

The Control of Anticoagulant Therapy: Current Dilemmas

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Since oral anticoagulants were introduced into clinical practice approximately 50 years ago, coumarin dosage monitoring has been achieved by the one-stage prothrombin time test, introduced by Quick in 1935. This test assesses the integrity of the extrinsic clotting pathway which comprises clotting factors II, VII and X - three of four vitamin K-dependent clotting factors which are inhibited by oral anticoagulants. The prothrombin time is uninfluenced by changes in factor IX levels since this is a component of the intrinsic coagulation pathway.

Tissue thromboplastin reagent is the most important variable in prothrombin time determinations. Although thromboplastin may be obtained from placenta or lung, brain tissue remains the main source of this material worldwide. Before the emergence of the human immunodeficiency virus and the subsequent recognition of its potential transmission by brain tissue, human brain was the source of most thromboplastins worldwide. However, in the early to mid-1980s, and on account of its potential infectivity, the human material was replaced in the UK by rabbit brain thromboplastin. The latter material has been used continuously in the United States, whilst many Scandinavian laboratories have favoured the use of bovine brain thromboplastin.

Although the therapeutic value of oral anticoagulants has been recognised for many years, there have been considerable difficulties in establishing risk-benefit assessments and in making comparisons between results of different studies, not only from different parts of the world but also from different centres in the same country. Two main factors were responsible for the confusion: 1) different methods of reporting prothrombin time results and 2) marked differences in the sensitivity of different thromboplastins to reductions of the vitamin K-dependent clotting factors. The shared control of a patient's oral anticoagulant therapy between hospitals using different reporting systems and also different thromboplastins therefore constituted a particularly difficult problem.

A number of different methods have been employed for reporting results of prothrombin time determinations in orally anticoagulated patients: a) the prothrombin index which is the reciprocal of the ratio expressed as a percentage; b) prothrombin activity, expressed as a percentage and derived from results of dilution curves; and c) prothrombin ratio, which is the patient's prothrombin time in seconds/normal control time in seconds.

The thromboplastin reagent is the most important determinant of prothrombin time results. Although all thromboplastins are sensitive to reductions in the plasma concentration of the vitamin K-dependent clotting factors II, VII and X, there are very marked differences in their individual sensitivity to these reductions.⁽¹⁻⁴⁾ The clinical implications of thromboplastin sensitivity were highlighted by Poller and Taberner,⁽⁵⁾ who reported that patients treated in centres using the more sensitive human brain thromboplastin for oral anticoagulant monitoring received lower doses of warfarin than those whose control was monitored with the less sensitive rabbit-derived materials. It should be stressed that differences in thromboplastin sensitivity are not solely a function

of their animal source, since there are also considerable differences in sensitivity to reductions of the vitamin K-dependent clotting factors between different reagents prepared from the same animal material.

In the 1950s, an attempt was made by the American Heart Association⁽⁶⁾ to standardise oral anticoagulant control by recommending a therapeutic range of 2.0-2.5 times the control value. From the above considerations, and particularly with respect to differences in thromboplastin sensitivity, there was little possibility that this could achieve its desired aim. Surprisingly, the recommendation persisted for the next 30 years, during which time a variety of different commercial rabbit brain thromboplastins were introduced into North America.

The first attempt to standardise actual prothrombin time determinations for oral anticoagulant control was made in the 1960s by Poller in the UK. The thromboplastin, which was used by many hospitals throughout the UK, was a standardised human brain thromboplastin, designated British Comparative Thromboplastin (BCT).⁽⁷⁾ In addition, in 1969, a national system of reporting was introduced based on the prothrombin ratio obtained with BCT (Poller, 1987, INR and the Therapeutic Range)

International Standardisation and Calibration

During the 1970s, a number of different reference thromboplastins were established by the International Committee on Thrombosis and Haemostasis (ICTH). In 1977, a research standard prepared in the UK on behalf of the ICTH and coded 67/40 was designated by WHO as the primary International Reference Preparation (IRP). This thromboplastin was prepared from human brain supplemented with adsorbed bovine plasma. Subsequently, following a series of international multicentre studies established under the joint direction of ICTH and the International Committee for Standardisation in Haematology (ICSH), WHO accepted, as secondary IRPs, batches of bovine, rabbit and human (plain) thromboplastins which had been calibrated against the primary IRP 67/40.⁽⁸⁾ These were designated OBT/79, RBT/79 and BCT/253, respectively⁽⁹⁾ (Fig.1).

HIERARCHY OF REFERENCE THROMBOPLASTINS

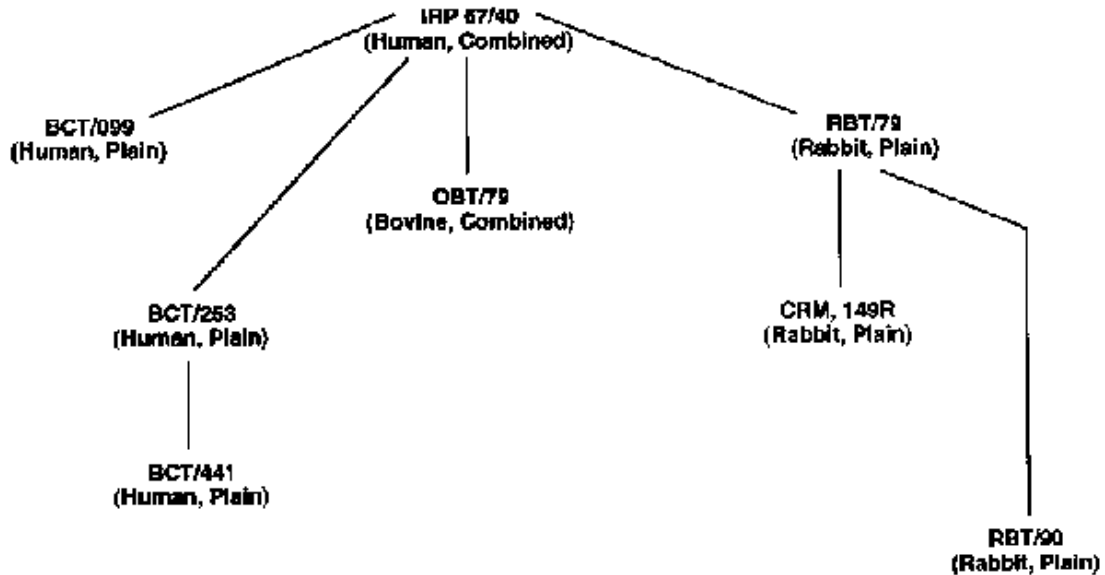


Figure 1. Hierarchy of reference thromboplastins.

The establishment of a hierarchy of thromboplastins is of great practical importance since it ensures that, as the supply of one thromboplastin becomes exhausted, it is replaced by another which is calibrated against its predecessor. An important principle, endorsed by the WHO Expert Committee on Biological Standardisation (1983), was the recommendation that IRPs of the same species should be used for the calibration of working thromboplastins.

International Sensitivity Index

In the initial calibration of the secondary IRPs against the primary IRP 67/40, the latter was arbitrarily assigned a calibration constant of 1.0. At this time, it was believed that a linear relationship would exist between prothrombin time ratios of coumarinised plasma samples determined by different thromboplastins. It was further assumed that this would permit the assignment of a calibration constant of any thromboplastin when prothrombin ratios derived from the latter were compared to those obtained with the primary IRP.⁽⁸⁾ Unfortunately the relationship between prothrombin time results obtained with different thromboplastins was not so predictable and calibration lines often failed to pass through the point of origin.

In 1983, important progress was made in thromboplastin calibration through the adoption of a model proposed by Kirkwood, who observed a more consistent linear relationship when the logarithms of the prothrombin times were used to construct the calibration plots.⁽¹⁰⁾ The Kirkwood model presupposes that a linear relationship exists between the logarithms of prothrombin times derived from the primary IRP and those from the thromboplastin under test. Moreover, the model requires that the same

relationship exists for normal plasma samples as well as for those obtained from stably anticoagulated patients.⁽¹¹⁾

The above relationships can be expressed in simple mathematical terms:

$$\log PT_{67/40} = a + c \cdot \log PT_{\text{unknown}}$$

PT = prothrombin time
a = intercept, c = slope of calibration line

This equation is readily converted to:

$$R_{67/40} = R^c,$$

where R = patient prothrombin time/mean normal prothrombin time

In 1985 the ICSH/ICTH Panel proposed that the calibration constant be replaced by the term International Sensitivity Index (ISI). The ISI is therefore the slope of the calibration line when the results obtained with the primary IRP 67/40 are plotted on the vertical axis. $R_{67/40}$ is now known as the International Normalised Ratio (INR). The previous formula now becomes

$$INR = R^{ISI}$$

The INR is thus the calculated prothrombin time ratio that would have been obtained with the primary IRP 67/40. This system has now been widely adopted for oral anticoagulant control.

External Quality Assessment of Oral Anticoagulant Control

The primary function of external quality assessment schemes (EQAS) is to enhance patient care through improved laboratory performance. This is largely achieved through analysis of individual laboratory results and the identification of those laboratories which are performing poorly in respect of any of the analyses offered by the scheme. EQAS with large numbers of participants have a considerable advantage over smaller schemes in that the large volume of data generated by participants also permits an analysis of reagent and instrument performance. This is of considerable value to the scheme participants.

EQAS in blood coagulation have now been established in a number of countries throughout the world. These vary considerably in respect of the number of analyses offered, the numbers of participants, the source of samples and the frequency of distribution.

In the UK National External Quality Assessment Scheme (UK NEQAS) there are approximately 500 registered participants for INR determination; these currently employ 17 different thromboplastins and 23 different methods of end-point detection. Plasma samples are obtained, by plasmapheresis from volunteer patient donors.

The laboratory control of oral anticoagulants is an important function of UK NEQAS. Participants receive six distributions annually and each of these includes two

lyophilised plasma samples, obtained from warfarinised patients, for INR determination. Participants are requested to determine the INR using their routine method and routine thromboplastin. They are also instructed to perform their own mean normal prothrombin time (MNPT) for their INR determination. This is the geometric mean prothrombin time of at least 20 fresh normal plasma samples, determined by the same method as that used for patients samples. Group median values are derived only from laboratories that follow this instruction. Although manufacturers provide MNPTs for their reagents, these are invariably different from those obtained by local determination. For this reason the use of manufacturers' MNPTs is not recommended.

The individual laboratory results are returned to the Scheme Organiser for detailed analysis. The analysed results are then related, using various statistical methods to both overall and individual group mean or median values. Performance analysis is determined by comparing individual participant's results against the median value for the thromboplastin group to which they belong. When there are less than ten users of any given thromboplastin, the result is compared against the overall median value. Results that are more than 15% above or below the median of the group with which they are compared are considered "outwith consensus". When this occurs in two consecutive surveys, a written offer of assistance is made by the Scheme Director to the participant.

As previously discussed, the theoretical advantage of the INR system is that, for any given warfarinised sample, the same INR will be obtained irrespective of thromboplastin used. However, the EQA schemes in both the Netherlands and also the UK have demonstrated thromboplastin-related differences in results of INR determination. In the UK the differences were evident over the entire therapeutic range but were most marked at its upper end, giving rise to some concern that the differences were sufficiently large to influence patient management.⁽¹²⁾

A possible explanation for such discrepancies in EQA surveys lies in the lyophilisation process or some other aspect of plasma collection and preparation. In order to exclude this possibility, Kitchen et al,⁽¹²⁾ using different thromboplastins, determined the INR of fresh plasma samples from warfarinised patients. The results of this exercise clearly demonstrated that the relationship between INRs determined with different thromboplastins is similar for both lyophilised and fresh samples. Possible reasons for the differences include the route of thromboplastin calibration, reagent quality and thromboplastin/coagulometer combinations.

Thromboplastin-related differences in INR might also occur as a consequence of incorrect or inappropriate ISI assignment. This was demonstrated by Poller et al,⁽¹³⁾ who reported that INRs determined with the rabbit brain thromboplastin RBT 79 were, on average, 7.35% greater than those measured with the human brain reference preparation BCT 253. Similar conclusions were drawn by van den Besselaar et al,⁽¹⁴⁾ who re-evaluated the relationship between the IRPs and noted that, compared with their original ISI values, there were now differences between the relative ISIs of BCT 253 and RBT 79 sufficient to produce differences in INR results of between 5-7%. As a direct consequence of these observations, it can be concluded that INRs determined by thromboplastins calibrated against RBT 79 would be expected to be higher than those derived from those calibrated against the human brain material BCT 253.

The magnitude of the differences in INR in respect of the two routes of thromboplastin calibration is not considered sufficiently large to influence clinical management. Nevertheless, the Scientific and Standardisation Committee (SSC) of the ISTH have recently addressed this problem through new recommendations on thromboplastin calibration.⁽¹⁵⁾ These include the novel approach of assigning a mean ISI value, determined through three separate species calibrations, of future IRPs.

Although the discrepant INR results observed in the UK NEQAS surveys could be explained on the basis of the route of thromboplastin calibration, as described above, the magnitude of the differences in INR was greater than would have been predicted by this explanation. The reasons for the marked thromboplastin-related discrepancies in INR remains unknown, but possible explanations could relate to the method of endpoint detection or to the assigned thromboplastin ISIs.

EQA and the Influence of Coagulometers

During recent years there has been a great expansion in the use of coagulometers for prothrombin time determinations. These instruments exert a measurable effect on the manually derived ISI of thromboplastins^(16,17) and as a direct consequence influence INR determinations. It is important to appreciate, therefore, that where coagulometers are employed the ISI should reflect the combination of instrument and thromboplastin.

A number of different approaches have been adopted to address this problem. These include the provision of a system-specific (coagulometer/thromboplastin) ISI and local system calibration. Local system calibration is performed using a series of plasmas with pre-assigned INR or prothrombin times (PT) values. A calibration curve is then constructed in which local PT results are plotted against the preassigned INR values. This calibration curve can then be used to convert local PTs into calibrated INRs. This approach has been applied in the French External Quality Assessment Scheme, where its potential value was confirmed by a substantial reduction in inter-laboratory variation.⁽¹⁸⁾ Other groups have also demonstrated improvements in relation to other reagents and instruments.^(19,20)

A local calibration exercise has recently been completed by participants of UK NEQAS for Blood Coagulation. Laboratories registered as employing some form of automation were invited to participate in the study. Of the 368 laboratories that agreed to participate, results were returned for analysis from 349. These centres employed 18 reagents and 21 different instruments in 60 combination. Each participant received five lyophilised plasma calibrants, labelled S1, S2, S3, S4 and S5, in addition to two NEQAS test plasmas. Sample S1 was a pool of citrated plasma from normal subjects. Samples S2 to S5 were pools of citrated samples from warfarinised patients selected to cover a range of INRs. Each participant performed triplicate PTs using the instrument and thromboplastin used routinely for INR determinations. A local calibration line was plotted by linear regression of the plasma prothrombin times against the pre-assigned INRs. The prothrombin times of the two NEQAS samples were then converted into INRs by reference to the local calibration line (Fig.2). These results were then compared with those obtained on the same sample determined in the conventional way using locally determined MNPT and reagent ISI. The potential value of local calibration was confirmed

by the demonstration of an improvement in overall between-laboratory agreement and particularly within specific reagent groups, where some CVs of less than 3.5% were achieved.

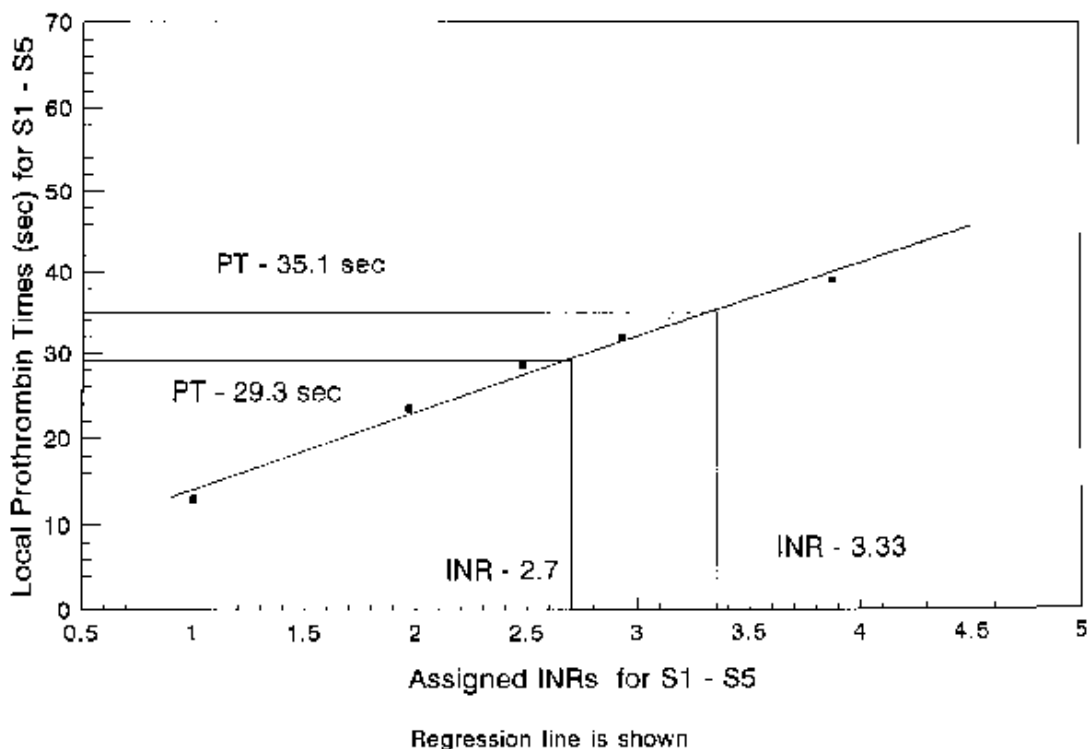


Figure 2. Example of an individual calibration curve allowing conversion of local prothrombin time into INR. Two examples are shown. (UK NEQAS Exercise-see text)

Heparin Dosage Monitoring by APTT: Which Reagent?

Unfractionated intravenous heparin is still widely used for the treatment of venous thromboembolism. Heparin dosage monitoring is usually achieved through the APTT test. Since there is evidence of an association between inadequate heparinisation, as determined by the APTT, and the recurrence of thrombosis, most laboratories have adopted a therapeutic range of 1.5-2.5. However, it is known that reagents vary in their sensitivity to heparin and the adoption of a uniform approach to heparin dosage monitoring, irrespective of reagent, would appear to be somewhat illogical. This view has recently been confirmed through analysis of results obtained on heparinised samples distributed to over 400 laboratories through UK NEQAS.

Data were reviewed from four surveys, using samples prepared by the addition of heparin to normal plasma in vitro and also from patients receiving intravenous heparin (ex vivo). For both in-vitro and ex-vivo samples, significant differences were observed in the sensitivity of different APTT reagents to heparin (Table 1).

Table 1. UK NEQAS heparin dosage assessment: APTT ratios analysed according to reagent used.

APTT reagent*laboratories	Average no. of laboratories	Median APTT ratio					
		73	75	77	79	82	84
A	13	1.69	2.02	4.01	2.81	1.63	1.67
B	38	1.49	1.89	2.89	2.31	1.30	1.37
DB	42	1.58	1.99	3.45	2.77	1.55	1.59
DK	65	1.31	1.60	2.69	2.47	1.45	1.53
IL	109	1.58	1.75	3.08	2.69	1.93	2.08
M	54	1.70	2.05	3.20	2.50	1.67	1.73
ML	9	2.0	2.65	4.50	4.60	1.92	1.96
OA	21	1.54	1.76	2.87	2.44	1.87	1.93
OP	7	-	1.67	2.83	2.49	1.75	2.36
All reagents	413	1.55	1.80	3.00	2.55	1.73	1.88

Surveys 73 to 79 used normal plasma heparinised in vitro. Surveys 82 and 84 used samples from heparinised patients.

Abbreviations: A - Actin FS, B - Boehringer Mannheim, DB - Diagen Bell and Alton, DK - Diagen KPS, IL - Instrumentation Laboratory, M - Manchester, ML - Manchester Low Opacity, OA - Organon Teknika Auto APTT, OP - Organon Teknika Platelin LS

For example, in one survey in which ex-vivo samples were distributed, the median ratio obtained by the users of one reagent was 1.37 compared with 2.36 for users of another. Statistical analysis revealed highly significant differences ($p < 0.0001$) between the least and most sensitive reagent in each of six surveys.

Of particular importance was the observation that there were also significant differences between in-vitro and ex-vivo samples with respect to the ranked sensitivities of the reagents. This clearly means that the use of samples to which heparin has been added are of little, if any, value in the EQA of heparin dosage monitoring.

The result obtained by this large number of laboratories clearly indicate that APTT reagents vary considerably with respect to their sensitivity to heparin, suggesting that reagent-specific therapeutic ranges are required. However, samples prepared by the addition of heparin to normal plasma should not be used for this purpose.

Summary

Heparin and the coumarin derivatives have vital and long-established roles in the management of patients with thrombovascular disease. For both, appropriate dosage

represents a balance between optimal anticoagulant effect and minimal haemorrhagic risk; in order to achieve this, precise and reliable laboratory monitoring is essential.

The introduction of the INR system for the monitoring of oral anticoagulant control represented a major advance, not only in terms of therapeutic efficacy but also in respect of patient safety. However, the system is only reliable if careful attention is paid to the many important variables which contribute to its overall design. These include the choice of thromboplastin, assignment of ISI, determination of the MNPT and the method of end-point detection. Participation in independent EQAS provides a unique opportunity for individual laboratories to identify, through laboratory performance analysis, problems relating to their own laboratory practice. Another major advantage, particularly of the larger schemes, is the identification of poor reagents and equipment and inconsistencies in their performance.

The increasing use of coagulometers and their influence on INR determinations has undoubtedly been a cause of some concern, which relates largely to some misunderstanding of the underlying principles of the INR system and a failure to appreciate that the ISI is a function of both thromboplastin and the instrument with which it is used. This focused attention on the necessity of assigning to thromboplastins an ISI that reflects the combined sensitivities of the reagent and coagulometer with which it is used. Another promising approach involves the use of calibrants and local calibration.

Dilemmas associated with heparin dosage monitoring are of a different nature. Currently, most laboratories employ the APTT for intravenous heparin dosage monitoring. However, the APTT reagents vary considerably in respect of their sensitivity, not only to heparin but also to other haemostatic changes which occur following acute venous thromboembolism.

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