Laboratory Monitoring of Heparin Therapy: Partial Thromboplastin Time or Anti-Xa Assay?

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Abstract

The activated partial thromboplastin time (PTT) is the principal method by which laboratories monitor unfractionated heparin therapy. A review of the experimental basis for heparin monitoring by the PTT reveals significant shortcomings of the assay. The availability of anti-Xa heparin assays on automated coagulation analyzers presents a seemingly logical alternative because the PTT therapeutic range is derived from anti-Xa measurements of plasma from heparinized patients. The anti-Xa assay is not susceptible to many of the preanalytical interferences affecting the PTT, and adoption of anti-Xa monitoring would eliminate the need for validating a PTT therapeutic range. However, anti-Xa heparin monitoring has not been rigorously validated by clinical outcomes studies, and decreasing clinical use of unfractionated heparin makes it unlikely that such data is forthcoming. Nonetheless, many laboratories may find themselves in the position of being unable to continue to validate their PTT therapeutic ranges according to current recommendations and accreditation requirements.

After reviewing this article, readers should be able to describe the physical characteristics and therapeutic uses of heparin and discuss the complexities associated with laboratory monitoring of heparin therapy.

Chemistry exam 20901 questions and corresponding answer form are located after this CE Update article on page **52**.

The activated partial thromboplastin time (PTT) continues to be the principal method by which laboratories monitor intravenous unfractionated heparin (UH) therapy.[1] However, the availability of anti-factor Xa (anti-Xa) assays on automated coagulation analyzers presents the opportunity to reassess the historical and scientific basis for the use of the PTT assay as the primary laboratory tool for monitoring heparin therapy. Ironically, the widespread availability of anti-Xa assays occurs at a time when the clinical indications for unfractionated heparin are narrowing because of the availability of low-molecular-weight heparin (LMWH) products.

Unfractionated Heparin and Low-Molecular-Weight Heparin

Heparin is a heterogeneous mixture of highly negatively charged, sulfated mucopolysaccharides (polysugars) also known as glycosaminoglycans. The molecular weights (MW) of heparin molecules in UH preparations range from 3,000 to 30,000 Daltons, with an average MW of 15,000 to 18,000 Daltons. This equates to polymers composed of approximately 45 to 50 monosaccharides. The dense negative charge surrounding large MW heparin molecules results in considerable nonselective binding of UH to cells and proteins reducing the anticoagulant effect. Unfractionated heparin is eliminated from the body by 2 mechanisms: 1) dose-dependent depolymerization, primarily of large MW molecules, mediated by endothelial cells and macrophages; and 2) dose-independent elimination of low MW molecules by the kidneys. Protein binding and saturable elimination kinetics produce significant variability in the anticoagulant effect of UH in individual patients.[2]

Low-molecular-weight heparin is a manufactured derivative of UH. It is prepared from UH by filtration or controlled depolymerization to yield polymers with an average MW of 3,000 Daltons. Low-molecular-weight heparin products are less likely to bind nonspecifically to proteins and are eliminated from the body by the kidneys. These properties result in a more predictable anticoagulant effect when weight-based dosing is used for patient therapy.[2]

Mechanism of Heparin Anticoagulation

Heparin's primary anticoagulant properties derive from its interaction with antithrombin (AT), a naturally occurring anticoagulant protein found in blood. Heparin binds to AT via a specific pentasaccharide sequence (**Figure 1**). Heparin binding induces conformational changes in the AT molecule resulting in a many-fold increase in the anticoagulant activity of AT. Antithrombin suppresses coagulation by inactivating proteins (serine proteases) involved in the coagulation cascade—primarily thrombin (FIIa) and factor Xa (FXa). Specific binding of the pentasaccharide sequence found in UH and LMWH to AT is sufficient for inactivation of FXa. Inactivation of thrombin occurs by nonspecific binding of the heparin:AT complex to FIIa and requires a polysaccharide chain of at least 18 monosaccharides (**Figure 2**). Consequently, heparin has roughly equivalent antithrombin and anti-Xa activity, while the antithrombin activity of the various commercially available LMWH products depends on the relative proportion of molecules containing 18 or more monosaccharides in each product.[2]

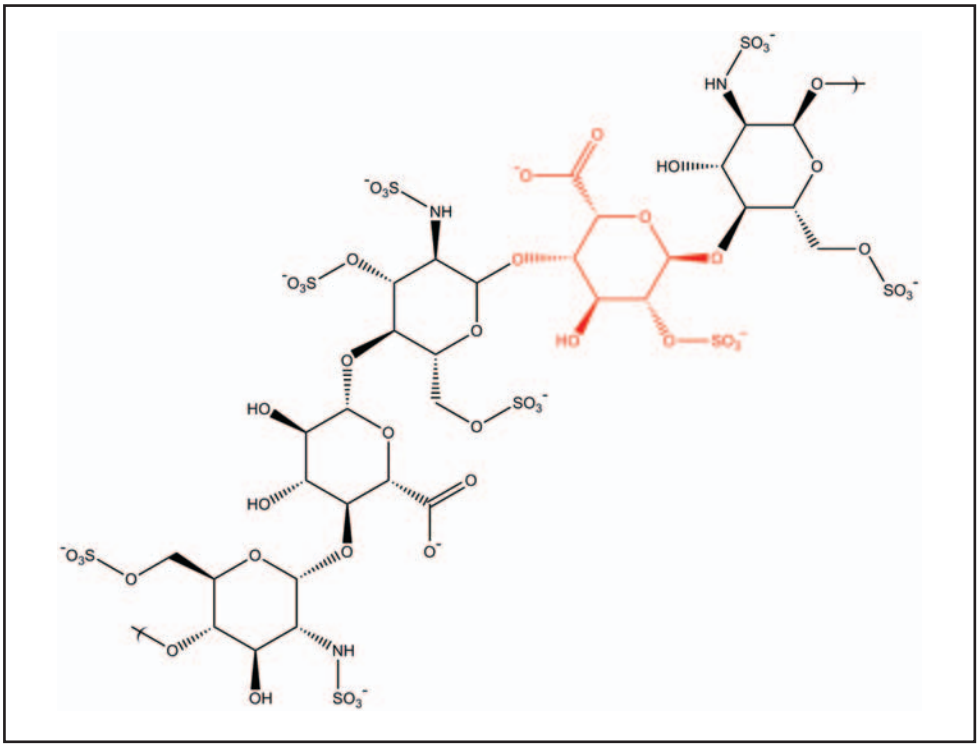

Figure 1_Pentasaccharide antithrombin binding site of heparin. A single saccharide unit is shown in red. The full polysaccharide heparin molecule contains numerous negatively charged groups that result in nonspecific binding of heparin to plasma proteins as well as blood and endothelial cells. This nonspecific binding decreases heparin activity and most likely accounts for the wide variability of anticoagulant effect observed in individual patients as measured by the PTT.

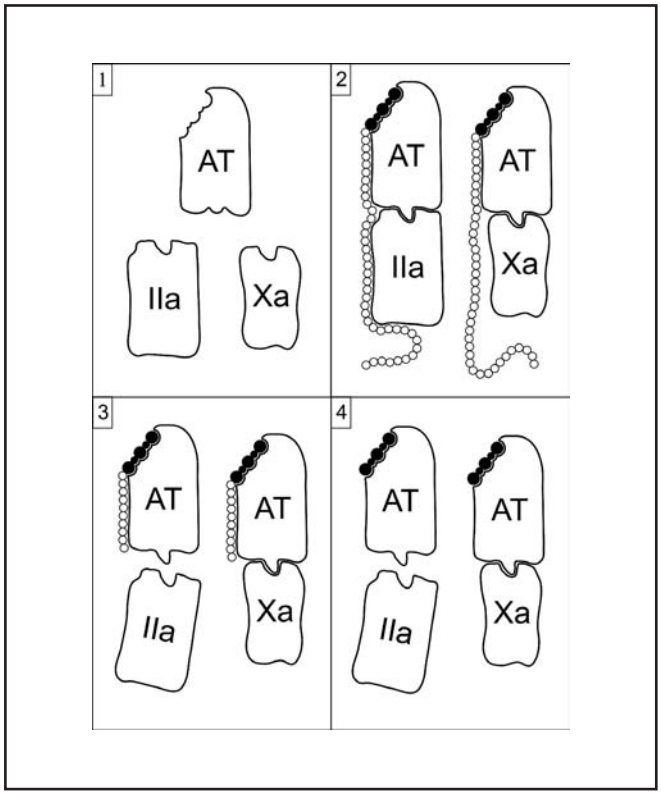

Figure 2_Formation of antithrombin complexes with factor IIa and factor Xa. (**1**) Antithrombin (AT), activated thrombin (FIIa), activated factor X (FXa). (**2**) Unfractionated heparin promotes the formation of both FIIa and FXa complexes with AT. (**3**) Polysaccharide chains shorter than 18 units promote AT complex formation with FXa but not with FIIa. (**4**) The pentasaccharide sequence promotes binding with FXa only. (Figure 10.3, p182. From: Bennett ST. Monitoring Anticoagulant Therapy. In: Bennett ST, Lehman CM, Rodgers GM, eds. *Laboratory Hemostasis: A Practical Guide for Pathologists.* 1st ed. New York: Springer; 167–205. Copyright 2007. With kind permission of Springer Science and Business Media).

Therapeutic Uses of Heparin

Traditionally, unfractionated heparin has been indicated for the treatment or prevention of spontaneous or iatrogenic (medical procedure-induced) venous or arterial thromboembolism (clotting). Heparin therapy has been demonstrated to be effective in reducing morbidity and mortality associated with established thromboemboli (eg, deep venous thrombosis, pulmonary embolism) and in reducing the risk of thrombus formation (eg, myocardial infarction, unstable angina, coronary angioplasty). Heparin may be administered to the patient by intravenous (IV) (in-patient) or subcutaneous (out-patient) routes depending on the clinical indication. Intravenous heparin therapy is initiated with a bolus dose followed by maintenance doses calculated to maintain the anticoagulation required for therapeutic benefit. Laboratory testing is considered essential for IV therapy, but is not indicated for subcutaneous heparin therapy. In current medical practice, primary clinical indications for UH therapy are decreasing as LMWH replaces UH as the heparin anticoagulant of choice due to its predictable anticoagulant response that makes routine laboratory monitoring unnecessary and a lower complication rate.[3,4]

Therapeutic Monitoring of Heparin

The PTT is the test of choice for monitoring low-dose IV heparin therapy. Data supporting the use of the PTT date to studies published in the early 1970s. A retrospective analysis of patient data published by Basu and colleagues in 1972 suggested a PTT equal to 1.5 to 2.5 times the mean control PTT reduced the risk of recurrent thromboembolism.[5] A subsequent paper published by the same group at McMaster University using the same PTT reagents in an experimental rabbit model of thrombus extension supported the 1.5 to 2.5 therapeutic range.[6] Thus the "1.5 to 2.5 times control" UH therapeutic range was born. Early clinical studies lent support to the concept that the PTT should be brought into the therapeutic range within 24 hours to avoid thrombosis.[7]

Correlation of elevated PTT values (>2.5 × control) with the incidence of bleeding has proven to be more problematic.[7, 8]

Partial Thromboplastin Times and Heparin Assays

The McMaster group also demonstrated a PTT of 1.5 to 2.5 times control (using their reagent) corresponded to a heparin level of 0.2 to 0.4 IU/mL using a protamine titration heparin assay.[9] As additional PTT reagents (and coagulation instruments) became available, it became clear that different reagents demonstrated varying sensitivities of the PTT to heparin. Kitchen and Preston measured therapeutic PTT ratios ranging from 1.61 to 2.60 at 0.4 IU/mL and from 1.93 to 3.94 at 0.6 IU/mL for 8 different PTT reagents.[10] Therefore, PTT therapeutic ranges derived from heparin levels of 0.2 to 0.4 IU/mL (by protamine assay) are, in fact, reagent specific. These data brought into question the use of a standardized PTT therapeutic ratio without consideration of the reagent:instrument combination employed for testing. A reexamination of clinical trials used different PTT reagents (with variable sensitivities to heparin) to maintain the therapeutic ratio of 1.5 to 2.5 times control demonstrated the effectiveness of heparin therapy, even when the PTT was sub-therapeutic.[7]

Once it was accepted that the PTT was not an accurate measure of successful heparin anticoagulation, consideration was given to improving the assay by creating reagent-specific therapeutic ranges. The use of therapeutic ratios was largely abandoned in favor of PTT therapeutic ranges calibrated by anti-Xa heparin measurements. Guidelines were developed using data from the McMaster group studies showing a heparin level of 0.2 to 0.4 IU/mL by protamine assay was equivalent to a level of 0.35 to 0.70 IU/mL using a factor Xa heparin assay.[9] This relationship formed the basis for recommendation of a 0.3 to 0.7 IU/mL therapeutic range for UH using an anti-Xa assay.[11] However, anti-Xa heparin assays are not harmonized. Assay comparison studies demonstrated that anti-Xa therapeutic heparin levels corresponding to a protamine assay concentration of 0.2 IU/mL ranged from 0.24 to 0.30 IU/mL, and anti-Xa therapeutic heparin levels corresponding to a protamine assay concentration of 0.4 IU/mL ranged from 0.38 to 0.60 IU/mL.[12]

College of American Pathologists (CAP) Requirements for a PTT-based Heparin Therapeutic Range

A laboratory monitoring heparin therapy with the PTT must establish a therapeutic range using an appropriate technique. For initial creation of a therapeutic range, the CAP recommends 1) collection of plasma samples from patients receiving IV heparin therapy (ex vivo samples) and 2) analysis by PTT and heparin assay.[13] A therapeutic PTT range can be calculated by identifying the PTT values corresponding to anti-Xa levels of 0.3 and 0.7 IU/mL. Changes in reagent lots and/or instrumentation require a revalidation of the therapeutic range. Laboratories may repeat the same validation process or analyze samples from patients receiving IV heparin therapy by the original PTT reagent lot (or method)

Table of Abbreviations and Terms

ACCP: American College of Chest Physicians.

Anti-Xa heparin assay: Laboratory assay that measures the activity of heparin against the activity of activated coagulation factor X.

AT: Antithrombin (formerly antithrombin III). A serine protease in blood that acts as a natural anticoagulant. AT activity increases many fold when bound to heparin.

CAP: College of American Pathologists.

FIIa: Thrombin.

FXa: Activated form of coagulation factor X.

INR: International Normalized Ratio defined as: (PTTest/PTMean normal)ISI, where the ISI is the International Sensitivity Index. A relative measure of the sensitivity of the PT reagent to the therapeutic effect of the anticoagulant coumadin.

IU: International Units. A unit of measurement of a biological substance based on its activity.

IV: Intravenous.

LMWH: Low-molecular-weight heparin.

MW: Molecular weight.

PT: Prothrombin time.

PTT: Partial thromboplastin time.

Polysaccharide: Multiple sugar molecules bound together. A pentasaccharide is composed of 5 sugar molecules.

Serine Proteases: Enzymes that cut peptide bonds in proteins.

UH: Unfractionated heparin.

and the new PTT lot and compare the results to determine clinically equivalent response. The mean difference between the lot used to establish the PTT therapeutic range and a subsequent lot must not exceed 7 seconds. Since each subsequent reagent lot is compared against the preceding lot, laboratories must monitor the sum of differences from the reagent lot used in the original validation to ensure that the cumulative mean PTT difference does not exceed 7 seconds.[11] Important preanalytical considerations for conducting validations include the following recommendations: 1) at least 30 samples should be collected from no fewer than 15 patients receiving heparin therapy (ex vivo samples); 2) samples should be collected no less than 4 hours following a bolus dose or change in dose (IV rate) to allow for drug equilibration; 3) samples collected from patients taking warfarin should be used only if the INR is <1.3, since warfarin treatment can prolong the PTT[14]; 4) the mix of sample results should span the heparin therapeutic range (ie, 0.3 to 0.7 IU/mL); and 5) preparation of study samples by spiking pooled normal plasma with heparin (in vitro specimens) is not recommended for testing since published data suggest a larger heparin effect in these samples than that observed for ex vivo samples.[13] Use of spiked samples will result in an artifactually elevated therapeutic range (**Figure 3**).

Validating PTT Therapeutic Ranges: Challenges for the Laboratory

Many laboratories will find it challenging to meet the recommendations for validating their laboratory-specific PTT therapeutic ranges. The most daunting problem is identifying a sufficient number of patients receiving UH therapy, since, as noted previously, LMWH is replacing UH as the heparin of choice for preventing or treating thromboembolism. This situation will be exacerbated as new anticoagulants are approved for use by regulatory agencies. In addition, it is difficult to collect samples from patients receiving warfarin who have an INR <1.3, since therapy with warfarin is frequently initiated simultaneously with UH therapy, thus narrowing the window of opportunity. The laboratory must rely on the clinical team to draw specimens at the appropriate interval following bolus doses or dose changes and, since PTT samples may be collected and sent for analysis at all hours of the day and night, identification of a patient receiving UH, sample retrieval, and anti-Xa analysis within sample stability time limits can be an issue. Finally, the degree of scatter found in a plot of PTT versus heparin concentration leads to very large confidence intervals around the estimated limits of the therapeutic range.

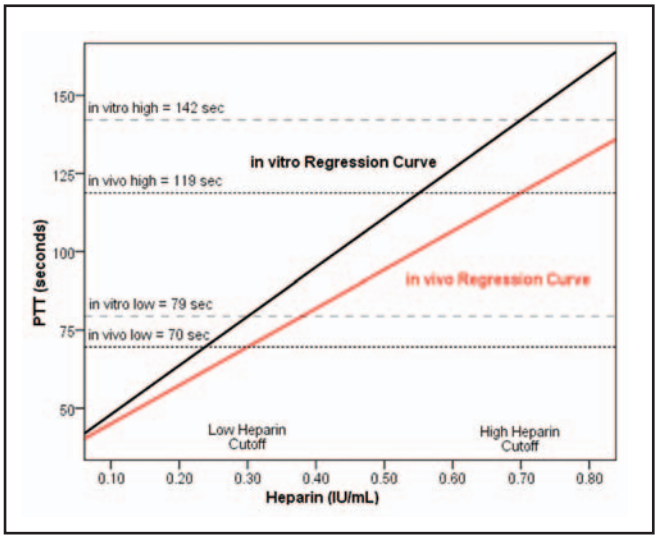

Figure 3_Hypothetical comparison of therapeutic ranges established from regression analysis of spiked plasma pools (in vitro response: 79 to 142 seconds) or specimens from heparinized patients (in vivo response: 70 to 119 seconds).

Advantages and Disadvantages of Using an Anti-Xa Heparin Assay for Monitoring UH Therapy

Abandoning the PTT in favor of the anti-Xa assay for monitoring heparin therapy would have the following advantages:

1) The anti-Xa assay is now available on many automated coagulation analyzers.
2) Unlike the PTT, the anti-Xa assay is not affected by underfilled collection tubes—a common preanalytic problem.
3) The anti-Xa assay is not susceptible to interference from elevated concentrations of factor VIII or fibrinogen that result from acute phase reactions.
4) The anti-Xa assay is not influenced by factor deficiencies, with the possible exception of AT deficiency (see below).
5) Most important, there would no longer be a need to establish a PTT therapeutic range, provided the laboratory has informed clinicians that UH therapy must be monitored using the anti-Xa assay rather than the PTT and the clinicians are also informed of the therapeutic range.

Unfortunately, there are some disadvantages to the anti-Xa assay:

1) Prompt sample processing (1 hour) is required to avoid heparin neutralization from platelet factor 4.
2) The assay is considerably more expensive than the PTT.
3) Despite the limitations of the PTT for monitoring adequacy of heparin therapy, it does represent a measure of the anticoagulant effect of heparin in patients.
4) The assay underestimates heparin concentration in the presence of significant AT deficiency, although the clinical significance of this finding is controversial.[15]
5) Though the authoritative recommendation for the anti-Xa therapeutic range is 0.3 to 0.7 IU/mL (ACCP), the published literature demonstrates the limitations of that recommendation.
6) There is limited published information on the use of anti-Xa assays for routine monitoring of UH therapy. One recent study identified patients in a medical intensive care unit who were receiving IV heparin but had no measurable heparin levels by 3 different anti-Xa assays.[15]
7) There are limited published outcomes data evaluating the safety and effectiveness of anti-Xa assays for managing UH therapy.

Conclusions

The PTT continues to be the primary test used by laboratories for monitoring IV heparin therapy in spite of known limitations for predicting adequacy of anticoagulation in the treated patient, and the difficulty of establishing and maintaining a validated therapeutic range with each reagent lot or instrument

change. Replacement of UH with LMWH and other new anticoagulants that do not require routine laboratory monitoring will increase the challenges labs face in validating their PTT therapeutic ranges. Unfractionated heparin therapy is not likely to vanish anytime soon, however, since there is a role for an anticoagulant whose effects are rapidly reversible in the event of bleeding (eg, intensive care patients). Therefore, many laboratories may find themselves in the position of attempting to validate their PTT therapeutic ranges but being unable to comply with current accreditation recommendations. In an attempt to acquire sufficient samples, laboratories might decide to 1) collect more than 2 samples from each patient or 2) make do with fewer than 30 samples. Either of these approaches would have the effect of increasing the inaccuracy of the estimated therapeutic range. Since an elevated PTT correlates poorly with heparin-induced bleeding,[7,8] the primary risk of an inaccurate therapeutic PTT range would be thrombosis secondary to inadequate anticoagulation (ie, underestimate the elevation of the PTT necessary to achieve therapeutic anticoagulation). However, the risk of thrombosis may be minimal if patients receive an adequate, weight-based bolus dose of UH, followed by maintenance doses, regardless of the PTT attained.[8]

Anti-Xa assays represent an attractive alternative to the PTT for UH monitoring; however, minimal outcomes data and greater expense are limiting factors. While the cost of Anti-Xa assays might decrease with higher test volumes, prospective clinical outcomes data are not likely to be forthcoming because of the waning utilization of UH. Nonetheless, laboratories may elect to switch to anti-Xa heparin monitoring based on the outcomes data currently available.[16] LM

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