A Critical Evaluation of the Prothrombin Time for Monitoring Oral Anticoagulant Therapy

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Key Words

International Normalized Ratio · International Standardization Index · Oral anticoagulant therapy · Quick prothrombin time · Thromboplastins · Vitamin K antagonists

Abstract

The Quick prothrombin time is the most common clotting test performed, principally for monitoring oral anticoagulant therapy. The International Normalized Ratio (INR) for comparing patient results from prothrombin time measurements and the International Standardized Index (ISI) for achieving greater consistency of results using different thromboplastins have made it possible to compare the results of vitamin K antagonist drug therapy that was impossible before the introduction of the INR and ISI. However, INR values obtained from the same patient plasma sample using different thromboplastins are significantly different. This is so even when the thromboplastins have nearly the same ISI values. We suggest that investigation of patient-specific differences can provide a means by which the INR discrepancies can be identified and understood and thus lead to better methods for monitoring oral anticoagulant therapy.

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Contemporary Monitoring of Oral Anticoagulant Therapy

Oral anticoagulant therapy is monitored almost universally using the Quick prothrombin time (PT) [1], or one of the variants such as the Owren prothrombin assay [2, 3]. Monitoring is necessary to balance the risk of thrombosis against the risk of excessive bleeding in the patients receiving vitamin K antagonists [4]. The currently performed Quick PT is virtually the same as the original assay [1]. Although our knowledge of the pathway by which thromboplastin-initiated coagulation has expanded from an original four components to more than twice that number, our thinking about the sources of variability in PT measurements seen with individual patient samples has not similarly advanced.

The introduction of the International Normalized Ratio (INR) [5–7] provided a substantial improvement to monitoring oral anticoagulant therapy, without which a comparison of results from different laboratories was impossible. The introduction of the International Standardization Index (ISI) enabled the comparison of thromboplastins from different sources. When used with the appropriate formulae [8] these two procedures enabled data from groups of patients in different laboratories and countries to be compared more readily.

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Fig. 1. a INR values (0.95–1.30) from stable anticoagulated patient's plasma samples measured using four different thromboplastins. ■ = Average INR values; --- = the highest and the lowest values obtained for the patient's plasma samples. The numbers shown at the peaks or troughs for some patient INRs indicate which thromboplastins produced the high or low INR values. The ISI values for the thromboplastins varied from 0.707 to 0.863; thus all are 'high sensitivity' thromboplastin preparations. Data for a fifth thromboplastin with an ISI of 1.139 are not shown. **b** INR values (2.4–4.5) from stable anticoagulated patient's plasma samples measured using four different thromboplastins. ■ = Average INR values; --- = the highest and the lowest values obtained for the patient's plasma samples. The numbers shown at the peaks or troughs for some patient INRs indicate which thromboplastins produced the high or low INR values. The ISI values for the thromboplastins varied from 0.707 to 0.863; thus all are 'high sensitivity' thromboplastin preparations. Data for a fifth thromboplastin with an ISI of 1.139 are not shown.

Inconsistency in INR Values for Single Patient Samples Measured with Different Thromboplastins

Although the INR and ISI have provided methods by which large groups of patients could be compared, however, when individual patient samples are assayed using

different thromboplastins, the limitations of this method are evident [9–11]. The most evident limitation is reflected in the fact that the INR values for a single patient sample are significantly different when assayed using different, albeit well-'standardized' thromboplastins. This is illustrated in figure 1a and b. Figure 1a shows that INR values for individual patients (subjects) in the 'normal'

Fig. 2. Relation between INR and ETP. ○ = Individual patients; ● = pooled plasmas of 20 patients at (near) identical INR; line = best-fitting hyperbola. If the ETP is determined in plasmas of anticoagulated patients, the correlation between ETP and INR is significant ($p < 0.005$) with a correlation coefficient of -0.68 . The therapeutic window of OAC as measured in the Netherlands [1] is INR 2–4, which corresponds to an ETP of 20–40%.

range using different thromboplastins are clearly not the same. Most notable, for some patients the INR values are very consistent with all thromboplastins (except for one lower-sensitivity thromboplastin); in other patients the INR values are clearly different. A closer look shows that for an individual patient, there is little or no relationship between higher or lower INR values and a particular thromboplastin. INR values obtained with thromboplastin A (ISI: 0.831) appear to be higher in more patients than any other single thromboplastin (ISI: 0.707–0.863), but there are patients in which the INR determined with thromboplastin A are lower. Similar patterns are seen in figure 1b for patient samples in which the INR is in the therapeutic range for oral anticoagulation. This variability thus does not appear to be related to the ISI value for any particular thromboplastin, but appears to be more likely to reflect differences in components of an individual patient sample.

Although insufficient replicate measurements are available to permit an appropriate statistical estimate to be made, we do not believe that mere experimental variability of clotting times can account for the data of figure 1a and b. Similar data can be found elsewhere, e.g. in manufacturer's comparisons of competitor thromboplastin preparations that can be found on the Internet. An explanation that cannot be ignored is that the ISI procedure for 'standardizing' thromboplastins, in spite of its success, does not succeed completely in normalizing the differences on the level of the individual patient sample and

thus alternative methods for monitoring vitamin K antagonist therapy must be investigated. An examination of any graph that is used to determine the ISI value for a particular thromboplastin, in spite of the logarithmic transformation of the data, shows substantial scatter among the points around the line from which the ISI is calculated. This observation similarly argues that the source of the variability is to be found in the samples from the patients. Therefore, the evaluation of these individual samples may be the most straightforward way to identify the factors responsible for this variability that is otherwise attributed to differences in the thromboplastins employed.

The measurement of the endogenous thrombin potential (ETP), described elsewhere in this volume, shows a reasonably good correlation with the INR (be it not a linear one) when pooled plasma of patients with approximately the same degree of anticoagulation are compared, but with individual samples the correlation is much weaker (fig. 2). The ETP values are however independent of the thromboplastin used and also independent of the thromboplastin concentration (if $>1\%$ of the concentration used in PT measurements [Hemker, pers. commun.]). However, in figure 2 because the INR is used as the parameter against which the ETP is plotted, variability is evident in this graph as well. Only an independent evaluation of the relationship between the ETP and the 'state of anticoagulation' will enable the ETP to be shown to be 'free' of the variability that is characteristic of INR values.

Fig. 4. Extent of anticoagulation measured for a single patient over a 2.5-month period using three different assay methods that are sensitive to the extent of γ -carboxylation of the Gla domain residues in the vitamin-K-dependent proteins.

molecules [29]. Thromboplastins prepared from recombinant tissue factor and synthetic phospholipids are almost entirely made of single bilayer lipid vesicles with maximum exposure of tissue factor, however, the thromboplastins prepared from brain and placenta are unlikely to be so.

Molecular Differences between Vitamin-K-Dependent Proteins in Normal Subjects and in Patients Receiving Vitamin K Antagonist Drugs

The vitamin-K-dependent proteins in plasma of individuals who are not being given oral anticoagulants are fully functional molecules that possess 10–12 Gla residues, i.e. glutamic acid residues (Glu) with an additional carboxyl group at the γ carbon [30–33]. Gla residues are formed posttranslationally in the liver in a vitamin-K-dependent process that is inhibited by oral anticoagulants [30]. Incompletely carboxylated proteins do not bind calcium in the same way as the normal molecules [34, 35] and therefore do not undergo the conformation change that is required for binding to the phospholipid surface [36–38]. More detailed descriptions of the action of the vitamin K antagonists and their effects on the structures and functionality of the proteins are found in the literature [30, 39–44].

The prothrombin time is a general measure of the rates of activation of all of the components that participate in

the reactions, beginning with tissue factor binding of FVII/FVIIa and ending with fibrin clot formation (fig. 3). Rates of activation of the proteins in this sequence depend on their binding to the phospholipid surface. The affinities of the under-carboxylated molecules are negligible compared to the fully carboxylated species [45]. The consequence of this is the slowing of the processes and the lengthening of the clotting time, although the thrombin that is formed is normal.

Generation of thrombin requires the conversion of three proteinase precursors into their proteolytically active forms: FVII to FVIIa, FX to FXa and prothrombin to thrombin (fig. 4). The most rapid activation of these precursors occurs in their respective complexes with their cofactor proteins: tissue factor for FVII to FVIIa and FX to FXa and FVa for prothrombin to thrombin. Although tissue factor is fully functional upon exposure, FV must be converted to its reactive form before it can participate [46]. The mechanism by which FV is converted to its reactive form is achieved by thrombin, possibly also by Xa. Both these proteins are vitamin K dependent and thus the rate of activation of FV is also influenced by oral anticoagulant therapy, because both the concentrations and rates of formation of these FV activators are decreased. Consequently, vitamin K antagonist drugs both directly and indirectly affect thrombin generation. Apart from the availability of vitamin K, various other processes govern the normal synthesis of individual vitamin-K-dependent proteins [47].

Fig. 5. Influence of anti-vitamin K treatment on the thrombomodulin sensitivity of the ETP thrombogram. In each set of two thrombin generation curves the lower one is obtained in the presence of 10 nM soluble recombinant thrombomodulin [from Hemmer et al., this issue, pp. 4–15].

The efficacy of the vitamin K antagonists is dependent on dietary conditions and the intestinal flora. Other drugs interfere with vitamin K antagonists, such as antibiotics that affect the intestinal flora and drugs that compete for the cytochrome P₄₅₀ system in the liver [48], as well as asparaginase [49, 50]. An example of such external influences is shown in figure 4. Over a period of more than 2 months, a patient on stable warfarin therapy was monitored at 5- to 7-day intervals by a PT measurement (Thrombotest, expressed as a percentage of the normal activity) and one-stage prothrombin assays in a medium with normal amounts of FX, FV and initiated by either Russell's viper venom or tiger snake venom [51]. Large changes in the apparent concentrations are evident with all assays but they do not vary in the same manner. The cause(s) for the variation are unknown but they must be due to phenomena not directly related to the intake of the vitamin K antagonist drug.

Effects on PT That Are Indirectly Related to Vitamin-K-Dependent Proteins

PT marks the beginning of explosive thrombin generation. Therefore, it is logical that it is insensitive to the decay phase of thrombin. Indeed PT is generally insensitive to antithrombin and heparin [52]. Some thromboplastins are formulated with heparin-neutralizing agents to ensure insensitivity to heparin. The greater sensitivity of clotting time measurements obtained with diluted

thromboplastin, and thus exhibiting longer clotting times and a sensitivity to 'lupus anticoagulants', suggest that the short clotting times characteristic of conventional PT measurements have effectively masked this source of variability.

When the PT assay was conceived, many of the currently known clotting factors were unknown. Perhaps most importantly, tissue factor pathway inhibitor (TFPI) [53–55], thrombomodulin and proteins C and S, which are vitamin K dependent, had not been discovered. These components, along with antithrombin and other inhibitors oppose the formation of thrombin [56, 57]. TFPI is a potent inhibitor of FVIIa and FXa when they are associated with tissue factor [54, 58, 59], thus its role as a regulator of the reactions involved in the PT assay merits further investigation. Thrombomodulin is assumed to have little influence on the INR [60], but the experimental basis for this assumption is limited.

The rationale for *in vitro* measurement of PT is that it is assumed suitable for assessing the hemostatic state of the patient *in vivo*. The discovery of the many important regulatory reactions that are mentioned above, and to which the PT appears to be insensitive, calls this assumed relationship between *in vivo* and *in vitro* into question. Especially because the influence of protein C activation on the hemostatic mechanism *in vivo* is important, as can be judged for example from the prothrombotic effect of the FV Leiden mutation, the assumption must be qualified, at least, or perhaps even abandoned.

It is interesting to note that the effect of thrombomodulin on the clotting system can be readily measured via thrombin generation (fig. 5). Although the effect of oral anticoagulants on protein C is considered to be less than on the other vitamin-K-dependent proteins [61], the presence of the des Gla forms of protein C apparently result in 'thrombomodulin resistance' as well as the reduction of thrombin generation.

One additional consideration deserves mention, namely the endpoint of PT, as is true for all clotting time measurements, is the gelation of fibrin to form the clot. Thus in monitoring anticoagulant therapy by PT, the influence of the fibrinogen concentration should not be ignored [62] also because thrombin inactivation is reduced through thrombin binding to fibrin [63], additional fibrin-related interactions also likely to be involved in patients with elevated fibrinogen concentrations.

Conclusions and Recommendations

It is important to recall that all assays that use clotting times as endpoints of a series of reactions provide only composite measures of elapsed time and do not provide clear information about individual reactions. In the interval between initiation of clotting and the formation of the fibrin gel, opposing reactions, e.g. activation of proteinase precursors and FV, are countered by inactivation of the proteinases by antithrombin and TFPI and inactivation of activated FV by activated protein C [64, 65]. Thus, like travel from one city to another in a fixed period of time, several paths are possible, several different delays but all with a fixed result that contains no information about the path or the cause of delays. This limitation is frequently unrecognized or unacknowledged in PT measurements with the consequence that apparently satisfactory INR values may hide information that, if it were available, might warn clinicians of impending bleeding or thrombotic risks. It is also worth recalling that all interpretations are based on the components known at the time. The discovery of protein C, thrombomodulin, TFPI and the possible involvement of protein Z [66–68] in some of the reactions of coagulation must remind us that interpretations are likely to be changed as new components and reactions are recognized.

In large groups of patients (~3,000 patient years), there is an excellent statistical correlation between the INR and thrombosis on the one hand and bleeding on the other [60]. The value of the INR and the ISI enabling comparison of different laboratory results cannot be un-

dervalued. However, a correlation does not necessarily imply a causal relationship, and an appropriate therapeutic intervention is more likely when the cause that provokes a need for a therapeutic intervention is correctly identified. It is clear that the PT in the individual patient shows so much thromboplastin-dependent variation that one might be led to think that each thromboplastin measures different quantitative contributions to the tissue-factor-initiated clotting process. Such a situation is clearly less than ideal for making clinical decisions.

Similarly, all thromboplastins, at the concentrations they are used in the PT assay, appear to be insensitive to most of the regulatory reactions of the hemostatic process. These regulatory reactions and components (antithrombin, the protein C system) certainly influence the occurrence of thrombosis and bleeding in patients and thus test(s) that are sensitive to them are needed for screening patients for risk of hemorrhage or thrombosis.

Discovery of new clotting factors, particularly those present in trace amounts in plasma and the reactions of the hemostatic system in which they are involved, has been possible because of the observations in the clinic on patients whose hemorrhagic predisposition could not be explained by the clotting factors known at the time. If only because of the historical value, it is recommended here that the differences in results from individual patients with different thromboplastin reagents be the focus for identifying the underlying cause(s) and thus developing better tests for monitoring vitamin K antagonist drug therapy in individual patients.

The essential question of what test, if any, accurately reflects the bleeding or thrombotic tendency of a patient remains at this moment unanswered. Thrombin generation is a very promising candidate because it has been shown to be sensitive to more phases of the blood clotting process than our existing tests. It is to be hoped that the ETP will provide more valuable information on the hemostatic status of the patient than the currently available tests.

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