A Reassessment of the Relationship between International Reference Preparations for Human and Rabbit Thromboplastins

A. M. H. P. van den Besselaar\(^1\), A. Tripodi\(^2\)

\(^1\)Haemostasis and Thrombosis Research Centre, Leiden University Medical Centre, Leiden, The Netherlands; \(^2\)A. Bianchi Bonomi Hemophilia and Thrombosis Centre, University and IRCCS Maggiore Hospital, Milan, Italy

Keywords

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Summary

Two established international reference preparations (IRP) for thromboplastins, i.e. RBT/90 (rabbit, plain) and rTF/95 (recombinant human, plain) have been calibrated against each other in a 7-centre exercise performed in 2000. The purpose of the study was to compare the calibration results with those of the original calibration study performed in 1995. The international sensitivity index (ISI) for rTF/95 was calculated relative to the ISI for RBT/90. The mean ISI for rTF/95 was 0.961 (between-lab SD 0.028) which was 2% greater than the historical value determined in 1995. There is no indication that the two IRP have deteriorated, but further monitoring of the calibration relationship is recommended.

Introduction

At present, there are three WHO international reference preparations (IRP) for thromboplastins, i.e. OBT/79 (bovine thromboplastin, combined), RBT/90 (rabbit thromboplastin, plain), and rTF/95 (recombinant human thromboplastin, plain). These lyophilized preparations are contained in sealed glass ampoules and are stored at –20°C. The international sensitivity index (ISI) for each IRP has been established by calibration against predecessor IRP in multicenter studies. However, there is no established programme to monitor the stability of these IRP.

In 2000, a multicenter study was performed for the calibration of a new thromboplastin preparation derived from cultured human cells. The primary purpose of that study was to determine the ISI for the new thromboplastin with the manual (tilt tube) technique and a number of different automated coagulometers. The results of ISI determination for the new thromboplastin will be reported elsewhere. Both rTF/95 and RBT/90 were included in the study. Opportunity was taken to determine the relationship between these IRP and to compare with the original relationship established in 1995. The results reported in the present paper can be considered as the first occasional stability monitoring of the recombinant human IRP.

Materials and Methods

The two IRP, i.e. rTF/95 and RBT/90 were provided by the W.H.O. and were stored at –20°C until use. The reconstitution fluid for rTF/95 was stored at 4°C. Calcium chloride (0.025 mol/l) for use with RBT/90 was obtained from Organon Teknika (Boxtel, the Netherlands). The IRP were used as recommended by the WHO.

The study was carried out by 7 laboratories in 6 different countries. Each laboratory used the two IRP by manual (tilt tube) technique. In each laboratory one expert operator performed the manual clotting time determinations. Fresh citrate plasmas were analysed by the operator over 10 working days. Fresh plasmas from 2 normal subjects and 6 patients on long-term oral anticoagulant therapy were used on each working day. Within each working day, the analyses were completed within 5 h from the start of blood collection. The order of manual testing with the different thromboplastins was changed each day.

Blood was collected with each laboratory’s routine equipment, i.e. Becton Dickinson Citrate Vacutainer Systems. The blood collection tubes contained either 0.105 or 0.109 mol/l sodium citrate solution. Nine volumes of blood were mixed with one volume of sodium citrate solution. Citrated blood was centrifuged at a minimal force of 2500 × g for 10 min at a controlled room temperature. Plasma was transferred into plastic tubes. The tubes were capped and stored at room temperature.

Statistical Analysis

The international normalised ratio (INR) was calculated from the clotting times measured with RBT/90 using ISI = 1.00 and each laboratory’s mean normal prothrombin time (MNPT) derived from the 20 normal samples. Patient samples with INR <1.5 or INR > 4.5 were excluded from the analysis. Log-transformed clotting times were plotted with RBT/90 on the vertical axis and rTF/95 on the horizontal axis. Orthogonal regression lines for log-transformed clotting times (normals plus patients) were calculated as described previously. Outliers were defined as samples with a perpendicular distance from the line greater than three times the residual standard deviation. Final orthogonal regression lines were calculated after elimination of outliers. The orthogonal regression lines for normals plus patients were compared with the lines for the patients only. The coincidence of the two lines was tested for significant deviation. The purpose of this procedure was to check the validity of one of the underlying assumptions of the ISI calibration model, i.e. that the log-transformed PT of normals and patients are linearly related and fit a single line. MNPT and ISI differences between the present and previous assessments were tested with Student’s t-test at the 5% significance level.

Results

All 7 centres returned a complete set of results. Between 1.7% and 8.3% of patient samples were excluded on the basis that they either exceed the INR bounds of 1.5 to 4.5, or were outliers, i.e. more than three standard deviations from the regression line.
The mean normal PT values for the 7 centres with the two IRP are given in Table 1. The SD comprising biological and measurement variability, for within and between-centre PT are provided. The MNPT values for the 7 centres were compared with the corresponding values for the 19 laboratories in the original study performed in 1995. In the original study the overall mean normal PT with rTF/95 (coded as X/95) was 13.14 seconds, which is not different from the present value (13.16 sec). The value with RBT/90 in the original study was 18.03 seconds, which is not different from the present value (17.78 sec).

The equations for the orthogonal regression lines (\( y = a + bx \) where \( y = \ln \text{PT}_{\text{RBT/90}} \) and \( x = \ln \text{PT}_{\text{rTF/95}} \)) of the individual centres are given in Table 2. An approximate test was used to examine the assumption that the mean logarithms of the PT of normals are found on the orthogonal regression line derived using patients’ samples. In one of the centres (nr. 4) a significant deviation from this assumption was found (\( 0.02 < p < 0.03 \)). The scatterplot of the data obtained by this centre is shown in Fig. 1. Despite the significant deviation, the INR difference between the orthogonal regression lines for patients only and for patients plus normals was less than 10%. In the other six centres no significant deviation was observed at the 5% significance level.

The mean slope (ISI) for the rTF/95 if it is assumed that the ISI for RBT/90 equals 1.00. The mean slope (ISI) for the 7 centres was only 2% greater than the corresponding value (0.942) for the 19 centres in the original study.

**Table 1** Mean and standard deviations (SD) of prothrombin time (seconds) for fresh normal plasmas (n = 20)

<table>
<thead>
<tr>
<th>Centre</th>
<th>RBT/90</th>
<th>SD</th>
<th>rTF/95</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.60</td>
<td>1.19</td>
<td>13.45</td>
<td>0.77</td>
</tr>
<tr>
<td>2</td>
<td>17.63</td>
<td>1.14</td>
<td>13.32</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>17.46</td>
<td>1.02</td>
<td>12.34</td>
<td>0.72</td>
</tr>
<tr>
<td>4</td>
<td>16.88</td>
<td>1.36</td>
<td>11.97</td>
<td>0.58</td>
</tr>
<tr>
<td>5</td>
<td>18.53</td>
<td>1.36</td>
<td>14.44</td>
<td>0.98</td>
</tr>
<tr>
<td>6</td>
<td>18.17</td>
<td>1.73</td>
<td>12.78</td>
<td>0.83</td>
</tr>
<tr>
<td>7</td>
<td>17.17</td>
<td>1.24</td>
<td>13.84</td>
<td>0.82</td>
</tr>
<tr>
<td>Mean</td>
<td>17.78</td>
<td>0.67</td>
<td>13.16</td>
<td>0.86</td>
</tr>
<tr>
<td>Between-lab SD</td>
<td></td>
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</tbody>
</table>
Discussion

According to WHO guidelines, the calibration of the IRP for thromboplastins, and their future replacements, should be carried out in international multicentre collaborative studies using fresh coumarin and normal plasmas, and manual techniques.

In the present study, seven centres performed a reassessment of the relationship between rTF/95 and RBT/90 in 2000, i.e. 5 years after the original calibration study of rTF/95.

The purpose of the present work was to compare the relationships between RBT/90 and rTF/95 as determined in the present and the original multicentre studies. The experimental conditions of the two studies were not identical. The original study included 19 centres whereas the present study was performed with the results from 7 centres. Only 4 of the centres were the same in the two studies. Operators performing the manual technique were not the same. Preanalytical conditions were not exactly the same because different lot numbers of blood collection tubes were used.

In 6 centres of the present study, there was no significant deviation from the ISI calibration model as the mean logarithms of the normal prothrombin times were found on the orthogonal regression lines calculated for patients' samples. In one centre (number 4, see Fig. 1), there was a significant displacement of the mean log normal PT from the patients-only line (0.02 < p < 0.03). When the ISI from this centre was used for calculation of the INR, the INR deviation in the range 2–4.5 was less than 10% which is still acceptable according to WHO guidelines (2).

Despite the different conditions of the two exercises performed in 1995 and 2000 respectively, there was good agreement regarding MNPT (Table 1) and the slope of the orthogonal regression line (Table 2). The mean slope of the orthogonal regression line (= ISI) in the present assessment was only 2% greater than the value originally obtained. Although this difference was not significant or of practical importance, it might be a sign of a small change in the relationship between the two IRP. The relationship between the two IRP is a function of their absolute stabilities and of the preanalytical conditions. There is no indication that the IRP have deteriorated during storage. Since the ISI and hence INR are ultimately dependent on the IRP, it is important to continue monitoring of their stability. Further multicentre studies are needed to monitor the relationship and stability of the IRP.

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References


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