Laboratory Monitoring of Unfractionated Heparin

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Heparin Monitoring

Unfractionated heparin is the most widely used anticoagulant for therapeutic purposes in hospitalized patients. Heparin is a heterogeneous mixture of negatively charged, highly-sulfated mucopolysaccharides commercially obtained from bovine or porcine tissue. It is not absorbed from the gastrointestinal tract and must be given by injection. A single intravenous dose has a half-life of approximately 60 minutes, with clearance occurring in the reticuloendothelial system, particularly the liver. Heparin does not directly inhibit coagulation, but exerts its potent anticoagulant effect by potentiating the antithrombotic effect of antithrombin. Heparin therapy requires rigorous laboratory monitoring with the activated partial thromboplastin time (aPTT), since its bioavailability is variably affected by binding to plasma and cellular proteins. Unfractionated heparin is gradually being replaced by low molecular weight heparin, which has a longer half-life and more predictable bioavailability.

Heparin Structure and Mechanism of Action

Unfractionated heparin is a complex mixture of glycosaminoglycans in the molecular weight range of 3,000 to 30,000 Da which is purified from bovine lung or porcine intestinal mucosa. The active components of this mixture contain a disaccharide repeat of either an iduronic (80%) or glucuronic acid and a glucosamine that is often disulfated. Only about a third of the glycosaminoglycans in the mixture inhibit coagulation at clinically achievable concentrations.

A functionally active heparin molecule contains a minimum of 18 saccharides, including a specific pentasaccharide sequence that binds antithrombin. Binding of antithrombin by the pentasaccharide causes a conformational change that augments inhibition of thrombin and factor Xa, and to a lesser extent factors IXa, XIa, and XIIa. A minimum length of 18 saccharides allows heparin to span between antithrombin and a binding site on the inhibited factor.

The augmented inhibition of thrombin is dependent on the ability of heparin to bind both molecules, whereas inhibition of factor Xa can be augmented solely by the conformational change associated with binding to antithrombin. The latter observation forms the basis for the development of low molecular weight heparins.

Heparin Pharmacokinetics

Heparin is not absorbed significantly through the gastrointestinal tract and must be administered either by intravenous infusion or subcutaneous injection. The bioavailability and clearance of heparin depends on a number of factors that make therapeutic dosing complicated.

Heparin binds to a number of plasma proteins and cells that complicate the clearance and anti-coagulant effects. At low doses, binding of endothelial cells, macrophages, and acute phase reactants rapidly removes
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Fig. 2. Heparin bound to thrombin and antithrombin. The complex between thrombin and antithrombin is shown as a surface model with thrombin colored green and antithrombin colored blue. A heparin mimetic is shown in spheres with the atoms colored by type.

Heparin from the circulation (at a dose of 25 U/kg, the half-life is ~30 minutes), but as these mechanisms become saturated the half-life extends (at a dose of 400 U/kg, the half-life is ~150 minutes). At the higher dosages, clearance is predominantly through a slower renal process. Therefore, a therapeutic dosage is determined by monitoring the anti-coagulant effect by measuring either the activated partial thromboplastin time (aPTT) or an anti-factor Xa assay (See separate documents).

Monitoring and Therapeutic Ranges

Traditionally, the aPTT has been used to monitor the therapeutic effect of heparin. This test remains the most readily available and practical method to follow anticoagulation by heparin. However, the relationship between the aPTT and the concentration of heparin varies between labs reflective of differences in reagents, methodologies, sample size, and other factors. The current recommendations are for each lab to standardize the aPTT therapeutic range to an anti-factor Xa assay of 0.3 to 0.7 U/mL or a direct heparin measurement by protamine titration of 0.2 to 0.4 U/mL. The current standardized recommended therapeutic ranges of aPTT are reported with each test result and are regularly updated for each new reagent lot, or other changes in laboratory reagents or instrumentation.

The “Brill-Edwards” protocol is the most widely used technique to measure the heparin-responsiveness of aPTT assay in a clinical laboratory. In the Brill-Edwards protocol, 50-100 plasma samples from different individuals receiving unfractionated heparin are obtained. The heparin concentration of each sample is determined by the chromogenic anti-Factor Xa technique, and the corresponding aPTT of each sample is measured with the laboratories reagents and instruments.

Fig. 3. A typical heparin response curve. The solid line represents the regression line of aPTT values plotted against heparin levels determined in fresh plasma specimens from at least 60 patients on heparin therapy by the anti-factor Xa assay. In this case, the therapeutic aPTT range corresponding to heparin levels of 0.3 to 0.7 U/mL is approximately 75-125 seconds.
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Instrumentation. A heparin response curve is constructed by plotting the aPTT values against the corresponding heparin concentrations (Fig. 2). Regression analysis is used to determine the range of aPTT values corresponding to UFH levels of 0.3-0.7 IU/mL (“heparin therapeutic range”). Theoretically, heparin dosing to maintain aPTT values within the therapeutic range will prevent over- or underdosing.

A “baseline” aPTT should be obtained prior to the administration of heparin. Regular monitoring should begin with the administration of heparin and continue regularly thereafter. Due to the complicated pharmacodynamics of heparin, and the potential for changes in clearance, the aPTT should be monitored daily, even after a therapeutic dose is found. Monitoring becomes a more difficult problem in patients with an abnormal baseline aPTT at baseline, those who require higher than average doses of heparin, and those who are undergoing transition to oral anticoagulation.

Monitoring Patients with an Abnormal Baseline aPTT

Prolongation of the baseline aPTT is present in receiving concomitant oral anticoagulation and in those with lupus anticoagulants, deficiencies of the common and intrinsic coagulation pathways, or specific coagulation inhibitors. The laboratory target aPTT cannot be used for monitoring in these patients. Alternatives include the use of low molecular weight heparin, or monitoring by measurement of heparin plasma levels using the antifactor-Xa assay.

Monitoring Patients with Heparin Resistance

Patients who require higher than average doses of heparin (usually >35,000 U/24 hours) to maintain aPTT values within the therapeutic range are said to be “heparin resistant.” This problem is relatively common, and occurs in approximately 25% of patients with venous thromboembolic disease. The usual causes of heparin resistance include:

- Heparin-induced thrombocytopenia (HIT)
- Elevations of factor VIII, fibrinogen, and/or platelet factor 4
- Antithrombin (AT) deficiency

HIT is discussed below. Factor VIII and fibrinogen are acute-phase proteins that undergo significant elevations in acute illness and pregnancy. The aPTT is shortened in these patients, and the heparin concentration-aPTT relationship is altered. Platelet factor 4 binds to and inactivates heparin, so that a higher dose is required to maintain an appropriate aPTT value. A plasma AT level >60% is required for the anticoagulant effect of heparin. Hereditary AT deficiency is an autosomal dominant thrombotic disease that occurs in 1:2000 to 1:5000 of the general population, usually associated with plasma AT levels of approximately 50%. Heparin consumes AT and also releases the antithrombin compound PF-4, causing a 15% to 30% decrease in AT levels within 12 hours of heparin administration. Further decreases in AT may be caused by increased consumption or clearance, decreased clearance, dilution, estrogen-binding in patients with liver disease, disseminated intravascular coagulation (DIC), nephrotic syndrome, or drug-induced diseases. Clinical conditions associated with heparin resistance, as compiled by Bharadwaj et al. (2003) include:

- Amyloidosis
- Antithrombin deficiency
- Autotransfusion
- Cancer
- Coronary artery disease, coronary artery bypass
- DIC, chronic/low-grade
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- Estrogen states: oral contraceptives, pregnancy
- Factor VIII, increased levels (>150%)
- Heparin therapy, previous
- Hypereosinophilic syndrome
- Infective endocarditis
- Intraaortic balloon counterpulsation
- Medications: Aprotinin, nitroglycerine, propylene glycol
- Medications, previous treatment: Heparin, streptokinase
- Neonatal respiratory distress syndrome
- Plasmapheresis
- Platelet levels, increased
- Platelet-rich plasma harvesting
- Postsurgical states
- Shock, burns, fever, thrombophlebitis
- Streptokinase, previous treatment
- Thrombosis, ongoing clotting and utilization of heparin

**Heparin Risks and Side Effects**

As one would expect, anti-coagulation with heparin leads to a risk of bleeding. The risk is greatest for those patients receiving a higher dose and is increased in patients with a history of heavy alcohol consumption, who are taking aspirin, and those with renal insufficiency. Severe hemorrhage is relatively infrequent, though, with an incidence between 3 to 5%. If needed, heparin therapy can be reversed with protamine sulfate.

A potentially life-threatening side effect of heparin is heparin-induced thrombocytopenia (HIT). A mild form of HIT, type I, occurs in many patients on heparin and leads to a mild decrease in platelet counts after administration of heparin but stays above 100,000/µL and less than a 50% decrease in baseline counts. This thrombocytopenia is generally self-limited, not immune mediated, and does not lead to a significant risk of bleeding or thrombosis. Type II HIT on the other hand leads to a dramatic drop in platelet counts (>50% decrease or a decrease below 100,000/µL) that usually develops between 3 to 15 days after initiating therapy but may take a month or more to develop even after heparin has been discontinued or may arise within a day in a patient that has been exposed to heparin previously. The drop reflects a platelet destructive process driven by the presence of antibodies against heparin bound to the platelet surface protein PF4. These anti-heparin/PF4 antibodies can be found in many patients on heparin, but only a subset of patients will develop HIT. Therefore, the diagnosis of HIT should be made if there is a drop in platelets temporally related to the initiation of heparin and confirmed by the presence of anti-heparin/PF4 antibodies. A serotonin release assay is more specific for HIT but depends on the use of radioisotope labeled serotonin and is not available in most laboratories. Once type II HIT has been recognized, all forms of heparin should be discontinued including low molecular weight heparin and heparin flushes of in-dwelling catheters. If the patient needs continued anti-coagulation, the direct thrombin inhibitors can be used as alternative therapy. Heparin must be stopped in order to avoid the serious yet paradoxical development of thromboses. The antigen:antibody complexes on the surface of platelets can stimulate aggregation and the formation of arterial and venous thromboses that can be quite difficult to manage and life-threatening.
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Selected References


