Tremendous improvements have been made in the outcomes of patients with acute venous thromboembolic events (VTE) since Murray et al. successfully administered heparin to postsurgery patients in 1937 [1]. Advances include accurate methods for diagnosis of deep vein thromboses (DVT) and pulmonary emboli (PE); effective regimens for initial and long-term anticoagulation; and wide acceptance of a system for standardization of thromboplastin sensitivities to the anticoagulant effects of warfarin (International Normalized Ratio; INR). In this issue of Clinical Chemistry, van den Besselaar et al. [2] address an unresolved aspect of anticoagulation therapy: standardization of activated partial thromboplastin time (APTT) reagents for monitoring heparin therapy.

Heparin is a glycosaminoglycan extracted from mast cells of porcine intestinal mucosa or bovine lung. It is composed of long chains of alternating d-glucuronic acid and N-acetyl-d-glucosamine sugar residues, which undergo a series of chemical modifications, primarily sulfation, leading to unique pentasaccharide sequences that serve as a binding site for antithrombin III (AT III) [3]. The anticoagulant effect of heparin is mediated through this interaction, which markedly accelerates the rate of AT III inhibition of thrombin (factor IIa) and factor Xa. Heparin polysaccharides are heterogeneous in length and anticoagulation activity and range in mass from 5000 to 30,000 kDa. Commercial preparations are calibrated in USP units and ranges were usually equivalent to plasma heparin activities between 0.2 and 0.4 USP unit/mL (based on protamine titration) and APTTs of 1.5 to 2.5 times baseline. Higher heparin concentrations produced minor additional reductions in fibrin accretion and longer bleeding times from jugular vein punctures.

Over the last two decades, clinical investigators have conducted a series of prospective, randomized clinical trials comparing different anticoagulation strategies for the management of patients with acute VTEs. Measured outcomes included recurrent thromboembolic events and bleeding. These studies have convincingly shown that:

1. Initial treatment with an oral anticoagulant alone leads to more recurrent VTEs than does combination therapy with heparin [10].
2. Recurrence rates are higher when subtherapeutic APTTs persist for >24–48 h after initiation of heparin [11].
3. Five days of therapeutic continuous intravenous heparin therapy is as effective as 10 days when warfarin is begun on day 1 rather than day 5 [12].
4. Nomograms for heparin dosing (intravenous bolus, then continuous infusion) increase the proportion of patients with APTTs above a minimal therapeutic threshold and improve outcome (lower recurrence rates) [13].
5. Weight-based dosing of SQ LMWH without laboratory monitoring is as efficacious as continuous infusion of unfractionated heparin with APTT therapeutic monitoring [14].

The efficacy rates of continuous heparin infusions were similar in these clinical trials (VTE recurrence ~6% [4], bleeding complications <5% [15]). APTT therapeutic ranges were usually equivalent to plasma heparin activities of 0.2–0.4 USP units/mL by protamine titration, and average initial rates of heparin infusion were ≥30,000 units/24 h [4].

However, reliance on a 1.5 to 2.5 times control PT target for
oral anticoagulation therapy. First, the APTT is a global test of coagulation; it is affected by many variables in addition to heparin, including coagulopathies, inhibitors, and increases of factor VIII and fibrinogen. Secondly, there is no agreement on what value should be used for the denominator of APTT ratios: mean or upper limit APTT of a reference range for normal, or a patient’s pretreatment APTT. Most importantly, commercial APTT reagent sensitivities to heparin vary widely [16, 17]. Factors involved are differences in the relative and absolute concentrations of polar phospholipids, as well as the use of different activators (kaolin, silica) in the APTT reagent. Finally, some investigators note that different instruments and clot detection methods contribute to the variability of APTT reagent sensitivity to heparin [18].

The most compelling demonstration of the potential risk for subtherapeutic dosing of heparin based on a 1.5–2.5-fold prolongation over mean normal control APTT was provided by Brill-Edwards et al. [19]. Five different commercial APTT reagents were calibrated against plasmas with ex vivo heparin activities of 0.2–0.4 USP units/mL by protamine titration to determine therapeutic ranges for APTT ratios. The ranges varied from 1.8–2.5 to 2.6–4.2 USP units/mL. Lot-to-lot variation of heparin sensitivity was also reported for one brand of APTT reagent.

Several approaches have been proposed to improve the therapeutic monitoring of heparin. Laboratories can calibrate the therapeutic range for APTT ratio for each reagent–instrument system to be equivalent to a heparin concentration of 0.2–0.4 USP units/mL by protamine titration or to 0.3–0.7 units/mL anti-Factor Xa activity [20]. There are obstacles to this approach, in addition to the required time, expense, and expertise. Addition of heparin in vitro to pooled normal plasma is not recommended because this produces unreliable and typically low APTT therapeutic ranges, compared with calibrations performed with plasma samples obtained from patients receiving heparin. Finally, laboratory directors must convince clinicians to change their practice of dosing heparin on the basis of a 1.5–2.5 APTT ratio in favor of a new, less familiar, range.

Newer automated coagulation analyzers can measure heparin activity directly (as anti-factor Xa activity). Their turnaround times are acceptable for patient management needs, but these assays are currently too expensive to consider as a replacement for the APTT. Finally, a reference APTT reagent and method could be established along with a formula to compare the heparin sensitivity of commercial reagents against the standardized values. This would permit the reporting of normalized APTT ratios.

Van den Besselaar et al. previously demonstrated that liposomes containing synthetic dioleoylphosphatidylserine, dioleoylphosphatidylcholine, and dioleoylphosphatylethanolamine could be produced that behaved like a commercial biological APTT reagent [21]. Now, they report their analysis of another synthetic APTT reagent, 91/558, that has been lyophilized and stored at −70 °C for 4 years [2]. Modifications include substitution of silica for kaolin, a relative decrease in the proportion of phosphatidylserine used, and an increase in total phospholipid concentration (from 40 to 67 nmol/L). Comparing this material with two biological APTT reagents for sensitivity to in vitro and ex vivo heparinized plasmas, the authors observed linear relationships with high correlation coefficients between 91/558 and Automated APTT (Organon Teknika) but not with 91/558 and “Manchester reagent.” They speculate that this may be due to a different activator, kaolin, in the Manchester reagent. However, it would be premature to assume that a synthetic APTT reagent can be used to calibrate the heparin sensitivity of other APTT reagents as long as the same activator is used. Other investigators have been disappointed when they attempted to calibrate heparin sensitivity of APTT reagents against an arbitrary commercial reference reagent [22, 23].

A synthetic phospholipid such as 91/558 could potentially be a reference APTT reagent. But is an APTT-INR system an urgent need? I do not believe it is. Instead, manufacturers should first work with clinical laboratories to develop simple, cost-effective strategies for determining reagent/instrument-specific APTT therapeutic ranges calibrated against ex vivo plasma heparin activity. The recent introduction of commercial synthetic APTT reagents with minimal lot-to-lot variation in heparin sensitivity is an important advance. Finally, LMWH will probably soon supplant unfractionated heparin for treatment of acute VTEs, pending FDA approval—a development that is desirable for both clinical [14] and economic reasons [24]. When that happens, standardization of APTT monitoring of heparin therapy may become an historical rather than urgent problem.

Nevertheless, cogent reasons support development of a reference APTT reagent. The APTT is a valuable screening test for detecting deficiencies of factor VIII and IX in patients with positive bleeding histories or for detecting nonspecific inhibitors (lupus anticoagulants) in patients with clinical findings suggestive of anti–phospholipid antibody syndrome. However, the lack of commercial APTT reagent standardization for these functions is frustrating. A reference synthetic phospholipid plus activator APTT reagent designed to be sensitive for the detection of patients with mild hemophilia, von Willebrand disease, and lupus anticoagulants would be an important advance. Manufacturers could then develop second-generation synthetic reagents nearly identical to the reference preparation. Sensitivity to the anticoagulant effect of unfractionated heparin could be a specification of a synthetic APTT reagent standard, as well. Although LMWH may eventually replace heparin or be replaced itself by a synthetic analog, such evolutions in clinical practice take longer than one might predict.

Van den Besselaar and colleagues have established the foundation for an important initiative that, when completed, will improve the quality of clinical laboratory hemostasis services.
References


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