Introduction

Unfractionated heparin therapy is widely used as a treatment for thromboembolic (obstructive blood clots) disorders. Heparin therapy, or the amount of heparin administered, is determined based on the result of the activated partial thromboplastin time or APTT. There are many pre-analytical and analytical variables that may affect the APTT, giving dramatically different APTT measurements from one laboratory to another. This results in a variance in the amount of heparin administered to the patient. In addition to the pre-analytical and analytical variables, clinical therapeutic range guidelines such as the standard “1.5 – 2 times the mean of the reference range”, almost always underestimate the amount of heparin required for adequate anticoagulation.²

This paper promotes standardization of heparin dosage by discussing the factors affecting an APTT result as well as the establishment of a heparin response curve. Establishing a heparin response curve or heparin therapeutic range within a laboratory lessens the analytical variability by determining the APTT range which corresponds to the known heparin therapeutic range measured, using a heparin assay.

Pre-analytical Variables

In addition to analytical variables such as the instrument type and the APTT reagent in use, and the type of heparin used, there are numerous pre-analytical variables that significantly affect the APTT measurement. The pre-analytical variables that affect the APTT result, which in turn affect the heparin therapy include sodium citrate concentration in collection tubes, sample volume in tubes, time between specimen collection and APTT testing, and centrifugation. Laboratories can minimize the affect of pre-analytical variables on APTT measurement by following published guidelines regarding the type and concentration of anticoagulant, temperature of sample storage, the maximal length of time before specimen processing and centrifugation speed and time³,⁴.

Type and Concentration of Sodium Citrate:
- Standard use of type and concentration of sodium citrate is recommended. NCCLS recommends a concentration of 3.2% (105 mmol/L).³

Sample Volume:
- Vacutainer tubes should be properly filled, as under-filled tubes have been shown to decrease the APTT values in heparinized samples.¹

Transport and Storage:
- Transport and storage requirements include keeping refrigerated at 2 – 4°C (preferable) or at room temperature (18 – 24°C) with centrifugation within 1 hour of collection. Testing may occur up to 4 hours after collection according to NCCLS guidelines³,⁴. Some published data suggests that delay in testing of more than 2 hours after collection may significantly underestimate the in-vivo heparin effect. Therefore, centrifugation and testing of APTT samples as soon as possible after collection is advisable.
Centrifugation:
- Adequate centrifugation at 1500 g for at least 15 minutes at room temperature (to produce platelet free plasma; platelet <10 x 10⁹/L) is recommended. Many studies would also suggest a second centrifugation of plasma to thoroughly remove platelets from the sample as residual platelets in samples collected for APTT measurement may neutralize the heparin present by release of platelet factor 4 (PF4).

Establishing a Heparin Response Curve to Determine the Heparin Therapeutic Range

There are two methods for determination of a heparin therapeutic range using a particular heparin-reagent-instrument system: the ex-vivo heparin response curve and the in-vitro heparin response curve.

Ex-Vivo Heparin Response Curve

Although this method is the recommended or reference methodology, it may not be possible for laboratories with a low volume of coagulation testing, because of the large number of blood samples required from patients on therapeutic unfractionated heparin.

This type of method requires that:
- individual patient blood samples are acquired from patients who are fully anticoagulated with unfractionated heparin;
- individual patient blood samples have APTT measurements performed on fresh plasma aliquots within 4 hours of collection;
- individual patient blood samples have heparin assays performed (residual plasma is rendered platelet-free, frozen, and stored until heparin assays can be performed on a batch of samples).
- Heparin assays include:
  - Protamine Sulfate Neutralisation:
    Protamine sulfate neutralisation measures the concentration of heparin by determining the amount of Protamine sulfate required to inhibit all the heparin activity in a sample.
  - Anti-Factor Xa assays:
    Anti-Factor Xa assays measure the residual Anti-Factor Xa activity in plasma, after addition of excess reagent Anti-Factor Xa to the assay. The amount of Anti-Factor Xa activity remaining is inversely proportional to the amount of heparin activity in a sample. Most Canadian laboratories currently use a factor Xa assay for measurement of heparin concentration.⁵

Note: A known therapeutic range for heparin concentration has been established for each of these assay types:
- the heparin concentration and APTT measurements from each of the test samples are graphically compared;
- the APTT therapeutic range is determined by reading the applicable APTT range from the graph that corresponds to the therapeutic heparin concentration.

Figure 1:
Figure 1: Individual, heparinized patient plasma has APTT measurement (Y-axis) as well as heparin concentration (X-axis) determined. The APTT range that corresponds to the heparin therapeutic range (0.2 – 0.4 U/mL by protamine titration) is the established heparin therapeutic range (56 – 84 seconds in this example).

For an in-vitro therapeutic range calculation, the X-axis would correspond to the known (spiked) concentrations of heparin in a pooled plasma preparation.

**In-Vitro Heparin Response Curve**

This method is performed more easily and does not require accumulation of samples from patients who are being treated with unfractionated heparin.

- A pool of plasma samples is prepared from normal, healthy individuals.
- The pool is “spiked” with known concentrations of the particular heparin brand and lot number in use in the facility where the therapeutic range is being calculated.
- The APTTs of the “spiked” platelet-free, pooled plasma are determined.
- The APTT and heparin concentration are graphically compared.
- The therapeutic APTT range is the APTT range in seconds that correlates with the therapeutic heparin concentration.

While this method is much easier to perform, it fails to account for the heparin activity in the blood stream (bioavailability) of those patients who may have underlying inflammation and acute phase changes in plasma.

Compared to the ex-vivo method, the in-vitro method usually underestimates the APTT therapeutic range that corresponds to the therapeutic heparin concentrations. Laboratories and their laboratory physicians may choose to increase the assayed in-vitro therapeutic range to compensate for this.
Low Molecular Weight Heparin (LMWH)

LMWH is being used more and more in the prevention and therapy of thromboembolic disorders. This drug is much less susceptible to individual variation in bioavailability than unfractionated heparin, and for most patients is given on a weight-adjusted basis without ongoing laboratory monitoring. Therefore, APTT heparin therapeutic range calculations are NOT required for LMWH. If required, LMWH monitoring is done by means of an anti-Xa assay.

Summary

Attention to preanalytical variables which affect APTT testing, and use of heparin response curve allows the laboratory to provide clinical staff with an APTT therapeutic range that is tailored to the particular heparin-reagent-instrument in use within a given laboratory. Confirmatory testing and/or redetermining a heparin response curve is required with each change of reagent, reagent lot number, or instrumentation.

References