The British system for anticoagulant control and Thrombotest

L. POLLER, JEAN M. THOMSON, AND M. R. ALDERSON

From the Haematology Department, Withington Hospital, Manchester, and the Department of Social and Preventive Medicine, University of Manchester

SYNOPSIS A study has been made to determine whether a reliable comparison can be made between the British Comparative Thromboplastin and Thrombotest using the nationally adopted standardization procedure. The method recommended appeared sufficiently satisfactory and statistically valid for the comparison between the British Comparative Thromboplastin and Thrombotest.

The degree of diversion in the location of the 'best lines', which should have been in an identical position at each centre, suggests that the standardization procedure at individual hospitals with Thrombotest is not usually a sufficiently reliable basis for clinical anticoagulant dosage. The degree of diversion almost certainly arises from technical error in the standardization procedure, and it is suggested that Thrombotest should be calibrated against the British Comparative Thromboplastin only in specialist coagulation centres thoroughly conversant with both techniques.

The national system for anticoagulant control, introduced in 1969, has been shown to be statistically valid when comparing one type of thromboplastin reagent with another using the Quick prothrombin time technique (Alderson, Poller, and Thomson, 1970). In a recent study of the interpretation of prothrombin results some difficulty was encountered at hospitals using the modified prothrombin technique with Thrombotest (Poller and Thomson, 1969). The present study was designed to determine whether hospitals which used the British Comparative Thromboplastin (BCT) as a reference preparation were able to perform the recommended standardization procedure with Thrombotest. For comparison a Quick test thromboplastin made from rabbit brain was also included in our study. The opportunity was also taken of comparing the results with our recent report on the statistical validity of the nationally adopted standardization procedure using the Quick test (Alderson *et al*, 1970).

Method of Study

Two groups of hospitals performed the following studies. In group 1 the recommended standardization procedure was performed with Thrombotest and in group 2 a rabbit brain thromboplastin (Diagen phenolized) was substituted for the British Comparative Thromboplastin and tested in parallel with Thrombotest.

Received for publication 30 July 1970.

GROUP 1

The same numbered batch (934) of Thrombotest reagent was sent, together with sufficient 3.2 mM calcium chloride solution and a fresh supply of the British Comparative Thromboplastin, to 20 hospitals. Each hospital was asked to test in parallel 20 patients who had been stabilized on oral anticoagulant treatment. They were also asked to test two fresh normal plasma specimens on each day they tested the coumarin specimens. The mean of the normals was used for the determination of prothrombin ratios.

Ten of the 20 hospitals invited to participate were known to use Thrombotest routinely, the other 10 used the Quick test. This was to discover if error resulted from unfamiliarity with the different technique using Thrombotest.

The detailed instructions were as follows:

Hospitals were asked to collect 4.5 ml of venous blood into 0.5 ml of 3.13% trisodium citrate in a plastic or siliconed container. Specimens were to be tested within one hour of collection where possible and if more than two hours old were not to be used. Blood was to be stored where necessary, at 4°C. To standardize contact activation each blood sample was to be tested individually with both reagents in turn. Results were recorded as clotting time in seconds. The procedure for Thrombotest was according to the manufacturer's instructions for the venous technique. An instruction sheet was also sent with the details of the Quick test procedure using the British Comparative Thromboplastin.

GROUP 2

The instructions were as above but the same batch of rabbit brain (Diagen phenolised) was substituted for the British Comparative Thromboplastin and sent to 14 hospitals, seven of which used Thrombotest routinely, the other seven the Quick test. The manufacturer's instructions were enclosed with the rabbit brain together with the recommended method for the British Comparative Thromboplastin.

The prothrombin ratios were calculated by us and we also plotted each set of results on arithmetical graph paper. We then drew the 'best straight line' between all the points by visual assessment in the usual way. Linear regression analysis was carried out and a calculated line inserted on the same graph.

It is important to determine whether the 'best straight line' is a fair representation of the data and the results were analysed from two points of view.

The difference between the 'best straight line' drawn by visual assessment and the calculated line was examined using the present results to see if there was a significant difference and also compared with the results from our recent study (Alderson *et al*, 1970), when the same method of comparison was used with two different Quick test procedures. The reliability of the 'best line' drawn by visual means was assessed by measuring the distance between the two lines at various points. Seven points with the following values on the scale of the Thrombotest and rabbit brain method were used: ratios of 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, and 3.0. In addition the vertical distance between the two lines was measured from the points on the calculated line equivalent to 1.8 and 3.0 with the British Comparative Thromboplastin. The latter two points are the usual extremes of the therapeutic scale with the British Comparative Thromboplastin.

The same batches of thromboplastin reagent were supplied to the hospitals and in theory the location of the lines should therefore not vary from centre to centre in each group. Any deviation almost certainly would represent individual differences in technique and a great variation with one type of reagent or technique would cast doubt on the reliability of the system when this was used. As a method of assessment in group 1, the ratios were recorded for Thrombotest equivalent to 3.0 on the British Comparative Thromboplastin scale. (This is the upper limit of the therapeutic range of the British Comparative Thromboplastin.) In group 2 Diagen was substituted for the British Comparative Thromboplastin. In our previous study (Alderson *et al*, 1970), using the same batch of rabbit brain, we found the ratio which corresponded to 3.0 on the British Comparative Thromboplastin scale was 2.23 for rabbit brain. The distribution of Thrombotest results equivalent to a Diagen

ratio of 2.23 was obtained using the calculated lines. This was used as a measure of the scatter of the lines, which could be compared with the measure of scatter for results in group 1.

Results

1 ANALYSIS OF RETURNS

Of the 20 hospitals in the first group, 15 sets of results were regarded as suitable for statistical analysis. The reasons for exclusion of the five others were as follows:

Four centres tested insufficient numbers of patients and one hospital did not perform the Thrombotest procedure in the way recommended. Of the 15 hospitals, eight of these used Thrombotest routinely, the other seven using the Quick test. In the second group statistical analysis was performed on 11 sets of results. Three hospitals had to be excluded because of incomplete data. Of the 11 analysed, five used Thrombotest as the routine method, the other six used a Quick test technique.

ANALYSIS OF RESULTS

The first point to be studied was the reliability of the line drawn by visual assessment when the British Comparative Thromboplastin was compared with Thrombotest. Table I gives the mean discrepancy between the two lines using both of these methods of assessment. Using the seven values for Thrombotest the overall discrepancies for the 12 centres varied between 0.04 and 0.36, with an average of 0.15. For the two therapeutic limits of the British Comparative Thromboplastin the mean discrepancy varied between 0.03 and 0.37 with an average of 0.17. The mean discrepancy at the upper and lower limits of the therapeutic range for all centres in each of the three

Hospital	Mean Discrepancy at Seven Values for Thrombotest	Mean Discrepancy at Upper and Lower Limits of Therapeutic Range for BCT
1	0.22	0.19
2	0.31	0.18
3	0.04	0.07
4	0.36	0.37
5	0.08	0.35
6	0.07	0.05
7	0.09	0.08
8	0.21	0.30
9	0.09	0.03
10	0.11	0.20
11	0.06	0.07
12	0.17	0.22
13	0.18	0.34
14	0.07	0.05
15	0.18	0.11
Mean	0.15	0.17

Table I Discrepancy between visual line and calculated regression line with Thrombotest compared with British Comparative Thromboplastin (BCT)

comparisons was: local human brain 0.10, rabbit brain 0.15, Thrombotest 0.17. One-way analysis of variance indicates that the difference between these three means is significant at the 1% level ($VR = 19.1$, $DF = 2$ and 35 , $P < 0.01$). Though the mean discrepancy for Thrombotest is largest for the three comparisons it is not very different from that for rabbit brain; both of these are considerably larger than the mean difference for the local human brain comparison reported in our previous survey (Alderson *et al.*, 1970).

In order to test whether the discrepancies result from a difference in method and not from technical errors, a further comparison was made between a rabbit brain Quick test reagent and Thrombotest in hospitals in group 2. Table II indicates the discrepancy between the plotted line and the calculated regression line for the comparison between Thrombotest and rabbit brain for the 12 centres that participated in this comparison. Table II shows a mean discrepancy for the seven values of Thrombotest which lay between 0.01 and 0.11 with an average of 0.06. The mean discrepancy for the therapeutic limits for rabbit brain varied between 0.01 and 0.23 with an average of 0.1. Thrombotest and the British Comparative Thromboplastin had shown an average difference of 0.17. A *t* test suggests that these results are not significantly different ($t = 1.83$, $DF = 24$, $P < 0.1$) indicating the reliability of the principle of drawing the 'best lines' in this comparison.

Hospital	Mean Discrepancy at Seven Values for Thrombotest	Mean Discrepancy at Upper and Lower Limits of Therapeutic Range for Rabbit Brain
16	0.02	0.06
17	0.10	0.15
18	0.03	0.14
19	0.03	0.02
20	0.03	0.02
21	0.07	0.07
22	0.02	0.07
23	0.11	0.23
24	0.11	0.20
25	0.01	0.01
26	0.09	0.12
Mean	0.06	0.10

Table II Discrepancy between visual line and calculated regression line with Thrombotest compared with rabbit brain

The second and more important aspect from the point of view of anticoagulant dosage is the reproducibility of the results at different centres. Samples of the same batches of reagents were sent to the centres operating in both groups. In theory the actual lines should be identical for each of the centres. For any fixed point on the vertical axis the same

Hospital	Estimated Thrombotest Ratio	
	Calculated Line	Visual Line
1	3.76	3.85
2	2.70	3.07
3	3.30	3.45
4	2.89	2.45
5	8.25	6.20
6	4.14	4.16
7	2.88	3.20
8	3.37	4.25
9	3.14	3.38
10	3.26	3.70
11	3.52	3.79
12	3.36	3.17
13	3.54	3.12
14	2.53	2.47
15	3.45	3.45
Range	2.53-8.25	2.47-6.20

Table III Thrombotest value at each centre for British Comparative Thromboplastin ratio of 3.0.

Fig. 1

Fig. 2

Figs. 1 and 2 Variation in location of the lines drawn by visual assessment using both the British Comparative Thromboplastin (Fig. 1) and Diagen (Fig. 2) v Thrombotest.

reading should be obtained on the horizontal axis for each hospital.

In the first group the range of values equivalent to 3.0 on the scale of the British Comparative Thromboplastin is seen to vary between 2.53 and 8.25 on the calculated line (2.47 and 6.20 on the line drawn through the points by visual assessment as shown in Table III). Figures 1 and 2 provide examples of this variation in location of the lines drawn by visual assessment using both the British Comparative Thromboplastin v Thrombotest and Diagen v Thrombotest comparisons. On each occasion the line was based on points with a near linear distribution. Table IV shows the values for Thrombotest ratios that appear equivalent at the different centres to a Diagen rabbit brain ratio of 2.23, which is the upper limit of the therapeutic range.

Hospital	Estimated Thrombotest Value (Calculated Line)
16	5.95
17	3.48
18	5.13
19	3.15
20	3.44
21	3.03
22	2.98
23	2.93
24	3.40
25	4.03
26	3.07
Range	2.93-5.95

Table IV *Thrombotest value at each centre for rabbit brain ratio of 2.3*

Discussion

In our previous paper (Alderson *et al.*, 1970) we showed that the recommended standardization procedure based on the national reference thromboplastin was statistically valid. The present study was designed to determine whether the same procedure would be valid with the Thrombotest technique which is a modification of the prothrombin time test. It appears from this study that when the calculated line was compared with the line drawn by visual assessment through the points obtained by plotting the prothrombin ratios, there was a significant degree of error introduced when the Thrombotest technique was used compared with human brain comparison in the Quick test method. These results, although statistically significant, were probably not of great clinical importance in drawing the 'best line' because there is usually a discernible trend of the points which indicates the correct position of the 'best line' even though the points may not lie as closely to the line with the Thrombotest correlation. In other words the standardization procedure recommended with the British Comparative Thrombo-

plastin is sufficiently satisfactory and statistically valid for the comparison between the British comparative thromboplastin and Thrombotest technique as well as for the British Comparative Thromboplastin and Quick test reagents.

More relevant clinically was the large variation in points equivalent to a prothrombin ratio of 3 with the British Comparative Thromboplastin, ie, the upper limit of the therapeutic range (see Table III). This is also shown by the location of the lines when Thrombotest was compared with either of the Quick test reagents. As the hospitals received the same numbered batch of British Comparative Thromboplastin, Thrombotest, and Diagen in the present trial, in theory their results should have been identical from centre to centre, and the degree of scatter of the ratios equivalent to 3 with the British Comparative Thromboplastin is a measure of the reproducibility of technique at the individual hospitals. The wide distribution of readings corresponding to 2.23 Diagen, which we have shown to be equivalent to 3.0 on the British Comparative Thromboplastin scale (Table IV), indicates that the difficulties encountered with Thrombotest cannot be explained by the differences between human brain and animal brain thromboplastin as both Diagen Quick test reagent and Thrombotest are made from animal brain extract. The only possible explanation is that the failure to obtain reproducible results with the standardization procedure, when Thrombotest is compared with the British Comparative Thromboplastin, is due to differences in laboratory technique. Few hospitals are sufficiently conversant with the technical pitfalls of both methods for an accurate comparison. The degree of diversion of the lines at the different hospitals was so great that one must question the feasibility of standardizing results using the nationally adopted standardization procedure at laboratories using Thrombotest. The present study suggests that it would be unwise to base patients' anticoagulant dosage on this 'standardization procedure' with Thrombotest except perhaps in a small number of expert coagulation laboratories. The reliance on a few 'expert' centres is not as good or reliable as performing the routine standardization procedure oneself, because local variations in technique in individual laboratories will not be controlled. There can be no certainty that the resulting British Corrected Ratio on which anticoagulant dosage depends is accurate.

References

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