EDUCATIONAL COMMENTARY: UNFRACTIONATED HEPARIN MONITORING: LIMITATIONS AND INTERFERENCE

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Learning Objectives

On completion of this exercise, the participant should be able to
- describe the optimum sample collection and preparation for heparin monitoring;
- describe the 3 main assay types used for monitoring heparin; and
- explain the causes of discordant partial thromboplastin time results during heparin monitoring.

Introduction

Unfractionated heparin (UFH) is an antithrombotic medication that is still widely used to prevent and treat venous and arterial thrombosis, particularly in the acute inpatient setting, owing to its rapid onset of action, rapid clearance, and ability to be neutralized with protamine. It has largely been replaced by low-molecular-weight heparin and direct oral anticoagulants for outpatient treatment of venous thrombosis. Dosages of heparin for different indications vary widely: 3.0 to 5.0 U/mL during cardiopulmonary bypass, 0.3 to 0.7 U/mL for treatment of thrombosis, and 0.2 to 0.4 U/mL for prevention of thrombosis in extracorporeal life support (ECLS) circuits.

The relationship between heparin dosage and heparin activity in blood is not simple. Heparin activity has been reported to be affected by age, sex, ethnicity, pregnancy, circadian rhythms, variations in the heparin clearance rate as a function of dosage, and binding of heparin to antithrombin and other cellular and plasma proteins, all leading to substantial differences in the heparin dosage required to achieve the same heparin activity. Monitoring heparin is not simple. Heparin is not a direct anticoagulant. It works by binding to antithrombin and accelerating antithrombin’s inhibition of activated coagulation factors including thrombin, factor Xa, and factor IXa. When antithrombin levels are low, heparin is less active. What is really being monitored is the concentration of heparin-antithrombin complex, which is affected by levels of both heparin and antithrombin in blood.

There are two critical assumptions when monitoring heparin: first, that the optimal level of the drug is known for each treatment use; and second, that the assay used to measure the heparin level is accurate and associated with improved outcome. As discussed below, these assumptions may not be true in all cases. Current UFH monitoring is based on functional assays designed to measure heparin activity. A
variety of heparin monitoring assays are available that use different methodologies (e.g. whole blood versus plasma, clot-based versus chromogenic, anti-IIa versus anti-Xa) with varying degrees of precision, accuracy, and standardization. No current UFH monitoring assay has a range that can accurately and precisely measure heparin activity from 0.1 to 10.0 U/mL, levels that can be seen across the clinical spectrum in situations from ECLS to cardiopulmonary bypass. Major assays used to monitor UFH and the limitations and interferences for each type of assay are discussed below.

Preanalytical Issues Related to Heparin Monitoring

Preanalytical errors are one of the most common sources of heparin monitoring problems. The optimum sample for heparin monitoring is peripheral blood drawn away from the site of heparin infusion and into a properly filled citrate tube. The tube is then centrifuged to prepare platelet-free plasma, with the plasma removed from the cells within 1 hour after centrifugation. Although a peripheral sample is optimal, in some patients only samples from catheters are readily available. When the sample is drawn through a catheter, there is always the possibility of dilution and contamination with the fluid in the catheter, with the worst-case scenario being that the same catheter is used to infuse heparin and to draw the sample. No single recommendation for catheter flushing is possible because the volume of blood needed to flush the catheter completely varies, depending on the catheter material, length, diameter, and fluid in the catheter. Catheter-drawn samples carry the risk of falsely low and falsely high heparin levels.

Citrate tubes, when properly filled, contain 1 part citrate and 9 parts whole blood. Underfilling citrate tubes can result in blood being diluted by the citrate and an inappropriate citrate level in the plasma sample, leading to falsely low heparin activity results, when using anti-Xa, assays and falsely prolonged clotting times when clot-based tests are used. If the sample is inadequately centrifuged or the plasma is left in contact with the cells for too long before removal, platelets can release proteins that neutralize part of the heparin in the sample, resulting in falsely low heparin activity results.

Anti-Xa Heparin Activity Assay

In anti-Xa heparin activity assays, plasma containing heparin is added to purified factor Xa. Heparin-antithrombin complex in the plasma binds to and inhibits factor Xa. The amount of residual factor Xa measured using a factor Xa–sensitive chromogenic substrate is inversely proportional to the heparin activity. Anti-Xa assays are sensitive to both the heparin and antithrombin levels in plasma (Figure 1). In the United States, more than 95% of laboratories use anti-Xa assays that do not include supplemental antithrombin. Anti-Xa assays that supplement with antithrombin can increase antithrombin and heparin-antithrombin complex levels in samples from patients with low in vivo antithrombin concentrations, resulting in an overestimation of heparin activity in the patient.
EDUCATIONAL COMMENTARY: UNFRACTIONATED HEPARIN MONITORING: LIMITATIONS AND INTERFERENCE (cont.)

Anti-Xa assays are not affected by changes in coagulation factor levels, contact system levels, or lupus inhibitors. They are more specific for heparin activity than clot-based assays such as the partial thromboplastin time (PTT) or activated clotting time (ACT), both measured in seconds. Compared with the PTT, when heparin is monitored with anti-Xa assays, it takes less time to reach therapeutic levels, the time in the therapeutic range is greater, there are fewer heparin dosage changes, and fewer transfusions are needed. Most therapeutic range recommendations are based on anti-Xa measurements. Anti-Xa heparin activity assays have become the standard of care for UFH monitoring and should be available on demand.

Limitations of anti-Xa assays include high levels of hemolysis or icterus, which can interfere with the chromogenic measurement of residual factor Xa activity, resulting in falsely low heparin activity results. If other anti-Xa inhibitors, such as low-molecular-weight heparin or direct oral anticoagulants, are present in plasma, they can interfere with the anti-Xa assay, producing falsely high heparin activity values. For samples that are above the linear range of the anti-Xa assay or where hemolysis/icterus is a problem, sample dilution has been suggested as a possible solution. However, dilution can result in
EDUCATIONAL COMMENTARY: UNFRACTIONATED HEPARIN MONITORING: LIMITATIONS AND INTERFERENCE (cont.)

heparin activity results above or below the level in the patient due to differences in antithrombin and other heparin-binding protein levels in the diluent versus patient plasma.

Partial Thromboplastin Time

In the PTT, partial thromboplastin (anionic phospholipids) and a contact system activator are added to plasma and incubated at 37°C to allow maximum activation of factor XII. These steps are followed by the addition of calcium and measurement of the clotting time. The PTT was originally developed for measuring factors VIII, IX, and XI. The PTT is sensitive to coagulation factor levels and inhibitors, contact system levels, multiple anticoagulants, and lupus inhibitors. The PTT assay works best when only one cause of prolongation is present (e.g. in heparin monitoring with normal coagulation factor levels and no lupus inhibitor). When only a single factor is changing, the PTT shows a predictable response, but when multiple causes of PTT prolongation are present at the same time, interpretation becomes complex (e.g. trying to monitor heparin with a lupus inhibitor present).

The PTT therapeutic range of 1.5 to 2.5 times normal was originally described for heparin monitoring in relatively uncomplicated patients with deep venous thrombosis. This range is not consistent as it varies with different instruments and reagents. The best approach for determining the UFH PTT therapeutic range is to correlate the PTT versus anti-Xa heparin activities between 0.3 and 0.7 U/mL (Figure 2). Using this type of correlation, the PTT ratio is typically 2.5 to 3.5 times the mean normal PTT rather than 1.5 to 2.5 as originally described. The optimal method is to collect samples from 20 to 30 patients receiving heparin, with less than 10% of samples from the same patient and less than 50% of samples with a prolonged prothrombin time (PT). Spiking plasma with heparin is not an acceptable method for determining the UFH PTT therapeutic range and it can result in more patients receiving inadequate heparin.

In hospitalized patients receiving heparin, the most common cause of discordance between the PTT and anti-Xa activity is a prolonged baseline PTT, often owing to coagulation factor or contact system deficiencies or lupus inhibitors. In hospitalized patients, 33% to 43% had a prolonged PT, indicating factor deficiency, and 13% to 25% of samples showed a prolonged PTT with normal PT, most often owing to lupus inhibitors. In one study, transient lupus inhibitors were seen in 53% of intensive care unit patients during admission. Most resolved spontaneously by the time of discharge and they were not associated with thrombosis or bleeding. In contrast to the common transient lupus inhibitors present in many hospitalized patients, antiphospholipid antibody syndrome is relatively rare and is characterized by the development of persistent, sometimes high–titer, lupus inhibitors that are associated with platelet activation and thrombosis (Table).
A shortened baseline PTT, often resulting from an acute-phase increase in factor VIII activity, is associated with discordant low PTT values, resulting in an apparent subtherapeutic PTT despite high doses of heparin given to the patient.33,34 The heparin responsiveness of different PTT reagents varies and the PTT is sensitive to multiple different factors in addition to UFH. Therefore, it is not surprising that PTT monitoring of UFH therapy has a limited ability to predict outcome in patients with thrombosis.2

Using the PTT to balance the risk of bleeding versus the risk of thrombosis is problematic.2,26,35 Without further testing, it is difficult to separate baseline prolongation of the PTT resulting from factor deficiency from prolongation caused by lupus inhibitor. In hospitalized patients, both may be present. When the baseline PTT is prolonged, the usual therapeutic range for the PTT may provide insufficient anticoagulation (low anti-Xa levels), particularly when there is a prothrombotic stimulus present, such as extracorporeal circulation or a circulatory assist device.25,35

One approach to mitigating the discordance between the PTT and anti-Xa is correcting the heparinized PTT for baseline PTT variations. The baseline PTT is determined after heparin is neutralized with polybrene, protamine, or heparinase.17,36 Baseline PTTs can vary during hospitalization; therefore, a single baseline PTT is inadequate. Separate baseline PTT measurements are required for each heparin monitoring sample. To adjust the heparinized PTT for baseline PTT variation, a baseline-corrected PTT was developed (Figure 2).17 This correction reduced the number of discordant samples by 64% and improved the correlation between PTT and anti-Xa activity. This correction is effective for samples with long or short baseline PTT values. Further work is needed to assess whether baseline-corrected PTTs show improved clinical effectiveness.

Hemolysis, icterus, and lipemia have variable effects on the PTT that are instrument and reagent dependent. In two studies, hemolysis resulted in shorter PTTs, potentially resulting in falsely low estimates of heparin effect.12,37 In some older optical instruments, hemolysis, icterus, and lipemia could interfere with clotting time detection. Mechanical clot detection and optical instruments that use wavelengths that are less affected by hemolysis or icterus show less interference.
Activated Clotting Time

The ACT is a point-of-care whole-blood clotting time that uses a contact system activator, typically celite or kaolin, which activates factor XII which, in turn, activates the coagulation system. The ACT has been used during open heart surgery to monitor UFH levels during cardiopulmonary bypass. The levels of heparin used during cardiopulmonary bypass are approximately ten times higher (3.0 to 5.0 U/mL) than the typical levels used for standard heparin therapy (0.3 to 0.7 U/mL), resulting in ACT values greater than 400 s after bypass heparinization. Activated clotting time has primarily been used as a threshold method to maintain adequate levels of heparin during bypass, not as a true measure of heparin activity.
During bypass, ACT is poorly correlated with heparin activity ($r^2$, 0.00-0.28). A modified ACT protamine titration method developed for estimating heparin activity shows concordance of 0.3 to 0.67 U/mL with anti-Xa assays. Although little data is available, use of ACT-based protamine titration assays would at least allow measurement of heparin activity, paving the way for studies of optimal heparin levels during bypass.

The ACT has also been used to monitor lower levels of heparin during angioplasty and ECLS. Recent studies have indicated that the ACT correlates poorly with anti-Xa heparin activity and heparin dosage during ECLS, and that ACT was not predictive of bleeding, need for circuit change, or survival. Under optimal conditions, the ACT is insensitive to UFH levels below 0.5 U/mL (Figure 3). The ACT is sensitive to changes in platelet count (the source of procoagulant phospholipid in the assay), coagulation factor levels, contact system levels, and lupus inhibitors. The ACT is highly variable, showing imprecision that is greater than the therapeutic range. Activated clotting times also show high variability between methods and are not interchangeable. Owing to inherent variability, low sensitivity to heparin activity, and sensitivity to multiple other factors, at lower heparin doses there is little or no correlation between the ACT value and heparin activity (Figure 3). Although useful for documenting adequate heparinization during high-dose UFH therapy associated with cardiopulmonary bypass, ACT is less useful for monitoring lower-dose heparin. There is little data to indicate the effect of hemolysis, icterus, or lipemia on the ACT. Because it is a whole-blood point-of-care test, these potential interferences are seldom, if ever, checked in the sample.

Viscoelastic testing has occasionally been used for heparin monitoring. It is essentially a whole-blood, contact-activated clotting time, similar to the ACT, and has all the ACTs limitations. In one study, there was no correlation between clotting times measured using viscoelastic testing and either bleeding or thrombosis, whereas both were predicted by anti-Xa levels.

Summary

Heparin activity primarily measures heparin-antithrombin complex levels, which are affected by age, sex, ethnicity, pregnancy, circadian rhythms, heparin clearance, and heparin-protein binding, making the relationship between heparin dosage and heparin level complex. To reduce preanalytical errors, blood should be drawn peripherally, away from heparin infusion sites, and the plasma removed from cells within 1 hour. Anti-Xa assays are the most specific because they are unaffected by coagulation or contact factor.
levels or lupus inhibitors but they are limited by interference from hemolysis and icterus. The PTT is nonspecific for heparin and it is sensitive to coagulation and contact factor levels, multiple anticoagulants, and lupus inhibitors. Unfractionated heparin PTT therapeutic ranges are best determined by correlating the PTT versus anti-Xa heparin activity in patient samples. The ACT is used to maintain adequate high-dose heparin levels during cardiopulmonary bypass but is poorly correlated with heparin activity. Because

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**Figure 3.** Activated clotting time (ACT) monitoring of low dose unfractionated heparin (UFH).6

A) ACT versus UFH spiked into normal whole blood.
B) Daily quality control results for ACT, horizontal lines indicated the heparin therapeutic range for the ACT.
C) ACT versus anti-Xa heparin activity in pediatric extracorporeal life support patients.
of its inherent variability, low sensitivity to heparin activity, and sensitivity to multiple other factors, the ACT shows little or no correlation with heparin levels at lower heparin dosages.

References


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