Laboratory Monitoring of Heparin: Challenges and Opportunities

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Abstract

Heparin, an anticoagulant used for the treatment and prophylaxis of thrombosis, is most commonly monitored using the activated partial thromboplastin time (APTT). Despite its widespread use, the APTT has limitations which stem from the coagulation factors it measures and the technical variability of the assay. Newer tests, such as the anti-factor Xa activity assay, provide an opportunity to improve laboratory monitoring of heparin and, most importantly, patient safety.

After reading this article, readers should become familiar with the pharmacologic profile of heparin, laboratory tests used to monitor heparin, and the potential use of the anti-factor Xa activity assay in heparin monitoring

Blood Banking Exam 60701 questions and corresponding answer form are located after the CE Update section on p. 503

Heparin, a potent anticoagulant, is widely used in both inpatient and outpatient settings for the prophylaxis and treatment of thrombosis (**Table 1**). Each year in the United States, approximately $3 billion is spent on therapeutic heparin administration alone.[1] Its efficacy and potential for reversal with protamine sulfate are some of the advantages of heparin; however, overanticoagulation may occur, which may result in clinically-significant bleeding. The balance of under- and overanticoagulation requires strict laboratory monitoring to ensure therapeutic dosing. Considering the consequences of not maintaining patients in the therapeutic range, improved laboratory monitoring of patients receiving heparin represents a new opportunity to improve patient safety. In this article, the authors review basic heparin pharmacology and discuss heparin monitoring from both a clinical and a laboratory perspective.

Table 1_Clinical Uses of Heparin

- Prophylaxis and treatment of deep-venous thrombosis.
- Treatment of pulmonary embolism or other clinically-significant thrombosis.
- Treatment of acute coronary syndromes such as unstable angina or non-ST elevation acute myocardial infarction.
- Prevention of stroke resulting from embolic source due to conditions such as atrial fibrillation.
- Intraoperative use during cardiopulmonary bypass surgery, coronary angioplasty, vascular surgery, or hemodialysis.

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The Pharmacology of Heparin

Heparin is composed of branched glycosaminoglycans derived from either porcine intestinal mucosa or bovine lung tissue.[2] The length of the polysaccharide chain varies, accounting for a range of molecular weights. Unfractionated heparin (UFH) ranges from 5 to 35 kDa molecular weight, compared with its low-molecular-weight derivative generated by depolymerization, which ranges from 3 to 5 kDa. The pharmacologic activity of heparin originates from a unique pentasaccharide sequence containing alternating residues of D-glucosamine and uronic acid. This specific sequence is required for the formation of a high-affinity bond with antithrombin (AT), a protease inhibitor that serves as an intrinsic anticoagulant.

Heparin exerts its anticoagulant affect by binding to AT, thus causing a conformational change allowing AT to expose its active site. Activated AT is then able to inactivate thrombin (factor II) and other proteases of the coagulation cascade, such as factor Xa, at an accelerated rate. Interestingly, inhibition of factor Xa requires only the pentasaccharide sequence, while that of thrombin requires at least 18 saccharides.[2] As a result, low-molecular-weight heparin (LMWH) preparations have greater factor Xa inhibition than anti-thrombin activity (**Figure 1**).

The efficacy of heparin is determined by its bioavailability and clearance. The bioavailability of heparin is affected by its route of administration.[2] Unfractionated heparin is typically administered intravenously; however, it may also be administered subcutaneously, which requires higher initial dosing due to a decreased bioavailability. Low-molecular-weight heparin is exclusively administered subcutaneously and has a bioavailability of 90% to 100%. Therefore, LMWH has more predictable pharmacokinetics and currently does not routinely require laboratory monitoring.
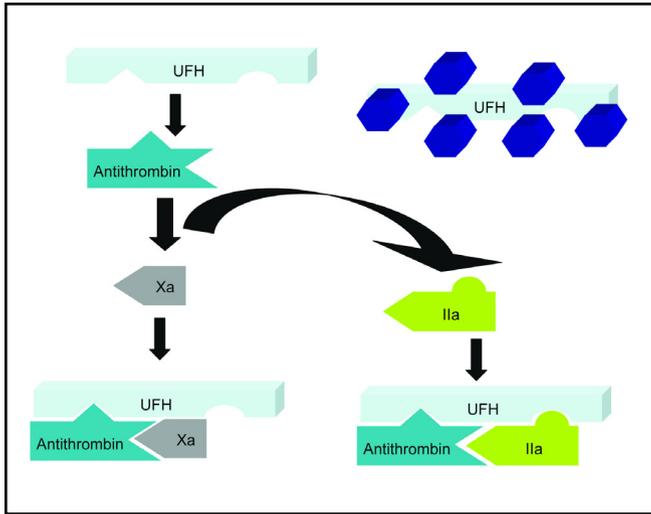
Figure 1A_Unfractionated heparin mechanism of action. When unfractionated heparin is not bound to plasma proteins, it binds to antithrombin (AT) creating the heparin-AT complex. This complex inactivates factor IIa (thrombin) and other proteases, namely factor Xa. The inactivation of thrombin is 10-fold greater than that of factor Xa.
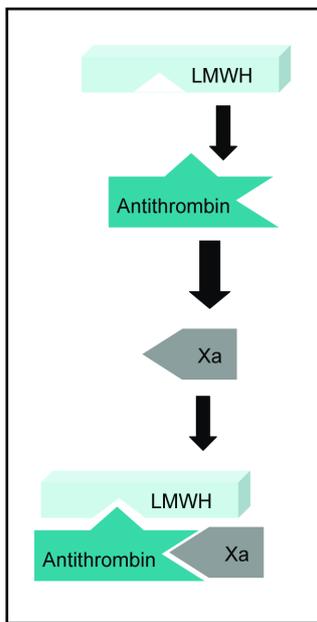

Figure 1B_Low-molecular-weight heparin mechanism of action. Low-molecular-weight heparin (LMWH) also complexes with AT forming the heparin-AT complex. However, LMWH has lost the ability to bind thrombin due to its molecular size (<18 saccharides). Reprinted with permission from the American Association for Clinical Chemistry from *Clinical Laboratory News*, Vol. 32, No. 12.

Heparin is cleared by 2 possible mechanisms.[2] First, heparin may bind to endothelial cells and macrophages causing depolymerization, which results in rapid, saturable, dose-dependent pharmacokinetics. This is the primary clearance mechanism of UFH that results in a half-life of 40 to 60 minutes when administered at typical intravenous doses. On the other hand, heparin may be cleared by a slower, nonsaturable, dose-independent route in the kidneys. Low-molecular-weight heparin is cleared primarily by this route and, if administered subcutaneously, has a 4-hour half-life. However, because of this clearance pathway, treatment with LMWH may require renal-adjusted dosing.

Heparin Monitoring: A Historical Perspective

As early as 1938, clotting assays, such as the Lee-White whole blood clotting time (WBCT), were used to monitor heparin therapy.[3] In 1955, therapeutic anticoagulation was achieved in a canine model using a Lee-White WBCT 2 to 3 times the normal reference range.[4] However, heparin monitoring using the Lee-White WBCT was time consuming and fell out of favor.

Since 1972, the activated partial thromboplastin time (APTT) has been used to monitor heparin. Early studies suggested prolongation of the APTT 1.5 to 2 times the normal reference range would be considered therapeutic for heparin therapy, thereby preventing recurrence of thrombosis while limiting the risk of bleeding.[5] Further studies showed this degree of prolongation correlated with a heparin level of 0.2 to 0.4 units/mL as measured by protamine sulfate titration or a level of 0.3 to 0.7 units/mL anti-factor Xa activity.[6] A recent College of American Pathologists (CAP) Q-Probes study found that 136 out of 140 surveyed institutions (97%) used the APTT as the test primarily used to monitor heparin.[7] Due to its wide availability, simplicity of performance (including automation), turnaround time, and cost, the APTT became the standard laboratory test to monitor heparin therapy.

Problems With the APTT

The APTT, an in vitro clotting assay, measures both the intrinsic and common pathways of the clotting cascade by adding activators of the contact factors and phospholipid to recalcified plasma. The resultant clot formation is then detected using an electromechanical or photo-optical device. For this reason, a major problem with the APTT is its sensitivity to factors involved in both the intrinsic and common pathways, which include fibrinogen, factors II, V, VIII, IX, X, XI, and XII, and the contact factors. Therefore, either congenital or acquired deficiencies of any of the above will cause prolongation of the APTT despite heparin treatment. In addition, inhibitors such as antiphospholipid antibodies that are often implicated as the underlying cause of arterial and venous thromboses, factor inhibitors such as those directed towards factor VIII, and simultaneous treatment with warfarin may also prolong the APTT making heparin monitoring more complex. Moreover, clinicians may encounter patients who require abnormally large amounts of heparin, which is necessary to achieve a therapeutic level of anticoagulation as measured by the APTT. These patients are referred to as heparin resistant. For example, increased levels of the acute phase reactants, factor VIII and fibrinogen, may shorten the APTT, thus requiring larger doses of heparin (**Table 2**).

The major concern with the APTT, however, stems from the variability of the assay.[8,9] Currently, over 300 commercial assays to measure the APTT exist, each using different instrumentation and reagents.[9] Unlike the international normalized ratio (INR), no universal standard for the APTT exists. As a result, the CAP and the American College of Chest Physicians

Table 2_Beware of Heparin Resistance

Mechanisms proposed that may result in the development of heparin resistance

- Antithrombin (AT) deficiency.
- Increased levels of factor VIII.
- Increased levels of fibrinogen.
- Increased plasma binding proteins.
- Increased clearance.
- Use of aprotinin or nitroglycerin.

Increased factor VIII levels result is a shortened APTT and will require larger amounts of heparin to prolong the APTT; however, the anti-factor Xa level will be unaffected. On the other hand, patients with congenital or acquired AT deficiency may have low anti-factor Xa activity.

(ACCP) proposed that clinical laboratories calibrate their therapeutic range.[10] Using plasma from a minimum of 30 to 40 patients receiving heparin, correlation of the APTT with a heparin level should be obtained using a protamine sulfate titration or anti-factor Xa activity assay. Unfortunately, this correlation, as suggested by other authors, is time consuming, and many clinical laboratories may not have access to these methods.

Sizing Up the APTT: Comparison With the Anti-Factor Xa Level

Anti-factor Xa activity is measured using a colorimetric assay that determines the amount of free factor Xa in a sample after heparin neutralization of factor Xa and AT. Therefore, the amount of free factor Xa is inversely proportional to the amount of heparin in the sample. As compared with the APTT, additional coagulation factors of the intrinsic and common pathways and inhibitors do not influence the result. In addition, the anti-factor Xa activity assay is less affected by other preanalytical variables such as anticoagulation, specimen collection, and processing.[11]

Multiple studies have shown that the APTT range corresponding to the therapeutic anti-factor Xa activity of 0.3 to 0.7 units/mL was highly variable and not consistently 1.5 to 2 times the reference range.[4,7,12] Moreover, most patients were subtherapeutic when the APTT was compared with the therapeutic anti-factor Xa activity assay. This was most often attributed to baseline prolongation of the APTT as a result of factor deficiency or inhibitor and simultaneous treatment with warfarin. However, no studies have shown a statistically-significant difference between both assays in terms of the incidence of a recurrent thrombotic event or bleeding episode.

In addition, monitoring with an anti-factor Xa activity assay has been demonstrated to result in decreased amounts of administered heparin, especially in heparin-resistant patients, fewer dosage changes, and decreased numbers of monitoring tests.[12,13] All of these have resulted in a cost benefit over the APTT, less nursing and laboratory time, and, most important, less chance for error. Even though the anti-factor Xa activity assay costs more to perform, it is an estimated $4.37 (16%) more than the APTT during a 96-hour hospital admission .[13]

Choice of Assay: APTT or Anti-Factor Xa Activity

Despite the limitations of the APTT, most laboratories continue to use this assay to monitor heparin therapy. Previously-cited reasons precluding the use of the anti-factor Xa activity assay included high cost and limited availability.

Studies have consistently shown the superiority of the anti-factor Xa activity assay to the APTT. At 24 hours, therapeutic APTT levels were obtained in 57% of 62 patients using a calibrated assay, or 52% of 5,000 patients using an uncalibrated assay, compared with 87% of 104 patients using the anti-factor Xa assay.[14] Furthermore, a UFH dosage protocol incorporating sex, age, height, and weight achieved a therapeutic level in 62% of 197 patients at 8 hours compared with 37% at 6 hours using a weight-based heparin dosing protocol.[14, 15]

In summary, the anti-factor Xa activity assay is a more accurate and precise assay to monitor heparin therapy. Interestingly, no studies to date have shown statistically-significant differences in clinical outcomes when comparing the anti-factor Xa assay with the APTT, although a randomized, controlled trial demonstrated a trend toward better outcomes.[12] In collaboration with nursing and pharmacy, the authors' institution has adopted a weight-based protocol using the anti-factor Xa activity assay to monitor heparin. Admittedly, introducing changes to the established heparin-dosing protocol was challenging and adjustments will occur as the authors continue to test their system. For example, decreasing variables such as sex, age, and height may prove to improve the authors' heparin-weight-based protocol as it has done for others.[14] Laboratories that choose to continue to employ the APTT as the primary test to monitor heparin should comply with the CAP and ACCP recommendations to calibrate the therapeutic range to a heparin level of 0.3 to 0.7 units/mL, and the authors advocate that patients selected for these studies be representative of the patient population receiving intravenous heparin. LM

1. Li W, Johnson DJ, Esmon, CT, et al. Structure of the antithrombin-thrombin-heparin ternary complex reveals the antithrombotic mechanism of heparin. *Nat Struct Mol Biol.* 2004;11:857–862.

2. Baglin T, Barrowcliffe TW, Cohen A, et al. Guidelines on the use and monitoring of heparin. *Br J Haematol.* 2006;133:19–34.

3. Rosborough TK. Comparison of anti-factor Xa heparin activity and activated partial thromboplastin time in 2,773 plasma samples from unfractionated heparin-treated patients. *Am J Clin Pathol.* 1997;108:662–668.

4. Wessler S, Morris LE. Studies in intravascular coagulation. IV. The effect of heparin and dicumarol on serum-induced venous thrombosis. *Circulation.* 1955;12:553–556.

5. Basu D, Gallus A, Hirsh J, et al. A prospective study of the value of monitoring heparin treatment with the activated partial thromboplastin time. *New Engl J Med.* 1972;287:324–327.

6. Bates SM, Weitz JI, Johnston M, et al. Use of a fixed activated partial thromboplastin time ratio to establish a therapeutic range of unfractionated heparin. *Arch Intern Med.* 2001;161:385–391.

7. Valenstein PN, Walsh MK, Meier F. Heparin monitoring and patient safety: A College of American Pathologists Q-probes study of 3,431 patients at 140 institutions. *Arch Pathol Lab Med.* 2004;128:397–402.

8. Baker BA, Adelman MD, Smith PA, et al. Inability of the activated partial thromboplastin time to predict heparin levels. Time to reassess guidelines for heparin assays. *Arch Intern Med.* 1997;157:2475–2479.

9. Eikelboom JW, Hirsh J. Monitoring unfractionated heparin with the aPTT: Time for a fresh look. *Thromb Haemost.* 2006;96:547–552.

10. Brill-Edwards P, Ginsberg JS, Johnston M, et al. Establishing a therapeutic range for heparin therapy. *Ann Intern Med.* 1993;119:104–109.

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11. McGlasson DL, Kaczor DA, Krasuski RA, et al. Effects of pre-analytical variables on the anti-activated factor X chromogenic assay when monitoring unfractionated heparin and low molecular weight heparin. *Blood Coagul Fibrinolysis*. 2005;16:173–176.

12. Levine MN, Hirsh J, Gent M et al. A randomized trial comparing activated thromboplastin time with heparin assay in patients with acute venous thromboembolism requiring large daily doses of heparin. *Arch Intern Med*. 1994;154:49–56.

13. Rosborough TK. Monitoring unfractionated heparin therapy with anti-factor Xa activity results in fewer monitoring tests and dosage changes than monitoring with the activated partial thromboplastin. *Pharmacotherapy*. 1999;19:760–766.

14. Rosborough TK, Shepherd MF. Achieving target antifactor Xa activity with a heparin protocol based on sex, age, height, and weight. *Pharmacotherapy*. 2004;24:713–719.

15. Rosborough TK. In unfractionated heparin dosing, the combination of patient age and estimated plasma volume predicts initial anti-factor Xa activity better than patient weight alone. *Pharmacotherapy*. 1998;18:1217–1223.