shown that there are important differences between the dose-response curve obtained with in vitro addition of heparin and the dose-response curve generated from performing aPTTs and heparin assays on samples obtained from patients receiving heparin.[6,7,32,33] In general, the in vitro–derived curve cannot be used to predict the dose-response curve for in vivo heparinization. In addition, the various aPTT test systems in clinical use differ in their dose-response characteristics to samples obtained from patients receiving heparin in vivo. Therefore, an interlaboratory ratio method is not an appropriate expression of the therapeutic range.

Proposed guidelines for aPTT performance were published more than 10 years ago.[35] In 1995, a definitive interlaboratory study was reported.[36] The goal was to identify a candidate reference reagent for utilization in a system analogous to the international normalized ratio (INR) for monitoring oral anticoagulant therapy. The study included several candidate reference aPTT reagents, but even with the limited reagents tested, efforts at standardization were only partially successful.[36] Other recent attempts to standardize the aPTT have also been unsuccessful.[37,38] At the present time, a method to standardize the aPTT analogous to the INR is not on the horizon.

Consequently, it is now apparent that laboratories must determine the appropriate therapeutic range for their own aPTT system used to monitor heparin therapy.[7] Ideally, this should be done by simultaneously determining the aPTT and heparin concentration for samples from patients receiving heparin for the treatment of thromboembolism. A dose-response curve can be calculated from the data using regression analysis, and the aPTT range corresponding to a heparin concentration of 0.3 to 0.7 U/mL

Table 3. Consensus Recommendations: For Manufacturers and Pharmacists

1. Manufacturers should provide the heparin responsiveness of reagents to be used for activated partial thromboplastin time. (Level 3) (By Consensus)
2. A hospital pharmacy should supply heparin of a single manufacturer and lot number for therapy. When the lot must change, the laboratory should be notified to reevaluate the therapeutic range of the test(s) being used for monitoring. (Level 3) (By Consensus)
3. Pharmaceutical heparin should be calibrated against an international standard (preferably the World Health Organization standard) using an anti-factor Xa assay.[83,85] (Level 2)

(by a factor Xa inhibition assay) can be derived. An alternative approach is outlined in the Appendix.

In contrast to the high degree of correlation between heparin added in vitro and aPTT prolongation, several published comparisons have shown more variability for paired data points comparing the aPTT to heparin concentration in ex vivo specimens,[6,7] yielding r^2 values less than 0.50 in many cases.[6,39–44] Less than 50% of the variation in aPTTs in heparinized plasmas is explained by differences in the heparin concentrations in those plasmas. In one study, the correlation was better for aPTT/anti-Xa comparisons than for aPTT/protamine titration comparisons,[6] suggesting that the functional anti-Xa assay may be preferable to the protamine titration assay for establishing the therapeutic range.

Although the use of the heparin assay may be preferred, the potential burden of such a recommendation should not be overlooked. For example, in the state of Iowa (United States), there are 130 hospitals, 85 of which have fewer than 100 beds and 42 that have fewer than 50 beds. Bed occupancy may be at the level of 50% in many of these small hospitals. These hospitals may perform only 1 or 2 aPTTs each day, yet a significant proportion of the anticoagulant monitoring is being performed in settings such as this. Laboratory validation of the therapeutic range by performance of simultaneous aPTTs and heparin assays may be overwhelming, if not technically infeasible, in such small hospitals. An alternative approach using ex vivo specimens, validation by comparison with the existing aPTT reagent, and control for reagent-to-reagent drift using cumulative summation is presented in the Appendix. Comparison with a heparin assay is preferable and should be performed where feasible. If a laboratory is unable to perform the less rigorous method, one could question whether it should be performing aPTTs at all. The difficulties for small laboratories could be helped considerably by the actions of reagent manufacturers and providers of proficiency testing. Manufacturers should provide calibrator plasma at the equivalent of 0.2 and 0.4 U/mL unfractionated heparin (ex vivo) for a variety of instruments to assist in standardization. With such data, the small laboratory would only need to validate the manufacturer's reported results. In addition, proficiency testing surveys could provide similar samples as a challenge to assist in both proficiency testing and validation of calibration.

The recommendations for manufacturers and pharmacies are shown in Table 3.

Preanalytic/Blood Sampling Issues

Blood specimens should be obtained every 6 hours until the patient is within the target therapeutic range; samples can be obtained daily thereafter.[1] The daily sampling time should be standardized, because there is a diurnal pattern to the aPTT-heparin interaction despite a constant infusion rate.[45,46] If the dose is changed, the response should be reevaluated every 6 hours until the response is again stable. Blood specimens should be drawn by venipuncture from the extremity opposite from where the infusion is being given to avoid artifacts due to possible contamination of the sample by heparin infusion.[47] Drawing through an indwelling catheter is to be avoided because of possible contamination of the sample with heparin from the catheter.

The National Committee on Clinical Laboratory Standards (NCCLS) has published recommendations for specimen collection and preparation for coagulation studies, including the aPTT.[48] Collection in evacuated tubes has become the standard practice and is suitable for coagulation assays. Tubes can be purchased with citrate in a final concentration of 3.2% (109 mmol/L) or 3.8% (129 mmol/L). The amount of citrate does influence the test result, particularly at higher heparin concentrations.[49,50] The NCCLS is proposing changing their recommendation to collect all samples for coagulation testing into 3.2% citrate. Adoption of this recommendation should facilitate further standardization of coagulation testing.

Transport and storage of specimens used for prothrombin time, aPTT, and heparin assays continue to be discussed. The aPTT and heparin assays do require transport and storage at 2°C to 4°C and processing to remove cellular components (especially platelets) within 1 hour. Ex vivo platelet activation occurring during specimen collection, transportation, processing, or storage can lead to significant release of PF4, a heparin-binding protein. Processing must minimize platelet release. In addition, the test plasma should be platelet poor; that is, the platelet count should be less than 10×10^9/L. This is particularly important when specimens are frozen for future testing.

Biological Variables Affecting the Use of the aPTT to Monitor Heparin

A variety of conditions may complicate the administration or monitoring of unfractionated heparin therapy. These conditions usually fall into 3 general groups as follows: (1) conditions that influence the pharmacokinetics or general bioavailability of heparin, (2) conditions that alter the characteristics of the aPTT dose response to heparin, and (3) conditions that cause an abnormal baseline aPTT. The group 1 conditions are more of a concern in terms of achieving the appropriate dose of heparin for an individual patient, whereas the conditions in groups 2 and 3 may interfere with the actual monitoring of therapy.

Group 1 conditions include (1) conditions that alter the effective intravascular volume (eg, obesity and aging),[25,51] (2) conditions that influence the production of heparin-binding proteins,[52–54] (3) conditions that influence the half-life of heparin (hepatic disease or renal disease),[55] and (4) general heparin resistance. Unfractionated heparin binds to a variety of plasma proteins in addition to antithrombin (AT). Whereas the binding to AT is dependent on a critical pentasaccharide sequence in heparin, the nonspecific binding to other plasma proteins appears to be a function of

Table 4. Consensus Recommendations: Evaluating Heparin Resistance

1. When heparin resistance is suspected (eg, high heparin dose), separate real from apparent resistance by performing a heparin assay.[67] (Level 2)
2. Using a functional method, assay the concentration of antithrombin acutely and, if decreased, during convalescence in patients with evidence of heparin resistance.[57,88] (Level 2)

molecular weight, with minimal protein binding exhibited by low-molecular-weight heparin and significant binding exhibited by unfractionated heparin.[54,56] Some of the proteins that bind heparin nonspecifically are acute phase reactants, and thus their plasma concentration is increased in patients with acute inflammatory reactions, a common condition among patients receiving heparin. These proteins compete with AT for heparin; thus, these alternate binding sites may need to be saturated before an effective concentration of heparin is achieved in vivo. An increase in the level of heparin-binding proteins is a common cause of ''heparin resistance,'' the inability to achieve a therapeutic response (either laboratory or clinical) despite administration of what is typically a therapeutic amount of drug.

Group 2 conditions, which alter the dose response of the aPTT to heparin, include (1) increased factor VIII and/or fibrinogen, (2) low AT, and (3) mild reduction of multiple coagulation factors. Factor VIII and fibrinogen are acute phase reactants, and their plasma concentrations are frequently increased in patients with thromboembolic disease. Increased factor VIII and/or fibrinogen are associated with a downward shift in the dose-response curve; that is, there is less prolongation of the aPTT at any given concentration of heparin than would be expected.[57] This phenomenon may also cause a picture of heparin resistance and needs to be distinguished from the problem caused by increased levels of heparin-binding proteins. Patients with resistance due to increased factor VIII do not need additional heparin, whereas patients with increased binding proteins do. The 2 entities can be distinguished by using a heparin assay or thrombin time to assess the concentration of heparin.

Antithrombin deficiency is a rare but important cause of heparin resistance. It may manifest by recurrent thrombotic events despite adequate plasma heparin levels.[58,59] It may or may not be reflected by laboratory evidence of heparin resistance (failure to optimally increase the aPTT), because not all patients with AT deficiency manifest heparin resistance. This situation is problematic because AT levels fall during thrombosis and heparin therapy even in patients who have normal baseline levels. Therefore, assays performed during the acute event may detect acquired deficiency and could lead to the inappropriate diagnosis of inherited AT deficiency. For this reason, low levels of AT observed during an acute episode of VTE need to be confirmed during convalescence. Despite these problems with interpretation, a functional assay for AT activity should be performed on patients who have unexplained resistance to heparin. Patients with congenital AT deficiency and thrombosis may benefit from administration of supplemental AT during episodes of acute thrombosis.[60] The long-term care of these individuals usually requires chronic oral anticoagulant therapy.[59]

Patients with liver disease or an evolving consumptive coagulopathy frequently have a mild reduction in multiple factors. The level of the coagulation proteins may be sufficient to maintain a normal or near-normal aPTT. However, the aPTT dose-response curve in such individuals may be increased; that is, the aPTT is more prolonged than would be expected for a given concentration of heparin.[61] A similar phenomenon has been noted during concurrent administration of oral anticoagulant therapy, presumably due to the reduction in vitamin K–dependent proteins. This response is related, in part, to the fact that the relationship between factor activity in blood and the aPTT is not linear, but logarithmic.[62] The longer the baseline aPTT (the lower the baseline factor activity), the less additional change is needed to further prolong the aPTT. The increased responsiveness of the aPTT in such patients will lead to a reduced dose of heparin to achieve an aPTT in the therapeutic range. On one hand, some clinicians argue that the added effects should be taken into account, believing that the result imparts protection from increased thrombus formation while reducing the risk of hemorrhage. On the other hand, some feel that the resulting reduced dose of heparin in this setting places the patient at risk for thrombosis. They believe that target concentration monitoring is safer in preventing thrombosis. The literature provides no guidance, and the ''best'' method to monitor heparin therapy in this setting is unclear.

Group 3 conditions are associated with a prolonged aPTT at baseline and include (1) lupus anticoagulants[63,64] and (2) factor XII, prekallikrein, and high-molecular-weight kininogen deficiency.[65,66] In each of these settings, there is a prolongation of the aPTT that is not associated with an in vivo abnormality of thrombin formation. Therefore, using a target prolongation of 1.5 to 2.5 times baseline could lead to an inappropriate dose of heparin. In patients with group 3 conditions, it is usually advisable to use an alternate approach to heparin therapy, such as monitoring with a heparin activity assay, thrombin time assay (see below), or switching to a low-molecular-weight heparin for therapy.

Finally, there may be settings in which the mild, multiple-factor deficiency or nonspecific (lupus-type) anticoagulant may produce a mild prolongation of the baseline aPTT. Some clinicians have confirmed the therapeutic level of heparin by target concentration of heparin and correlated this with an aPTT performed on the same sample. If the corresponding aPTT is not excessively prolonged (<120 seconds), they then monitor using the aPTT that has been determined to be therapeutic in the patient.

The recommendations regarding heparin resistance are shown in Table 4.

Summary of Utilization of aPTT for Monitoring Heparin Therapy

Although there are several advantages to the aPTT for monitoring unfractionated heparin, there are also significant disadvantages, including (1) the assay is not standardized, (2) individual laboratories need to determine the appropriate therapeutic range for their specific system, and (3) there are a number of biological variables that can interfere with the monitoring of heparin by the aPTT. The widespread use of the aPTT suggests that most users still

Table 5. Consensus Recommendations: Monitoring With the Activated Partial Thromboplastin Time (aPTT) and Thrombin Time

1. A pretreatment aPTT and platelet count should be performed in all patients prior to initiating therapy. (Level 3) (By Consensus)
2. The therapeutic range of unfractionated heparin for the aPTT reagent-instrument system should be determined with each change in reagent (lot number or manufacturer) or instrument.[7,33,34] (Level 2) This may be accomplished by
    a. Comparison of ex vivo specimens with an appropriately validated heparin assay (anti–factor Xa or protamine sulfate neutralization).[7,33,34,39] (Level 1)
    b. Comparison of ex vivo specimens to a previously calibrated aPTT, using a method to control for reagent drift. (Level 3) (By Consensus)
3. The therapeutic range of the thrombin time reagent-instrument system should be determined using in vitro heparin when reagent, instrument, or lot of heparin changes.[89,90,92] (Level 2)

perceive sufficient value in using this assay. Of greatest importance at this time is the understanding of the limitations of the test, using it only in clinical settings in which it is most likely to provide valid clinical information.

The recommendations for the use of the aPTT in monitoring heparin therapy are presented in Table 5.

MONITORING UNFRACTIONATED HEPARIN WITH HEPARIN ASSAYS

The target concentration strategy has been used successfully to monitor unfractionated heparin therapy in a variety of clinical settings.[1,67] However, as recently as 1995, only 1% of laboratories participating in the College of American Pathologists CG2 survey were using a heparin assay and a target concentration strategy as the primary method to monitor heparin therapy. Only 110 of 782 participants reported results for a quantitative heparin assay in the 1997 CG2 survey. Most of these 110 laboratories use the aPTT as the primary test to monitor heparin, using the quantitative assay for selected clinical circumstances. Stated another way, based on this sample, 86% of laboratories in the United States would be unable to respond to the recommendation to use a heparin assay when the clinical setting renders the aPTT ineffective. In contrast, more than 4000 laboratories in France perform an assay for heparin, demonstrating that widespread availability of the assay can be achieved. New, multipurpose coagulation analyzers can provide fully automated heparin assays, making heparin assays in the routine monitoring of therapy open to consideration.

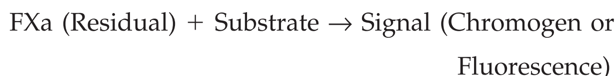
Methods for measuring heparin concentration in body fluids fall into the following 3 categories: (1) chemical assays that measure the concentration of sulfated polysaccharides, (2) methods that measure the amount of substance required to neutralize the anticoagulant effects of heparin, and (3) methods that measure the functional activity of heparin in terms of inhibition of specific coagulation factors. The methods for the chemical determination of heparin are based on dye-binding, photometric, and radioisotope techniques.[68–73] Although available for decades, these methods have not gained much acceptance in the clinical laboratory because they are difficult to standardize, cumbersome to perform, subject to interference, and not automated. In addition, determining the chemical concentration of unfractionated heparin is unlikely to be clinically useful because a significant portion of heparin is inert in the inhibition of coagulation.

Heparin neutralization assays estimate heparin activity by determining the concentration of material that just blocks heparin prolongation of a coagulation test. The 2 most commonly used substances for heparin neutralization assays are protamine sulfate[23] and polybrene. The protamine and polybrene titration methods are reliable and reproducible assays that have been used for many years. The heparin activity in the test plasma is estimated by determining the lowest titer of protamine that neutralizes the heparin-induced prolongation of the thrombin time and comparing this effect with the effect of protamine addition to the standard plasmas containing a known heparin concentration. These assays are inconvenient to perform and are not readily automated because multiple dilutions and mixtures must be prepared and measured. Consequently, these assays are usually not used for routine monitoring in an acute care environment.

Methods for determination of the functional activity of heparin are based on the ability of heparin to accelerate the inhibition of a standard concentration of an activated coagulation enzyme, such as factor Xa or thrombin, in the presence of enough AT to complex all available heparin. The ease, accuracy, and precision of these assays have been improved by the development of enzyme-specific chromogenic and fluorogenic substrates.[40,74–78] The basic scheme for these assays is as follows:

Free AT + Heparin → AT-Heparin

AT-Heparin + FXa (in Excess) → AT-Heparin-FXa + FXa (Residual)


FXa (Residual) + Substrate → Signal (Chromogen or Fluorescence)

The signal generated is proportional to the amount of residual factor Xa (or thrombin) remaining after neutralization by the AT-heparin complex and is thus inversely proportional to the concentration of heparin. The signal may be measured by endpoint or kinetic techniques. Functional heparin assays should be simple, reliable, reproducible, highly sensitive, and easily adapted to automation. These assays also should be relatively easy to standardize among laboratories; however, there is still variability reported.[79] Some of this variability may be due to preanalytic differences. Many of the newer multipurpose coagulation instruments can perform heparin assays concurrently with other routine coagulation assays without requiring major instrument adjustments or reagent preparation. The chromogenic assays based on factor Xa inhibition have also have been shown to be effective for the measurement of low-molecular-weight heparin.[80]

The Heptest (Haemachem, St Louis, Mo) is a clot endpoint heparin assay that reflects anti-Xa but also measures anti-IIa activity. It has been reported to be easy to use, highly sensitive, and well correlated to the anti-Xa assay.[81] Of interest, the Heptest is relatively insensitive to the newer heparinoids, potentially making it less useful in the clinical laboratory.

Therapeutic Range

There appears to be wide agreement in the literature that the therapeutic range of heparin in plasma is 0.2 to 0.4 U/mL when assayed by protamine titration. Despite this agreement, the primary evidence for this range is based on a single study using a thrombogenic model in rabbits.[23] Subsequent large clinical studies have indirectly validated this range by showing that clinical outcomes are acceptable when the patients' aPTTs are kept in the range that, on average, corresponds to 0.2 to 0.4 U/mL of heparin by titration. These studies, however, did not consider alternative ranges for comparison. Since the results of paired protamine determinations and chromogenic assays may diverge, the therapeutic range for chromogenic assays may be different than that for protamine assays. Hirsh and colleagues[1,6] have reported that the range of 0.35 to 0.7 U/mL by the anti-Xa assay is equivalent to the range of 0.2 to 0.4 U/mL by the protamine assay, but subsequently different findings have also been reported. The cause for this difference is unclear at this point. It may relate to the heparin preparation,[77] the specific chromogenic method,[6] differences in response to low-molecular-weight heparin or, possibly, to the high statistical error in determining the intermethod equivalence.

Preanalytic/Blood Sampling Issues

Functional heparin assays are susceptible to the same problems outlined for use of the aPTT. In vitro platelet activation may release PF4, which can bind to and neutralize the functional activity of heparin. Therefore, careful acquisition, processing, and storage of samples for heparin assays is critical.

Biological Variables Affecting the Use of Heparin Assays to Monitor Heparin

The group 1 conditions described under utilization of the aPTT will affect the results of heparin assays as well. In contrast to the aPTT, variations in the level of coagulation factors (group 2 conditions) usually do not affect heparin assays. Thus, the results are not affected by increased levels of factor VIII and fibrinogen or decreased levels of procoagulant proteins. A very low concentration of plasma AT may affect some assays for heparin determination; this can usually be obviated by addition of purified AT to the test system. Heparin assays may also be valid in patients who have prolonged aPTTs at baseline (group 3 conditions). This is true in patients with a lupus anticoagulant, with contact factor deficiency, and for patients receiving oral anticoagulant therapy. The effectiveness of their use remains to be demonstrated in patients with liver disease or consumptive coagulopathies.

Calibration of Unfractionated Heparin Assays

Appropriate dosing and monitoring of heparin is dependent on accurate and reliable assignment of heparin activity for the administered drug and the material used to establish the reference curve for the assays. Unfortunately, labeling of heparin activity has not been standardized. Units of antifactor activity, United States Pharmacopeia (USP) units, and International Units (IU) are all different measures of heparin anticoagulant activity. The USP unitage, used for labeling the therapeutic product, is derived from the amount of heparin needed to inhibit the clotting of sheep blood and therefore is not a rigorously defined measure of activity.[82] The potency is stated to be ''not <90.0% and not more than 110.0% of the potency stated on the label in terms of USP heparin units.'' One can see that such variation in the therapeutic heparin potency can cause significant problems with efforts for therapeutic monitoring. Sheep are almost certainly as variable as humans in their response to heparin. An international standard preparation of heparin has been prepared, and its biologic activity value has been assigned by rigorous assessment in both factor Xa and thrombin-based assays.[83] More recently, an alternative technique for assignment of heparin activity has been proposed; this technique has the advantage of being relatively free of the biological variables that have affected other value assignment methods.[84]

Commercial heparin preparations vary and are usually assayed for only the USP or IU value.[77,85] When assaying for USP activity in clinical samples, the calibration of an assay is against the same heparin preparation that has been used to treat the patient.[77] However, when assaying for specific anti-factor activity (eg, anti-Xa activity), it is important to use a calibrator that has been assayed for that activity against an appropriate international standard. When performing assays for nonstandard heparins, like low-molecular-weight heparin, some believe that the heparin used in preparation of the reference curve should be of the same or similar composition as the nonstandard heparin.[86,87] Alternatively, assay of all varieties of therapeutic material against a single international standard heparin has considerable appeal, allowing meaningful comparison of data and ease of communication regarding treatments.

Heparin assays have been used successfully and may be strongly indicated in settings where the aPTT may not be effective in reflecting anticoagulation with heparin.[88] Such settings include failure to achieve an adequate response to more than 1.5 times the expected dose,[67] or less than 0.5 times the expected dose is required to achieve the therapeutic range; when the baseline aPTT is prolonged; or in the presence of lupus anticoagulant even if it is not reflected in the aPTT.

Summary of Utilization of Heparin Assays for Monitoring Heparin Therapy

There are several advantages to utilization of heparin assays for monitoring therapy with unfractionated heparin, including (1) the assays are simple to perform, (2) only routine collection and prompt preparation of the sample are required, (3) results from the assays can be available in a timely fashion 24 hours a day, (4) there is minimal interference from biological variables, and (5) it should be possible to standardize heparin assays. Disadvantages of heparin assays at this time are as follows: (1) they are not yet widely available, (2) they are more expensive than an aPTT, and (3) titration assays are not well suited for he acute care setting.

The recommendations regarding target concentration strategy in monitoring heparin therapy are presented in Table 6.

USE OF THROMBIN TIME TO MONITOR HEPARIN THERAPY

The thrombin time is a clot-based assay used by some laboratories to monitor unfractionated heparin therapy. This assay is performed by adding a known concentration of thrombin to platelet-poor plasma and measuring the time to clot formation. Under the appropriate assay con-

Table 6. Consensus Recommendations: Monitoring by Target Concentration

1. The target concentration strategy may be used to monitor unfractionated heparin therapy.[67,88] (Level 1)
2. The heparin used for the calibration of the heparin assay should be linked to an approved international standard heparin, preferably the World Health Organization standard.[77,83,85] (Level 2)
3. Monitoring heparin by target concentration should be considered when
   a. Heparin dosage is elevated (>50%) above that needed to produce the expected activated partial thromboplastin time (aPTT) effect, particularly when treating venous thromboembolic disease.[19,22,23,88] (Level 1)
   b. The baseline aPTT (or activated clotting time) is prolonged by lupus anticoagulants, contact factor deficiency, or oral anticoagulant effect. The optimal method for monitoring unfractionated heparin in other acquired coagulopathies remains unclear.[63–66] (Level 2)
   c. A nonspecific (lupus-type) anticoagulant is present, even with a normal aPTT. (Level 3) (By Consensus)

ditions, heparin produces a dose-dependent prolongation of the thrombin time, which is semilogarithmic.[89,90] The origin of the thrombin (bovine vs human), thrombin concentration, and calcium concentration are critical variables for this assay.[22,24,41,89–95] Despite the fact that results are reported in seconds, the thrombin time conforms to the target concentration strategy because it does not reflect the physiologic effect of heparin in the patient specimen.

It is recommended that a recalcified thrombin time be used when monitoring heparin therapy. In one study, a recalcified thrombin time (2.3 U/mL human thrombin, 0.033 mol/L calcium chloride) produced adequately prolonged thrombin times (11–93 seconds) in the clinically useful heparin range of 0.1 to 0.6 U/mL.[89] In retrospective studies, the recalcified assay was more useful than the non-recalcified assay in predicting resolution of deep venous thrombosis, the development of pulmonary embolism, and major bleeding.[22,24] No attempts have been made to standardize thrombin time assays among laboratories.

The preanalytic and biological variables affecting the thrombin time are similar to those affecting heparin assays. The thrombin time is independent of increased factor VIII, decreased coagulation factors, and conditions associated with a prolonged baseline aPTT. Occasional patients exposed to topical thrombin develop antibodies directed at bovine thrombin. These antibodies can cause significant prolongation of a thrombin time based on bovine thrombin and occasionally show cross-reactivity with human thrombin.[96,97] Thus, the presence of such antibodies could interfere with thrombin time assays for heparin. The thrombin time is not readily automated, although some newer coagulation instruments are capable of performing the test.

The responsiveness of the thrombin time to heparin in patients with lupus anticoagulants has not been studied. At low concentrations of heparin (<1 U/mL), preliminary data indicate a possible interference (E. M. VanCott, MD, oral communication, 1997). The effects at high concentrations of thrombin are not available.

Summary of the Use of Thrombin Times for Monitoring Heparin Therapy

There are some advantages to the thrombin time assay for monitoring heparin, including (1) the test is simple to perform, (2) results of the test can be readily available 24 hours a day, (3) no special collection or handling of the sample is required, (4) there is little interference from biological variables, and (5) the test is relatively inexpensive to perform. Disadvantages of the assay include (1) recalcified thrombin time assays are not widely available, (2) the assay is not automated, and (3) there is potential interference by anti–bovine thrombin antibodies.

The recommendations for the use of the thrombin time in monitoring heparin therapy are presented in Table 5.

USE OF THE ACTIVATED CLOTTING TIME TO MONITOR HEPARIN THERAPY

The activated clotting time (ACT) is performed by adding a particulate activator to blood (often non-anticoagulated) and determining the time until clot formation.[98] The test is analogous to the aPTT because it is sensitive to changes in the intrinsic cascade of coagulation, including the presence of heparin. The dose-response range is significantly wider for the ACT than it is for the aPTT, permitting assessment of high heparin levels, such as those used to maintain extracorporeal circulation.[98]

In the College of American Pathologists proficiency survey for the ACT (CTA-1997) the participants (>1700) responded as follows concerning the clinical setting for the primary use of the ACT: vascular catheterization (including cardiac catheterization), 31%; cardiopulmonary bypass, 31%; intensive or coronary care unit, 17%; and hemodialysis, 10%; the remaining 11% was distributed among other clinical settings.[28] Fewer than 10% of ACTs are performed in central laboratories, and fewer than 1% are being used for monitoring heparin therapy of VTE.

Because the ACT is commonly performed on whole blood that is not anticoagulated, technique is critical in its reliability. The ACT is subject to many of the same biologic variables as the aPTT and offers no advantage over the aPTT in this regard. The ACT is generally not as precise as the aPTT and is significantly affected by the time between specimen acquisition and testing and by changes in the ambient temperature. Like the aPTT, ACT performance varies among the available methods, and no method for standardizing these differences has been described.

Summary of the Use of ACTs for Monitoring Heparin Therapy

Advantages of the ACT include the following: (1) it has a wide dose-response range, making it suitable for monitoring high concentrations (>0.8 U/mL) of heparin; (2) it may be performed at the patient's bedside; (3) results are generally available within a few minutes; (4) it is relatively easy to perform; and (5) there is extensive clinical experience with using the ACT to monitor high-dose heparin. Disadvantages include (1) it is not as precise as other assays, (2) testing must be performed immediately after sample acquisition, (3) there is no procedure to standardize the various methods of performing the ACT, (4) the test is moderately expensive, (5) the correlation between ACT and heparin concentration is poor, and (6) it may be difficult to enter results into the laboratory information system or the patient's chart. In view of the lack of specific

advantages over the aPTT for monitoring therapy of VTE, the ACT is not highly recommended for use in this setting.

WHOLE BLOOD aPTT AND POINT-OF-CARE MONITORING OF HEPARIN THERAPY

The whole blood aPTT was developed to improve test turnaround time by eliminating the need for sample centrifugation. Several instruments have been recently introduced to the clinical market, with an emphasis on utilization in the point-of-care setting. Performance data have been reported in the literature for 3 instruments, namely, the CoaguChek Plus (Boehringer Mannheim Diagnostics, Indianapolis, Ind), the Thrombolytic Assessment System (Cardiovascular Diagnostics, Raleigh, NC), and Hemochron Instruments (International Technidine Corporation, Edison, NJ).[99–113] These instruments perform an aPTT on a single drop of non-anticoagulated and/or anticoagulated whole blood.

Although the whole blood aPTT is prolonged by heparin in a dose-dependent fashion, whole blood and plasma aPTT results on the same sample should not be considered equivalent. One study showed clinically significant discordance with respect to heparin therapy decisions in 22% of samples using a 3-way decision algorithm (subtherapeutic, therapeutic, and supratherapeutic).[100] The appropriate therapeutic range must be determined for each whole blood aPTT system. Point-of-care whole blood and centralized laboratory plasma aPTTs should not be used interchangeably on the same patient unless the 2 systems have been calibrated against each other.

A whole blood aPTT cannot be recommended in patients receiving concomitant heparin and either oral anticoagulants or aprotinin. Oral anticoagulants and aprotinin are significant interfering factors for at least 1 test system (CoaguChek Plus), producing more prolonged aPTTs in the presence or absence of heparin.[105,108] In addition, caution should be used in interpreting whole blood aPTTs that are clinically suspicious, since poorly performed fingerstick collection can frequently produce erroneous results. The error rate due to traumatic fingersticks was 14% in one study.[99]

Only a limited number of studies have evaluated point-of-care whole blood aPTTs for routine monitoring of heparin therapy. Whole blood aPTTs were associated with improvement of some secondary outcomes, such as aPTT turnaround time (3 vs 45–126 minutes), time to clinical decision making (14.5 vs 180 minutes), and time to reach therapeutic heparin levels (8.2 hours vs 181 hours).[103] No data are available regarding the influence on length of stay, rethrombosis, bleeding, or mortality. Until such primary outcome data are available, this testing modality cannot be strongly recommended for routinely monitoring unfractionated heparin therapy.

Summary of the Use of Whole Blood aPTTs for Monitoring Heparin Therapy

It is difficult to assess the appropriate role of whole blood aPTTs, owing to the relatively limited clinical experience with these assays. Potential advantages of the whole blood aPTT include that (1) the test is easy to perform, (2) the test may be performed at the patient's bedside, and (3) results are available within minutes. Potential disadvantages include that (1) the test is subject to the same biological variables as the plasma aPTT; (2) the appropriate therapeutic interval needs to be determined for each system; (3) the assay may be difficult to standardize; (4) the test is susceptible to technical errors, such as traumatic fingerstick; (5) the test is more affected by oral anticoagulants and antifibrinolytic medication; (6) the test remains more expensive; and (7) it may be difficult to enter results into the laboratory information system or the patient's chart. The role for this approach to monitoring heparin therapy needs to be carefully assessed with well-designed clinical studies.

MONITORING HEPARIN THERAPY FOR EXTRACORPOREAL CIRCULATION

Cardiopulmonary Bypass

Patients undergoing cardiopulmonary bypass regularly receive high doses of heparin. Loading doses in this clinical setting range from 300 to 400 U/kg of body weight with an additional dose in the circuit as high as 10 000 U, depending on the equipment being used. The goal is to achieve an ACT that exceeds approximately 480 seconds prior to initiating extracorporeal circulation.[114] The concentration of heparin in blood averages 4 to 5 U/mL to achieve this ACT; this is approximately 10 times the concentration of heparin used to treat VTE. The ACT is performed every 30 to 60 minutes, and additional heparin is given as needed to maintain the ACT above the threshold level (480 seconds).[114] An abundance of clinical experience supports this approach to anticoagulation during cardiopulmonary bypass. Recent studies have shown that there is a relatively poor correlation between the ACT during bypass and heparin concentration.[115,116] Thus, this may be one of the few clinical settings in which a global coagulation assay is not being used as a surrogate heparin assay but as an assay to achieve a certain physiologic goal.

Alternatives to the ACT have been reported. These include instruments that use protamine sulfate neutralization to provide a target concentration, with the target being a heparin concentration of 3 to 4 U/mL.[115] Another approach is to perform a thrombin time using a high concentration of thrombin; this modified thrombin time essentially serves as a surrogate test for heparin concentration.[117–119]

In cardiopulmonary bypass the concentration of heparin used during the procedure renders the patient's blood essentially incoagulable. The risk of hemorrhage in the postoperative period, if the anticoagulation is not reversed, is such that the circulating heparin is neutralized. This is most commonly achieved by the administration of protamine sulfate, although the use of recombinant PF4 has been reported.[120] Protamine sulfate in excess is an anticoagulant, and the use of higher doses of protamine sulfate has been associated with increased use of blood products during the postoperative period.[116,121] The goal, therefore, is to administer only the amount of protamine sulfate required to neutralize the patient's heparin. It has been suggested that an optimal dose of protamine sulfate is 1.3 mg/100 U heparin.[114] However, determining the units of heparin that need to be neutralized is difficult owing to a number of variables, including (1) most of the methodologies for determining the dose of protamine sulfate change the use of the ACT from a physiologic test of overall anticoagulant effect to a surrogate assay for heparin; (2) when, in the estimation of the surgeon, there is continued bleeding from the operative site, it is common, regardless of test results, to administer additional prot-

amine sulfate; (3) aprotinin is an agent that is commonly administered during cardiopulmonary bypass to decrease transfusion requirements, and it also prolongs the ACT (celite activated > kaolin activated), interfering with interpretation of results[122–125]; (4) thrombocytopenia and/or abnormal platelet function can prolong the ACT, an issue in both cardiopulmonary bypass and hemodialysis[125]; (5) cardiopulmonary bypass is often performed with significant hemodilution, which can have an effect on the ACT and its response to heparin[126]; and (6) protamine sulfate is an anticoagulant, and in excess it can prolong the ACT as well as other tests.[127]

The dose of protamine sulfate necessary to neutralize the circulating heparin can be calculated in a variety of ways.[71,128,129] All of the available date indicate the critical importance of using a method to determine the appropriate dose of protamine sulfate for heparin neutralization because of the consequences of both residual heparin and excess protamine sulfate in the patient.

Other Extracorporeal Devices and Vascular Catheterization

When heparin is used during vascular catheterization, hemodialysis, or extracorporeal membrane oxygenation, it is used at substantially lower concentrations than are used in cardiopulmonary bypass. The heparin dose in these settings is frequently based on a standard nomogram adjusted for the patient's baseline ACT. Heparin administration is subsequently adjusted to maintain a desired level of anticoagulation by intermittent measurement of the ACT. In comparison to cardiopulmonary bypass, these procedures are associated with significantly less tissue trauma and lower heparin concentrations; therefore, most clinicians do not routinely neutralize the heparin at the conclusion of the procedure. The natural clearance of circulating heparin is often monitored with the ACT, keeping the patient under observation until the ACT has fallen below a threshold regarded as safe for the patient depending on the procedure.

In hemodialysis it is common to establish a dosing and infusion protocol for a patient by careful monitoring of the patient with the ACT at 30-minute intervals. Once the regimen is established, the heparin protocol is not changed or monitored during subsequent procedures unless there is a significant change in the patient's medical condition or clinical response to the protocol.[130,131] The target for the therapeutic range is somewhat variable because of differences in performance of various ACT systems. In one carefully performed study using a manual ACT, a therapeutic range of 132 to 234 seconds was established.[132]

HEPARIN-INDUCED THROMBOCYTOPENIA

Heparin-induced thrombocytopenia (HIT) is an uncommon but clinically significant complication of heparin therapy. The syndrome is due to the development of an antibody, usually immunoglobulin (Ig) G, that binds to and activates platelets in the presence of heparin.[133] The pathogenic antibody appears to be directed at an immunogenic complex formed by heparin and a basic protein, most commonly PF4.[134] The immune complex formed by IgG, heparin, and protein then binds to and clusters the platelet Fcγ receptor (FcγRIIa), resulting in platelet activation and the generation of procoagulant platelet microparticles.[135] The antibody may also bind to damaged endothelial cells, leading to the expression of tissue factor.[136] It is hypothesized that the intense surge of procoagulant activity may overwhelm the anticoagulant effect of the administered heparin, resulting in the thrombosis that is associated with this syndrome.[135]

The true incidence of HIT is uncertain. Well-designed prospective studies would suggest that the incidence varies from less than 1% to about 3% of patients exposed to unfractionated heparin, while the incidence is significantly lower in patients exposed to only low-molecular-weight heparin.[137,138] Assays used to measure antibody are widely variable in sensitivity. Screening patients prior to cardiopulmonary bypass surgery demonstrated antibody in 8% of patients (platelet aggregation/release method) to as high as 50% of patients (anti-PF4 method).[139] There is a clinical spectrum, which includes (1) asymptomatic antibody formation; (2) antibody formation associated with thrombocytopenia; and (3) antibody formation associated with thrombocytopenia and thrombosis. The clinical significance of the presence of antibody without thrombocytopenia or thrombosis remains unclear.

The onset of thrombocytopenia and/or thrombosis usually occurs 5 to 12 (range, 4–20)[140] days after treatment is initiated in patients who are exposed to the drug for the first time. Symptoms may occur more rapidly in patients who have had previous exposure to heparin, particularly if the exposure has occurred within the last 3 months. Rarely, the onset of thrombocytopenia and/or thrombosis may be delayed, up to 22 days, in neonates.[141] Patients with HIT usually develop mild to moderate thrombocytopenia, with the nadir ranging from 20 to 150 $\times 10^9$/L.[142] However, HIT should be suspected whenever an unexplained decrease of greater than 50% from the baseline platelet count occurs during heparin therapy, even if the platelet count remains in the reference range or if the count falls below 100 $\times 10^9$/L. Thromboembolic complications may be either arterial or venous and may involve large vessels, for example, the femoral artery. When patients develop skin necrosis during combined heparin and oral anticoagulant therapy, the diagnosis of HIT should be considered. Development of thrombosis in patients with HIT is a serious complication, because the incidence of serious morbidity is in excess of 50% and the mortality as high as 15%.[133]

For these reasons, all patients receiving heparin, including use of heparin-coated indwelling vascular catheters, should be monitored for the development of HIT. Proposed diagnostic criteria for a diagnosis of HIT include the following: (1) onset of unexplained thrombocytopenia or thrombosis following initiation of heparin therapy, and (2) positive test for the presence of a heparin-dependent antibody by a sensitive and specific diagnostic assay. In addition, recovery of the platelet count into the reference range after stopping heparin therapy is a helpful diagnostic finding. Laboratory evaluation is indicated in these settings to differentiate HIT from other causes of thrombocytopenia, including nonimmune heparin–induced thrombocytopenia (formerly called HIT type I).[143]

There are now 2 types of assay for HIT, namely, (1) platelet-based or functional assays and (2) antibody recognition of the heparin-PF4 complex. Platelet-based assays depend on activation or lysis of platelets in the presence of test sample and heparin. Washed normal platelets provide a sensitive test system when either ^{14}C-serotonin release or platelet aggregation is used as an end point.[144–146] The ^{14}C-serotonin release assay has been evaluated in a

prospective clinical study, and a positive assay was strongly associated with HIT (odds ratio, 78.2; 95% confidence intervals, 12.0–818.8; $P < .001$) with a specificity of 96%.[137] The specificity of these assays may be improved by performing the assay at low (0.1–0.3 U/mL) and high (10–100 U/mL) concentrations of heparin.[147,148] High concentrations of heparin dissociate the heparin-PF4 complexes from the platelet surface and interfere with HIT antibody interaction with platelet FcγRIIa and subsequent platelet activation. Thus, platelet activation or lysis, manifested by platelet aggregation or ^{14}C-serotonin release, at low, but not high, concentrations of heparin is regarded as confirmatory of the presence of antibody and diagnostic of HIT.[148]

Experience with these test systems has also confirmed the need for careful selection of platelet donors and the use of weakly positive HIT control samples. Substantial variation in the sensitivity to FcγRIIa-mediated platelet activation may be found among normal donors.[149,150] Thus, it is essential to use donor platelets known to be responsive to HIT samples. The use of weakly positive HIT control samples may help control for this and the loss of sensitivity during platelet washing.[151] The difficulties with platelet washing procedures and the inconvenience of using radioactive materials have led many laboratories to use platelet-rich, plasma-based aggregation assays.[149,152] It has been suggested that such assays are less sensitive. This, however has not been confirmed by comparison with optimized test systems.[147,148] It is possible that, with appropriate attention to methodologic detail, platelet-rich plasma assays may provide a convenient and more rapid method for diagnosis of HIT.

The second laboratory approach to the diagnosis of HIT is use of assays that detect antibodies to the PF4-heparin macromolecular complex. Enzyme-linked immunosorbent assays have been developed that permit identification of antibodies reacting with PF4-heparin.[153,154] Microtiter plate wells are coated with PF4-heparin and allowed to incubate with a patient sample. Bound antibody is then detected by appropriate antisera to IgG, IgA, or IgM by standard techniques. Although the concordance between platelet-based activation and enzyme-linked immunosorbent assays is high, the results are discordant in approximately 10% to 20% of cases.[153,155] Several factors may contribute to this. First, the enzyme-linked immunosorbent assay may detect IgA and IgM antibodies that are incapable of mediating FcγRIIa-induced platelet activation.[156] Second, some antibodies appear to be directed at complexes of heparin and other proteins, such as interleukin 8 and neutrophil-activating peptide 2.[157] Third, the enzyme-linked immunosorbent assay method may detect low-titer or avidity antibodies that are not associated with clinical manifestations.[139] To date, there are no data from prospective clinical trials delineating the comparative performance of these 2 approaches to the laboratory confirmation of HIT. Therefore, it is difficult to recommend one technique over another, although there is level 1 evidence correlating the serotonin release assay with clinical HIT.[137]

Summary of Heparin-Induced Thrombocytopenia

Once a diagnosis of HIT has been documented, it is important that all sources of heparin, including heparin-coated catheters, be discontinued.[133] Alternate anticoagulant strategies should be used if the patient requires anticoagulation for management of a thromboembolic problem. While it was thought that low-molecular-weight heparin may be effective in such patients, clinical studies have shown a high rate of cross-reactivity; thus, low-molecular-weight heparin is not recommended for patients with HIT.[133] Heparinoids, may be very effective and show a low rate of clinical cross-reactivity.[158] Some of the newer specific thrombin inhibitors, such as hirudin and argatroban, may be effective in patients with HIT.[159]

In patients receiving heparin for the first time, the platelet count should be determined at beseline and on alternate days (at least) after 4 days of therapy. In patients with a history of previous exposure to heparin, the platelet count should be monitored from the outset of therapy. Determination of the platelet count every other day should be adequate unless there is a high degree of clinical suspicion for HIT. Should a fall in the platelet count occur, the clinical context is important, because a fall in the platelet count during the first 4 to 5 days after commencing therapy and without prior exposure to heparin is unlikely to be due to HIT. It is important to keep in mind that in this setting, other, non–heparin-related causes of thrombocytopenia are far more common. However, owing to the severity of the consequences of a failure to recognize HIT, physicians will frequently request diagnostic studies in such settings and err in the direction of overdiagnosis, electing to institute alternate forms of anticoagulation.[160] Consequently, laboratory studies to confirm the diagnosis of HIT should be undertaken in any patient suspected of having the syndrome. It is suggested that this should be done at the time of clinical suspicion if possible, but testing up to 6 weeks after heparin exposure may be useful.[142,144]

A negative laboratory study does not exclude HIT, as 5% to 10% of patients with potentially clinically significant HIT may not be detected by currently available assays. A repeat assay performed 2 to 3 days after discontinuing heparin may help identify patients with HIT with initially negative laboratory results. Recovery of the platelet count after stopping heparin therapy or recurrence of thrombocytopenia upon reexposure to heparin are also helpful in confirming the diagnosis. A diagnosis of HIT should be carefully documented in the medical record, and the patient should be informed of the results so that future exposures may be prevented. Alternatively, if the clinical diagnosis is not confirmed by laboratory evidence, and further review of the clinical data indicates a low probability of HIT, then these findings should be recorded in the patient's record to prevent inappropriately withholding heparin when it is clinically indicated. The sensitivity of the available assays is problematic. It must be emphasized that the most important diagnostic criterion remains the clinical setting, and clinicians should treat patients based on their presentation and not on test results. In general, a positive test associated with the appropriate clinical sitting is helpful, whereas a negative test is less so.

The recommendations regarding HIT are presented in Table 7.

COMMENT

The aPTT is, by far, the most commonly used laboratory test for monitoring heparin therapy of acute thromboembolic disease. The College of American Pathologists 1995 CG2 survey revealed that 95% of participants used the aPTT as their primary monitoring tool. However, the popularity of the aPTT belies its many deficiencies as a mon-

Table 7. Consensus Recommendations: Heparin-Induced Thrombocytopenia (HIT)

1. In patients receiving adjusted-dose and full-dose heparin, monitor for and evaluate HIT by performing platelet counts pretreatment and, at least, on alternate days for 14 days beginning on day 4 of therapy in the naive patient and beginning on day 1 in patients with prior heparin exposure.[141,143] (Level 2)
2. Confirm the clinical diagnosis of HIT by
   a. Performing an assay for heparin-mediated antibody if the platelet count falls below 50% or drops below 100×10^9/L, preferably on an acute specimen or within 6 weeks of the initiation of therapy.[141,143] (Level 2)
   b. Performing an assay for heparin-mediated antibody if there is development or progression of arterial or venous thrombosis, even if the platelet count does not fall[134,141,143] (Level 2)

itoring tool. The discordance in responsiveness between the many available aPTT systems has been well documented for a number of years.[5–7,30–32,161–166] The differences in responsiveness indicate that the use of fixed ratios for expressing the therapeutic range is inappropriate and may result in dosage miscalculations.[6,7,32,41,61,166–168] Attempts to reduce intermethod and interlaboratory variability by implementing a calibration scheme that would allow laboratories to obtain a standardized result, such as the INR, have repeatedly failed.[169–171] It is now apparent that each laboratory should determine the appropriate therapeutic range for its own aPTT method. In-house calibration of the aPTT using heparin added in vitro is hindered by the substantial differences in the aPTT response to in vitro and ex vivo heparinized plasmas.[7,32] The most appropriate method for determination of the therapeutic range is through direct comparison of the aPTT with heparin concentration in samples from patients receiving heparin.

Effective use of the aPTT is also hampered by the biological variables that may interfere with interpretation of results. Recent reports suggest that the aPTT may erroneously indicate a state of heparin resistance, and therefore the risks of over-anticoagulation and bleeding are troubling.[172,173] This effect is thought to be due to the effect of increased concentration of some acute phase reactant proteins, such as factor VIII.[41,172,173] The aPTT cannot differentiate between the effect of such proteins on the aPTT and the effect of increased levels of heparin-binding proteins, which may cause a true heparin resistance. When a patient demonstrates an apparent resistance to heparin, as reflected by the failure of the aPTT to prolong with an appropriate dose of drug, changing to a target concentration of strategy (heparin assay) is recommended. In addition, the aPTT cannot be reliably used in patients with prolonged aPTTs at baseline, such as patients with nonspecific (lupus-like) anticoagulants.

Despite these drawbacks, the aPTT seems to perform satisfactorily in the majority of clinical settings, and clear-cut advantages of direct heparin assays have not been demonstrated in clinical trials.[174] However, a few studies have shown that among patients heparinized for VTE, those who were monitored by a heparin assay protocol reached therapeutic levels more rapidly than did a control group monitored by an empiric aPTT protocol.[175,176] In a prospective clinical trial, heparin concentration more clearly differentiated patients at risk of bleeding than did the aPTT.[24] In addition, heparin assays are less affected by diurnal variation in results and many of the biologic variables that hamper the aPTT.[46,177] On the other hand, since most chromogenic heparin assays have AT added in excess, these tests are unable to detect heparin resistance due to very low levels of AT.[178]

In view of these aspects of the 2 methods for monitoring heparin, many argue that the direct heparin assay should replace the aPTT for routine monitoring of heparin therapy for VTE. Counterbalancing this logic is the known reliability and clinical acceptability of the aPTT. Moreover, the clinical benefits and cost-effectiveness of widespread use of heparin assays remain to be established by direct clinical studies. Others have also advocated the secondary physiologic goal of endogenous thrombin generation. Commonly used tests (aPTT, prothrombin time, ACT, and others) do not reflect this activity well and have the limitations described above when used to monitor unfractionated heparin. Unfortunately, more effective physiologic methods await the development of user-friendly assays.[179,180] Until then, the aPTT will probably remain the major assay for monitoring heparin, with the heparin assay likely to assume an increasingly important role.

Delineation of the optimal therapeutic range for treatment of acute thromboembolic disease still presents some challenges. While it is apparent that the currently available methods rely on the target concentration technique for therapeutic drug monitoring, there have not been direct clinical studies that determine the correct therapeutic range. Furthermore, there are differences in apparent heparin activity that depend on the assay used, with anti-FXa methods generally giving somewhat higher levels than protamine titration/thrombin time methods. The reasons for the discordant results are still uncertain, but they may relate to assay methodology, differences in heparin preparations, or the differential clearance of large and small heparin molecules from the circulation. The clinical data currently available would suggest that the appropriate therapeutic range for protamine titration assays is 0.2 to 0.4 U/mL, while the corresponding range for anti-Xa–based assays would be 0.3 to 0.7 U/mL.

The ACT was the first, and remains the most widely used, point-of-care test of hemostasis. Clinically, it is used almost exclusively for the monitoring of heparin therapy in high doses. In contrast to the aPTT, the ACT is used effectively in the manner of the second goal of therapeutic drug monitoring when initiating anticoagulation. In contrast, monitoring of the neutralization of heparin with protamine sulfate at the conclusion of high-dose therapy is done by changing the role of the ACT to one of estimating heparin concentration, a role for which it is ill-suited. There is still a substantial need for a rapid and effective method for determining the appropriate dose of protamine sulfate.

It is clear that monitoring of the platelet count in patients who receive unfractionated heparin is indicated, possibly even in those whose exposure is limited to flushing of intravenous lines. There appears to be a developing consensus that those patients who develop a significant fall in the platelet count or new thromboembolic problems during exposure to heparin should be tested for the presence of heparin-dependent antibodies. It must be remem-

bered that pathogenic antibodies may be missed by any of the available tests, and thus a negative laboratory result does not rule out clinically significant HIT. Overt clinical evidence of HIT, such as recurrence of thrombocytopenia upon reexposure to heparin after a negative laboratory result, should lead to a clinical diagnosis of HIT and appropriate intervention. While current assays may be helpful in confirming the diagnosis of HIT in the correct clinical setting, assays of improved specificity and sensitivity are needed, particularly if they are to be helpful for clinical decision support in the acute setting.

Recent studies have documented the effectiveness of low-molecular-weight heparin for the therapy of VTE, including pulmonary embolism.[181] There are many advantages to low-molecular-weight heparin that will affect the problems currently associated with monitoring of heparin therapy. In most patients, low-molecular-weight heparin can be administered by a defined protocol without any laboratory monitoring. The incidence of HIT also appears to be significantly lower than with unfractionated heparin. The increased utilization of low-molecular-weight heparin may thus resolve many of the issues that currently surround the monitoring of unfractionated heparin. Unfractionated heparin will be used clinically for some time, and it appears that monitoring it in the clinical setting will remain as much art as science.

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APPENDIX

Validation of Heparin Sensitivity of the aPTT Using Ex Vivo Heparin Specimens: Comparison With an Existing, Validated aPTT Reagent

When changing the activated partial thromboplastin time (aPTT) method, the laboratory has essentially 2 options available. The first is to determine heparin responsiveness and to inform clinicians of the change that has occurred. This method implies that all clinicians will change their behavior with each change of reagent. Changing the behavior of a diverse group of clinicians can be difficult.

The alternative is not to ask clinicians to change. To do this, the laboratory must select a reagent that has the same (or nearly the same) heparin responsiveness as the one currently in use. By doing so, the behavior of clinicians need not change. It is important to control for (and prevent) drift with multiple changes over time.

We present a method for reagent evaluation that will identify reagents that are sufficiently similar for clinical purposes. It uses the cumulative summation method[182] to control for drift. The method has been adapted from one used at Laboratory Control Ltd in Ottumwa, Iowa, and is presented as a useful example consistent with the recommendations presented in the text. The method is as follows:

1. Accumulating Patient Specimens.—There are no data to dictate the number of patient specimens that should be used to perform comparative testing of 2 aPTT reagents on ex vivo heparin specimens. In the examples provided in the table at the end of this Appendix, more than 30 specimens were used. Not more than 2 specimens from a single patient were included in this study. Specimens can be collected during the period of time that an aPTT reagent is being used in production in the laboratory. They are carefully centrifuged to remove all platelets and frozen (−20°C) in aliquots for future aPTT reagent comparisons. Doing so should not be a particular burden, even for the smallest laboratory.

2. Selecting a New Partial Thromboplastin Time Reagent to Test.—This report has recommended that manufacturers provide laboratories with the heparin responsiveness of varying lots of their aPTT reagents. Although the instrument contribution to variability is small, providing information specific to a variety of instruments would certainly be useful. Laboratories could then obtain reagents with a responsiveness to heparin that would be predicted to be similar to the reagent that is currently being used in their laboratory. The determination of heparin sensitivity could be performed in the laboratory if assays are available or could be supplied to the laboratory by the reagent manufacturer.

3. Comparison Testing.—Once a potential replacement reagent has been selected for testing, split specimens can be used to perform the aPTT using each of the 2 reagents on the instrument(s) used in production. The comparison data are plotted with the old reagent on the x axis and new on the y axis. Visual or regression analysis can be used to judge the acceptability of the comparison data and to identify discrepant and outlier results. The data for each aPTT reagent are summed, and the mean and standard deviation are determined. The difference between the means of the new and old aPTT reagents are then recorded for future reference.

4. A Cumulative Summation of Differences.—Each time there is a change in reagents or instrument, comparison testing should be performed. In addition to recording the difference in the mean, the laboratory should prepare a cumulative summation of the differences that have occurred in the past. In doing so, the cumulative shift in the reagent performance in the presence of heparin can be determined. A cumulative change of 5 to 7 seconds is reason for concern, while a cumulative change of more than 7 seconds necessitates action. Suitable actions include evaluating a different reagent to find one with an acceptable level of variation, informing all clinicians using heparin of the change, recommending that clinicians change their thresholds, or reverification of the aPTT with heparin concentration.

This example is taken from the data of Laboratory Control Ltd. During the course of the collection of these data, there were 4 changes involving 5 reagents. The protocol described above was used for the generation of all of the data points given in the table below. Different sets of patient specimens were analyzed with each reagent change, which explains the variation of mean values for each patient data set. Comparisons are made within a patient data set (a row in the table), not between data sets (columns in the table).

It is of interest that the cumulative summation has remained within a range that has not required testing of a different reagent or informing clinicians of the need to

change the therapeutic range for the aPTT. These data were collected over approximately 5 years and demonstrate the simplicity of the method. The simplicity was facilitated by using aPTT reagents from a single manufacturer. In addition, there was an instrument change that occurred during this time period.

Cumulative Summation of Reagent Mean Differences

Patient Data Set* No.	Mean Old Lot, s (Reagent No.)	Mean New Lot, s (Reagent No.)	Difference, s†	Cumulative Summation Difference, s
1	78.6 (1)	73.9 (2)	−4.7	−4.7
2	61.5 (2)	64.8 (3)	+3.3	−1.4
3	71.9 (3)	72.3 (4)	+0.4	−1.0
4	62.8 (4)	60.3 (5)	−2.5	−3.5

* Reagent change.
† New minus old.