Considerations on an international standard for the one-stage prothrombin time in the control of anticoagulant therapy

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10. Considerations on an International Standard for the One-Stage Prothrombin Time in the Control of Anticoagulant Therapy

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On the basis of the work of one of us (H. c. H.) on coagulation factor kinetics and our experience at the Thrombosis Service, the following remarks are offered as a contribution to the Subcommittee's considerations.

Let me begin by discussing the work done by Dr. Biggs and Dr. Denson. As a primary basis for the standardization of the one-stage prothrombin time they took the obvious rectilinearity of the curve of correlation between the clotting time (or clotting ratio) and the reciprocal of concentration (i.e., the dilution) of normal plasma (1, 2). This rectilinear correlation has been confirmed statistically by our results (3) and holds not only for the one-stage prothrombin time, but for all the so-called extrinsic test systems, irrespective of whether a single coagulation factor or the factor II, VII, and X complex is tested (3, 7, 9). For Thrombotest (which measures the complex II, VII, and X), for instance, the curved correlation line given by the manufacturer on double logarithmic paper is perfectly rectilinear when presented as the aforementioned correlation (Fig. 1). In this test, factor X appears to be rate-limiting as long as the three factors are lowered to a similar degree (3).

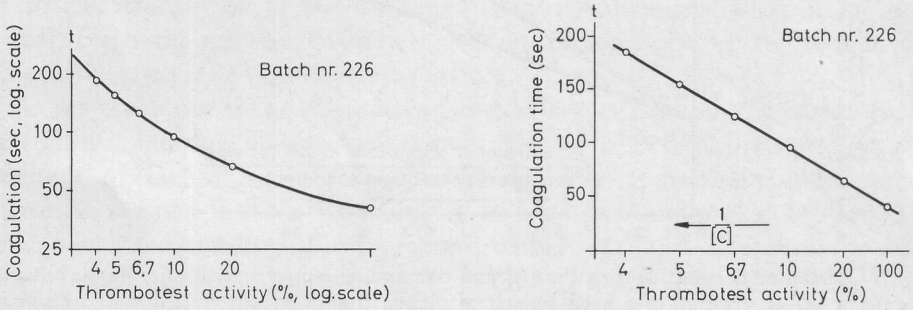

Fig. 1. Correlation between clotting time (t) and concentration of normal plasma (c) as obtained with the original Thrombotest. — The curve at the left gives the plot of log t against log c, that at the right the correlation of t against the inverse of c.

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The second basic concept taken for the standardization put forward by Biggs and Denson is the assumption that dilution of coagulation factors in whole plasma from patients treated with vitamin K antagonists will also be rectilinear in the same kind of graph. This assumption, according to the authors, is supported by the observation that in patients treated with coumarin congeners the correlation between the clotting time ratios found with different methods is rectilinear when plotted against each other. We do not think that this argument is valid. A rectilinear correlation curve between clotting times found with two different thromboplastin reagents in coumarin plasmas containing varying amounts of clotting factors tells us nothing except that the underlying time/concentration curve displays the same shape. It gives us no information at all about the shape itself, particularly with respect to rectilinearity.

Nevertheless, we are of the opinion that Biggs' and Denson's assumption is valid. Not only have we confirmed the rectilinear correlation between clotting time ratios demonstrated by these authors, but we also have two sets of data that provide substantial evidence for the correctness of their assumption: first, dilution of normal plasma in the presence of plasma from a coumarin-treated patient results in a rectilinear correlation between the coagulation time ratio and the dilution (Fig. 2); second, and more important, the correlation between Thrombotest coagulation times of blood of patients treated by our

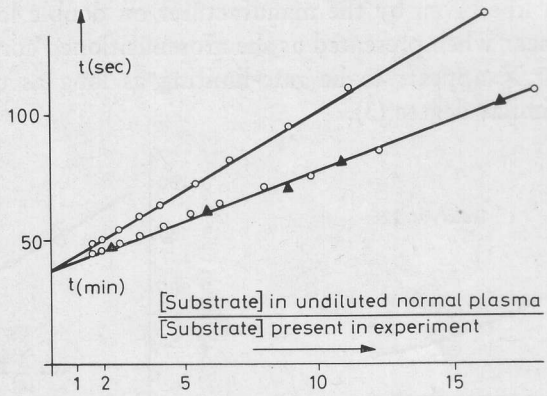

Fig. 2. Correlation between clotting time (t) and coagulation factor concentration (c) as obtained in absence and in presence of a fixed amount of plasma from a patient treated with a coumarin congener (so-called coumarin plasma). — The concentration range of the factors II, VII, IX, and X was prepared by mixing normal plasma with $Al(OH)_3$-adsorbed plasma in varying proportions. To each of these mixtures a fixed amount of $Al(OH)_3$-adsorbed normal plasma (o — lower curve), coumarin plasma (o — upper curve) or $Al(OH)_3$-adsorbed coumarin plasma (▲ — lower curve) was added. The ratio which is the independent variable of the x-axis was calculated from the known concentration of the factors II, VII, IX, and X in normal plasma (100%), $Al(OH)_3$-adsorbed normal or coumarin plasma (0%) and the coumarin plasma (5%).

thrombosis service on a long-term basis and the inverse of the activity of the coagulation factors present in the blood of these coumarin-treated patients (as determined in plasma diluted 1/40 and 1/80) is probably rectilinear (Fig. 3).

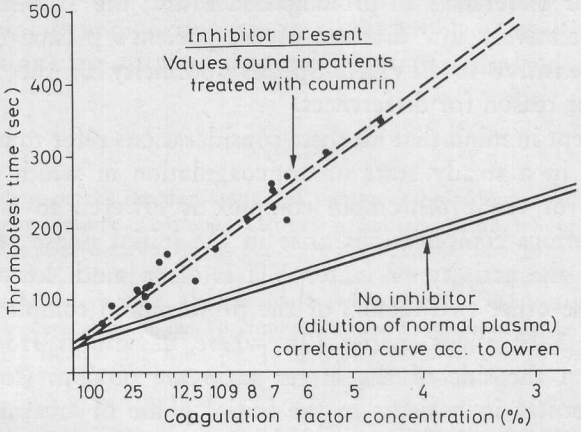

Fig. 3. Correlation between Thrombotest time (t) and coagulation factor concentration (c) as assessed in patients treated at the Thrombosis Service. — Each dot represents the correlation found in an individual patient under long-term anticoagulant treatment. The regression line through these points differs markedly from the regression line obtained with dilutions of normal plasma, but appears to maintain its rectilinearity.

Fig. 3 shows, in addition to rectilinearity, that coagulation times found with dilutions of normal plasma (Owren's standard reference curve) are much shorter than those found with patients' undiluted plasma containing the same amount of coagulation factors as the respective dilutions of normal plasma. In other words, coumarin treatment causes a relative prolongation of the coagulation time at the same level of coagulation factors.

As far as we can see at the moment, the difference between the curves found in patients and for dilutions of normal plasma is caused by a competitive inhibitor of factor X. This inhibitor is probably a metabolic precursor of factor X, released into the blood-stream in cases of vitamin K deficiency. The inhibitor was initially called preprothrombin (7) and has recently been denominated in a more general way as PIVKA (*P*rotein *I*nduced by *V*itamin *K* *A*bsence or *A*ntagonists) (8).

The amount of the competitive inhibitor circulating in patients with different levels of anticoagulation is, as a rule, independent of this level and scatters around a constant mean for all patients. As an inhibitor of the competitive type, it causes only a difference in the steepness of the correlation curve and not a deviation from rectilinearity. Hence, for Thrombotest, the real percentage of

coagulation factors present in a given patient's plasma can be calculated by multiplying Thrombotest percentages by a constant (approx. 2.0).

The PIVKA-sensitivity of the test system is, according to our experience, one of the reasons for differences in prolongation ratios; the Thrombotest, perhaps because of the relatively low dilution of the patient's plasma (roughly 1/10), is particularly sensitive to PIVKA. Species specificity of the thromboplastin reagent is another reason for differences.

It should be kept in mind that all these considerations refer to blood or plasma of patients kept in a steady state of anticoagulation in which the activity of the four factors of the prothrombin complex is lowered to a similar degree (4, 5). Rather serious complications arise in the initial phase of anticoagulant treatment, when the activity of factor VII is often much lower than that of factor X (and the other two factors of the prothrombin complex). This is illustrated by Fig. 3 of Biggs' paper (2), where deviations from the straight regression line on the side of the larger ratio are obvious (long coagulation times are seen mostly in patients in the initial phase of treatment or in cases of acute change of tolerance).

To summarize our theoretical considerations concerning standardization of the one-stage prothrombin time for the control of anticoagulation therapy: evidence put forward by us supports the correctness of the conclusion reached by Biggs and Denson. Hence, the calibration table presented by these authors provides a unique key to the comparability of results obtained with different thromboplastin reagents, at least as far as patients under steady-state anticoagulation are concerned.

For practical purposes, however, besides the introduction of a reference preparation and a calibration table, the proposal made by Poller, namely the introduction of a reagent to be used in a wide area, should also be seriously considered. In the Netherlands, in spite of many prejudices, the Scientific Committee of the Thrombosis Services of the Netherlands Red Cross already proposed the use of Thrombotest 6 years ago, because of its outstanding quality control by the manufactures and the fact that it can be obtained all over the world. This year, about 75,000 Dutch patients have been checked with Thrombotest, 3,500 of these belonging to our Thrombosis Service. There have been almost no complaints concerning the reagent.

Differences between batches are so small that in practice we use coagulation times only for the adjustment of the dosage. Sensitivity to the initial factor VII decrease is an important advantage of Thrombotest. Moreover, Thrombotest has proven to be useful in assessing and irrefutably defining the intensity of treatment during clinical trials. In two Dutch studies on the value of long-term anticoagulant treatment in patients suffering from coronary thrombosis, both of

which gave results favoring long-term anticoagulation treatment, the range of Thrombotest values aimed at was between 5% and 13% (10).

As a concluding remark I may add that in discussing the introduction of an international standard, the questions of the quality control of the ultimate reference preparation and of coping with all the problems arising in the hundreds of laboratories using the standard should not be underestimated.

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