Has the Time Arrived to Replace the Quick Prothrombin Time Test for Monitoring Oral Anticoagulant Therapy?

The Quick (1) and Owren (2) prothrombin time (PT) tests remain the basis for monitoring anticoagulant therapy worldwide. The use of these tests is remarkable because the Quick PT preceded the discovery of the anticoagulant effect of dicoumarol in spoiled sweet clover and both tests were in use long before there was any understanding of the mechanism of the action of oral anticoagulants or which of the plasma factors were affected by them. Both PT tests are conceptually based on the four-factor theory of coagulation proposed by Morawitz in 1905 (3), and the two tests are similar. The reactions of the Quick PT test depend entirely on coagulation proteins present in the patient plasma sample; the Owren PT test adds components of bovine plasma to compensate for variability related to the coagulation proteins fibrinogen and factor V. Efforts to standardize the Quick PT have provided a common means for expressing patient PT results, the International Normalized Ratio (INR), and have provided a method for quantitative comparison of thromboplastin reagents (4). These efforts have improved interlaboratory comparability of mean values and standard deviations for groups of patient samples.

In this issue of Clinical Chemistry, Horsti et al. (5) report PT results from testing the same samples, from patients receiving oral anticoagulants, on the same instruments but with different thromboplastins. They demonstrate that intrapatient comparability has not been achieved by use of the current standardization procedures (4). Their systematic comparison of seven different thromboplastins provides compelling evidence that neither the Quick nor the Owren PT test provides suitably comparable data for monitoring individual patients receiving oral anticoagulants if more than one thromboplastin reagent is used. Direct comparisons of the INR results from an individual patient’s samples measured with use of different thromboplastins indicate that the INR values can vary dramatically, certainly far more than is desirable for maintaining the balance between the risk of hemorrhage and the risk of thrombosis for the patient. An even more dramatic indication of this laboratory testing problem is seen from the slopes of Bland–Altman plots, which can be positive or negative when the data from pairs of thromboplastins are compared. The inescapable conclusion from the Bland–Altman plots is that the current procedure (4) for thromboplastin standardization is not achieving the desired result for patient monitoring, although it can make interlaboratory comparisons appear better. The importance of improving the results from PT testing is obvious when it is recognized that 800 million PT tests are performed each year throughout the world. In addition, somewhat frightening is the fact that not only the PT but also the other commonly performed clotting test, the “(activated) partial thromboplastin time (PTT or aPTT),” is an in vitro procedure that is frequently interpreted as if the difference between a glass or plastic tube and the vascular endothelium with its many receptors can be ignored.

Since the inception of the PT test, at least 20 new components of the coagulation process have been discovered (6,7), and an entirely new pathway has been described that opposes the reactions that promote clotting (8). The present-day model for explaining the prolongation of the PT by oral anticoagulants is based on the structural and functional properties of the vitamin K-dependent proteins and the posttranslational modifications inhibited by warfarin that were discovered during the 1970s. These discoveries included identification of γ-carboxyglutamic acid (Gla) and its requirement for Ca²⁺ binding, and the folding of the domain that contains the Gla residues. This protein folding enables the vitamin K-dependent proteins to bind to the membranes of damaged cells, platelets, and, in vitro, to the phospholipid components of the thromboplastin. A contemporary scheme, which also illustrates the interactions among the coagulation system components, is shown in Fig. 1. The vitamin K-dependent factors involved in the PT test (factors VII, X, and II as well as factor V) are illustrated in color.

Standardization of PT tests presents a challenge in addition to that inherent in the complexity of the reactions involved in clot formation in those tests. Warfarin acts indirectly by inhibiting the vitamin K epoxide reductase (7) in the liver and thus blocks regeneration of vitamin K; regeneration of the vitamin is required for converting Glu residues in vitamin K-dependent proteins to Gla residues. As a consequence, proteins that are missing the extra COO⁻ group of the Gla residues are secreted into the circulating blood (7). Although not established experimentally, these proteins might, in theory, act as inhibitors of some coagulation interactions. Of greater possible importance is the fact that there are Gla-containing proteins in the anticoagulant pathway and that this pathway is therefore also functionally impaired during anticoagulant therapy (7). The effect of this pathway is the inactivation of factor Va by activated protein C, a key reaction in shutting down thrombin formation and thus clot formation. The importance of this reaction is most evident from the added risk of thrombosis in individuals with the factor V Leiden mutation. Additional evidence comes from the assay for factor VII (9), which is essentially a PT assay using factor VII-deficient plasma. This assay is sensitive to the concentration of protein C, which in turn regulates the concentration of factor Va, which is rate limiting for the conversion of prothrombin to thrombin (IIa) by Xa.

Thus, the clotting time of the plasma from anticoagulated patients, recorded in a PT assay, results from two opposing reactions: the reduction in the activity of the
procoagulant factors (VII, X, II, and IX) balanced by the reduction of the anticoagulant factors (proteins C and S). Other modifiers of the rate of thrombin generation are the inhibitors: tissue factor pathway inhibitor, antithrombin, and other proteinase inhibitors. These are components for which specific effects might be linked to different thromboplastins, but such influences have not been extensively characterized.

Yet another complication to the PT assay arises from the use of different species of tissue factor (TF). TF may be derived from extracts of rabbit, bovine, or human brain or human placenta, or it may be prepared from a recombinant human protein and synthetic phospholipids. Early work (10) showed that the reactivity of TF with human factor VII varied depending on its animal source. This complication is further illustrated by a subsequent finding (11) that there is a variant (R304Q) of factor VII that fails to react with rabbit thromboplastin, whereas the reaction with recombinant human TF is essentially normal. This is but one more example of the subtlety of intermolecular recognitions that exist in the coagulation process. Clearly then, from the theoretical standpoint, the Quick PT, as currently performed with a variety of thromboplastins, is clearly a case of “one size does not fit all”.

It has been suggested (12–14) that to minimize the effect of some of these theoretical problems, the Owren PT assay, calibrated with dilutions of pooled plasma, provides a simple and reliable alternative procedure to the Quick PT assay. Comparability among laboratories is better in the Owren PT test, almost certainly the result of the use of a reagent that contains both factor V and fibrinogen from bovine plasma (13). However, the extent to which this version of the PT test with its added bovine proteins solves problems without creating new ones has not been much studied. The Owren PT is used almost exclusively in the Nordic and Benelux countries and Japan. Results from several years of external quality assessment schemes in these countries should provide a
large database for a retrospective evaluation of the limitations and comparability of results obtained with the Owren PT test. A procedure for expressing Owren PT results as INR values has also been published (12).

If more extensive studies that include consideration of what is currently known about coagulation using an "Owren-like" method confirm the results already published (12–14), then adoption of this method could be merited. For the clinicians who have adopted the INR as the indicator of appropriate anticoagulation, however, expressing PT results as INR is important, and relearning is generally an invitation for error, particularly during the early stages of change. However, such Owren-like methods should not, in our opinion, be used without study of possible improvements in comparability that might be obtained by comprehensively investigating the components of the reagents. The phospholipid composition is clearly an important variable (15), as are the factor V and fibrinogen (better from human plasma?) and the animal source of TF. Moreover, any consideration of a replacement for the Quick PT should include a thorough evaluation of the influence of concentrations of other plasma proteins on the results of the test.

Given our knowledge of the coagulation system and its components today, it is imperative to ask whether there might not be better measurement procedures than the PT tests for monitoring anticoagulant therapy. Although it remains to be established, one alternative is the “endogenous thrombin potential” test (16). This test has potential advantages, although it is more complex than the PT tests and requires a longer time before a result is available. Another is a proposal for an alternative algorithm for standardizing thromboplastins (17).

It may be fitting to conclude this editorial by quoting William Kingdom Clifford (1845–1879) in his essay “The Ethics of Belief”. Clifford states: “It is wrong in all cases to believe on insufficient evidence; and where it is presumptive to doubt and to investigate, there it is worse than to believe.” Horsti et al. (5) have provided the evidence to compel investigation. These authors must be congratulated for carrying out not only such a detailed and clear analysis of the performance of the various commercial thromboplastins but also emphasizing that it is time for a reappraisal of the PT assay.

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