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BACKGROUND: The need to perform local International Sensitivity Index (ISI) calibrations and in particular the requirement for a manual method for prothrombin time (PT) determination, have proved to be obstacles to application of the WHO scheme for PT standardization.

METHODS: We used international normalized ratio (INR) derived with a set of only 5 European Concerted Action on Anticoagulation (ECAA) lyophilized calibrant plasmas, certified manually by expert centers with reference thromboplastins, to determine a local PT/INR Line. We compared results of an independent set of validation plasmas with INRs from conventional ISI calibrations and with manually certified INRs.

RESULTS: The mean certified INR of 5 lyophilized validation plasmas was 2.41 with human thromboplastin, 2.04 with bovine/combined, and 2.80 with rabbit. With 42 human reagents, the mean observed INR of the validation plasmas was 2.68 (11.2% deviation from certified INR). Deviation was reduced to 0.4% with both local ISI calibration and the PT/INR Line. Eight results based on bovine/combined thromboplastin gave an INR deviation of 4.9%, becoming 0.5% after ISI calibration and 2.4% with the PT/INR Line. Six results with rabbit reagents deviated from certified INR by 2.5%. After ISI calibration, deviation became 1.1%, and with the PT/INR Line, 0.7%. The PT/INR Line gave similar results with both linear and orthogonal regression analysis. The total proportion of validation plasmas giving INR within 10% deviation from certified values was 42.5% with uncorrected INR, which increased to 92.1% with local ISI calibration and 93.2% with the PT/INR Line.

CONCLUSIONS: The PT/INR Line procedure with 5 ECAA calibrant plasmas successfully substitutes for local ISI calibrations in deriving reliable INRs.

Improved safety and effectiveness of oral anticoagulant treatment resulted from the WHO’s introduction more than 25 years ago of the scheme for prothrombin time (PT) standardization using international normalized ratios (INRs) (1, 2). Thrombotic and bleeding events were found to increase exponentially below INR 2.0 and above INR 4.5, respectively (3).

Since the introduction of the WHO INR scheme, the original manually derived PT scheme has been replaced almost universally by automated methods. The recommended WHO International Sensitivity Index (ISI) calibration derived from manual PT testing is therefore no longer feasible at most centers. In WHO ISI calibrations, all blood samples are tested in parallel with the local method using the manual PT technique and the same-species thromboplastin international reference preparation (IRP). Furthermore, even if successfully derived, manual ISI may be greatly changed by coagulometers (4–10), and manual calibration ISI may not be relevant. To overcome this, some manufacturers provide a range of ISIs for different coagulometers when used with their thromboplastins (“system ISI”), but the effects on INR of individual coagulometers even from the same manufacturer vary. Because of these difficulties, important clinical trials of warfarin continue to be published without evidence of validation of stated INRs (11).

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INR Derivation Using the PT/INR Line

The problems of manual PT testing have led to the use of lyophilized plasmas with certified INRs to derive local ISIs and hence dependable INRs (8–10). One such scheme was approved by the U.S. Food and Drug Administration (FDA) for local ISI calibrations following a series of reports (12–19). Even this procedure has been little used, either because it has proved too complex for most laboratories or because the necessary sets of 20 abnormal and 7 normal lyophilized certified plasmas have not been available.

The PT/INR Line described here is a development of the “Direct INR” method of Houbouyan and Goguel (20), later modified in the Scientific and Standardization Committee (SSC) Guidelines and in the Clinical and Laboratory Standards Institute document (21, 22). In the present report, we evaluate the possible value of a rapid method for local INR derivation, which we have termed the PT/INR Line, obtained from a small set of European Concerted Action on Anticoagulation (ECAA) INR-certified plasmas (see Acknowledgments for source information). This was part of a multicenter international randomized study of computer-assisted dosage. The measurement of INRs at 28 participant centers was the subject of ongoing external quality control during the 5-year duration of the study (23). The value of a selected set of 5 lyophilized ECAA certified INR plasmas had previously been established in external quality assessment of CoaguChek point-of-care testing PT meters (24–26).

The latter study investigated whether the use of the PT/INR Line from a small set of 5 ECAA calibrant plasmas certified with manually determined INRs could reliably replace local ISI calibration. We therefore compared INRs derived from the PT/INR Line by use of an independent set of 5 validation plasmas with the certified manual INRs on the same plasmas obtained with the relevant WHO reference thromboplastins and also with results from local ISI calibrations with the FDA-approved method (12).

METHOD OF STUDY

We conducted this assessment on a series of 56 multicenter ISI calibrations over a 5-year period at 28 participant centers. We compared the INR results with certified values. Fig. 1 gives an overview of this study.

CERTIFIED PT AND INR

Certified measurements of PT and INR were provided by 3 experienced ECAA Certifying Centers (Leiden, Manchester, and Milan), obtained with their manual PT testing with 3 different species of IRP, WHO human recombinant plain rTF/95 (ISI 0.94), WHO bovine/combined OBT-79 (ISI 1.0), and EUTHR-1 (ISI 1.67). With the nonavailability of WHO rabbit IRP, we used ECAA rabbit plain EUTHR-1, which was used in previous ECAA multicenter studies and certified in terms of WHO rabbit IRP RBT/90 (10, 27–29).

CERTIFIED INRs

We chose a set of 20 ECAA artificially depleted lyophilized and 7 lyophilized normal plasmas to provide a spread over the 2.0–4.5 INR range with the 3 reference thromboplastins. For certification, all plasmas were tested on a single occasion on 3 different days at each center.

Certified INRs for each of the 20 artificially depleted plasmas were obtained with 7 lyophilized normal plasmas to derive the local mean normal PT (MNPT):

\[
INR_{cert} = \left( \frac{PT_{cert}}{MNPT_{cert}} \right)^{ISI_{ref}}
\]

where \(MNPT_{cert}\) is the geometric mean PT of the 7 lyophilized normal plasmas and \(ISI_{ref}\) is the ISI for the 3 reference thromboplastins. The certified INRs for each of the 3 reference thromboplastins were the mean of the 9 determinations at the 3 certifying centers.

An additional independent set of 5 lyophilized plasmas was also certified. These were single-donation, artificially depleted plasmas tested on 3 different days. We used them as validation plasmas—they provided certified INRs to validate the local INR results before and after correction by ISI calibrations and the local PT/INR Line.

LOCAL ISI CALIBRATIONS

Local ISI calibrations were performed on the set of 20 artificially depleted and 7 lyophilized normal plasmas provided by all centers and tested in duplicate on the same day. Centers also stated their local MNPTs and the thromboplatin manufacturer’s ISI used to determine the INRs of the 5 validation plasmas before correction. ISI calibrations were performed to obtain the local ISI to derive corrected INRs for the 5 validation plasmas (8–10).

LOCAL PT/INR LINE CORRECTION

Local PT results with the calibrant plasmas were plotted against their certified INR values obtained by both orthogonal and linear regression analysis for comparison. We used the regression lines obtained to derive a new set of corrected INRs for the 5 validation plasmas, which were compared with their certified values. The calibrant plasmas and the 5 validation plasmas were tested in duplicate on the same day by the participants.

OPTIMUM NUMBER OF PLASMAS REQUIRED FOR THE PT/INR LINE

The optimum number of calibrant plasmas required for reliable INR derivation with the PT/INR Line was
first determined, and INRs were compared with the certified values for the 5 validation plasmas and with results from parallel multicenter local ISI calibrations. From the 20 ECAA calibrant plasmas, 3 were selected, with a range of 2.0–4.5 INR. Their PT/INR Line was estimated, and the local INR for the 5 validation plasmas was then calculated from the PT/INR Line results. These were compared with the certified values and with results from local ISI calibrations. Additional calibrant plasmas were chosen, giving intermediate values and an even distribution between 2.0 and 4.5 INR. These were included with the first 3 to give sets of plasmas ranging from 4 to 10. The PT/INR Line for these was then determined to establish the minimum number required in a set for reliable INR derivation. All plasmas gave INR ranges of 1.7–4.0, and no normal plasma was included.

LINEAR VS ORTHOGONAL REGRESSION
To determine whether the WHO recommended orthogonal regression analysis for ISI derivation could be reliably replaced by simpler linear regression, PT/INR
Lines were derived with both regression methods and compared. Linear regression was shown previously to be inappropriate compared with orthogonal regression in conventional ISI calibrations of coagulometer PT systems (30). In a linear regression model, we assumed the certified INRs (x axis) to have no error and to represent true values. All the error is contained in the local PT (y axis). In orthogonal regression, we assumed that error is contained on both axes.

**STATISTICAL ANALYSIS**

**Certified INR.** To assess the reliability of results from the 3 ECAA certifying centers, we performed a 1-way ANOVA to test for significant differences in INRs across centers.

**Local ISI calibrations.** Local ISI calibrations were based on plotting local PT with the set of 20 lyophilized plasmas and 7 normal plasmas on a natural logarithm scale (ln) against their mean certified PT. A calibration line was fitted by orthogonal regression obtaining the intercept (a) and slope (b). The precision of the slope (b) was determined according to WHO protocol by its CV, and CV(b) = 100 × se(b)/b, where se(b) is the standard error of b.

The ISIs of the local thromboplastins were derived as follows:

\[
ISI_{local} = b \times ISI_{ref}
\]

where ISI_{ref} is the ISI of the IRP.

For each participant center, we determined local INRs of the 5 validation plasmas using the local ISI (ISI_{local}) and the MNPT of the 7 lyophilized normal plasmas, where

\[
INR_{local} = \frac{PT_{local}/MNPT_{local}^{ISI_{local}}}{1}.
\]

For example, a local PT of 36 s, MNPT of 12 s, and local ISI of 1.0 would give a local INR of \((36/12)^{1.0} = 3.0\).

We calculated the mean INR of the 5 validation plasmas for all centers and examined the dispersion, expressed as % CV. In addition, we examined INR deviation (as a percentage) from certified values before and after local ISI calibration and tested it for significant difference with Cochran Q test. Table 1 shows results from the validation plasmas, giving overall mean INRs of the certified values together with results before and after correction obtained with the PT/INR Line and local ISI calibration. Table 2 shows INRs of the individual validation plasmas and their certified values.

**CERTIFIED INR**

The mean certified INRs on the 5 validation plasmas with human reagents ranged from 1.71 to 3.01 (overall mean 2.41, SD 0.50). With bovine/combined reagents, INRs ranged from 1.48 to 2.59 (overall mean 2.04, SD 0.45). With rabbit reagents, the INRs were higher, ranging from 1.81 to 3.81 (overall mean 2.80, SD 0.72).

A 1-way ANOVA test for differences in INR between the certifying centers was not significant (P = 0.39).

**INR CORRECTION WITH LOCAL ISI CALIBRATIONS**

**Human reagents.** The mean manufacturers’ ISI with human reagents was 0.94 (range 0.79–1.06). After local ISI calibration, mean ISI became 0.93 (range 0.77–1.15, mean CV of slope 1.90%). Average stated MNPT was 10.94 s (range 8.7–12.9), and mean local MNPT (from 7 lyophilized normals) was 11.85 (9.5–13.4). Through use of the manufacturers’ ISI and MNPTs provided by participants, the absolute mean deviation from mean certified INR with the 5 validation plasmas was 11.2%
After local ISI calibration, the absolute mean deviation became 0.4% (mean INR 2.40; 95% CI 2.34 –2.48). Percentage deviation from the certified INR plotted against the certified values for human thromboplastins is shown in Fig. 2A. The total proportion of validation plasmas within 10% of certified values was significantly increased after local ISI calibration ($P < 0.001$).

**Bovine/combined reagents.** The mean manufacturers’ ISI with bovine/combined reagents was 1.02 (range 1.0 –1.04), and after local ISI calibration, mean ISI was 1.11 (range 1.0–1.39; mean CV of slope 2.26%). Average MNPT stated was 37.7 s (range 32.1–47.5), and mean local MNPT was 37.9 (32.8–52.7). With the manufacturers’ ISI and MNPTs, the absolute deviation from mean certified INR was 4.9% (mean INR 1.94; 95% CI 1.80–2.07). After local ISI calibration, deviation was reduced to 0.5% (mean INR 2.05; 95% CI 1.90–2.19). Percentage deviations plotted against the certified values are shown in Fig. 2B. The total proportion of validation plasmas within 10% of certified values showed a nonsignificant increase ($P = 0.73$).

**Rabbit reagents.** The mean manufacturers’ ISI with rabbit reagents was 1.22 (range 1.07–1.72), and after local ISI calibration, the mean ISI became 1.44 (range 1.25–1.89; mean CV of slope 2.37%). Average stated MNPT was 12.8 s (range 12.1–13.5), and mean local MNPT was 13.2 (12.9–13.5). The absolute mean deviation from certified INR with the 5 validation plasmas before correction was 10.4% (mean INR 2.51; 95% CI 2.27–2.76). After local ISI calibration, the deviation was reduced to 1.1% (mean INR 2.83; 95% CI 2.50–3.16). Percentage deviations plotted against the certified values are shown in Fig. 2C. The total proportion of validation plasmas within 10% of certified values significantly increased after local ISI calibration ($P = 0.02$).

**INR CORRECTION WITH THE PT/INR LINE**

**Minimum number of plasmas required.** Table 1 shows absolute mean INRs of the 5 validation plasmas when derived from the PT/INR Line with increasing numbers of artificially depleted calibrant plasmas from 3 to 10. Testing with 3 ECAA plasmas gave a deviation of 1.66% from the certified INR with the PT/INR Line, compared to 0.4% with the local ISI calibration INR for the human reagents. The absolute deviation from certified INR was 0.4% by use of both 4 and 5 plasmas, and with no added benefit with increasing numbers up to

### Table 1. Results of validation plasmas required for the PT/INR Line.\(^a\)

<table>
<thead>
<tr>
<th>INR derivation</th>
<th>Human</th>
<th>Bovine/combined</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean INR (% CV)</td>
<td>INRs within 10% deviation from certified values (SE)</td>
<td>Mean INR (% CV)</td>
</tr>
<tr>
<td>Certified values</td>
<td>2.41</td>
<td>2.04</td>
<td>2.8</td>
</tr>
<tr>
<td>Before correction</td>
<td>2.68 (11.7)</td>
<td>34.3 (3.3)</td>
<td>1.93 (4.0)</td>
</tr>
<tr>
<td>Local ISI calibration</td>
<td>2.40 (4.7)</td>
<td>94.3 (1.6)(^b)</td>
<td>2.05 (3.2)</td>
</tr>
</tbody>
</table>

**Orthogonal regression**

<table>
<thead>
<tr>
<th>Plasmas</th>
<th>Mean INR (% CV)</th>
<th>INRs within 10% deviation from certified values (SE)</th>
<th>Mean INR (% CV)</th>
<th>INRs within 10% deviation from certified values (SE)</th>
<th>Mean INR (% CV)</th>
<th>INRs within 10% deviation from certified values (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Plasmas</td>
<td>2.37 (5.3)</td>
<td>90.5 (1.6)(^b)</td>
<td>2.13 (3.6)</td>
<td>82.5 (6.1)</td>
<td>2.84 (8.3)</td>
<td>70.0 (8.5) (^c)</td>
</tr>
<tr>
<td>4 Plasmas</td>
<td>2.40 (5.1)</td>
<td>94.8 (1.5)(^b)</td>
<td>2.12 (3.0)</td>
<td>82.5 (6.1)</td>
<td>2.83 (8.0)</td>
<td>76.7 (7.8) (^c)</td>
</tr>
<tr>
<td>5 Plasmas</td>
<td>2.40 (4.6)</td>
<td>97.6 (1.1)(^b)</td>
<td>2.09 (2.9)</td>
<td>90.0 (4.8)</td>
<td>2.82 (8.0)</td>
<td>73.3 (8.2) (^c)</td>
</tr>
<tr>
<td>6 Plasmas</td>
<td>2.38 (4.1)</td>
<td>97.1 (1.2)(^b)</td>
<td>2.10 (2.6)</td>
<td>92.5 (4.2)</td>
<td>2.83 (8.1)</td>
<td>76.7 (7.8) (^c)</td>
</tr>
<tr>
<td>7 Plasmas</td>
<td>2.39 (4.0)</td>
<td>96.7 (1.2)(^b)</td>
<td>2.11 (2.3)</td>
<td>87.5 (5.3)</td>
<td>2.83 (8.0)</td>
<td>76.7 (7.8) (^c)</td>
</tr>
<tr>
<td>8 Plasmas</td>
<td>2.40 (3.8)</td>
<td>96.2 (1.3)(^b)</td>
<td>2.11 (1.9)</td>
<td>92.5 (4.2)</td>
<td>2.83 (7.0)</td>
<td>80.0 (7.4) (^c)</td>
</tr>
<tr>
<td>9 Plasmas</td>
<td>2.40 (3.6)</td>
<td>97.1 (1.2)(^b)</td>
<td>2.11 (2.0)</td>
<td>92.5 (4.2)</td>
<td>2.84 (6.6)</td>
<td>80.0 (7.4) (^c)</td>
</tr>
<tr>
<td>10 Plasmas</td>
<td>2.41 (3.6)</td>
<td>96.7 (1.2)(^b)</td>
<td>2.12 (1.8)</td>
<td>92.5 (4.2)</td>
<td>2.84 (6.6)</td>
<td>76.7 (7.8) (^c)</td>
</tr>
</tbody>
</table>

**Linear regression**

<table>
<thead>
<tr>
<th>Plasmas</th>
<th>Mean INR (% CV)</th>
<th>INRs within 10% deviation from certified values (SE)</th>
<th>Mean INR (% CV)</th>
<th>INRs within 10% deviation from certified values (SE)</th>
<th>Mean INR (% CV)</th>
<th>INRs within 10% deviation from certified values (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Plasmas</td>
<td>2.40 (4.5)</td>
<td>96.7 (1.2)(^b)</td>
<td>2.10 (2.8)</td>
<td>92.5 (4.0)</td>
<td>2.83 (7.8)</td>
<td>73.3 (8.2) (^c)</td>
</tr>
</tbody>
</table>

\(^a\) Absolute mean INR (% CV) and the proportion of INR within 10% deviation from mean certified INR of the 5 independent validation plasmas before and after correction with local ISI calibration and with increasing numbers of ECAA calibrant plasmas from 3 in a set up to 10 for the PT/INR Line.

\(^b\) Statistically significant compared to INR before correction using Cochran Q test: $P < 0.001$.

\(^c\) Statistically significant compared to INR before correction using Cochran Q test: $P < 0.05$.  

10. With bovine/combined reagents, deviation from 3 calibrant plasmas was 4.4% with the PT/INR Line and 0.5% with local ISI calibration. The deviation became 2.5% with the PT/INR Line from 5 calibrant plasmas. With rabbit reagents, deviation with 3 ECAA plasmas using the PT/INR Line was 1.4% and 1.1% with local ISI calibration. With the PT/INR Line derived from 5 calibrant plasmas, deviation was 0.7% and similar with increasing number of plasmas from 6 to 10.

**Linear vs orthogonal regression analysis.** Fig. 3 shows an example of 2 PT/INR Lines obtained by orthogonal and linear regression estimated by the use of 5 calibrant plasmas at 1 participant center. Linear regression gave an intercept of 2.74 and a slope of 1.14. Orthogonal regression gave similar results, with the intercept being 2.73 and the slope 1.12.

This analysis was performed with results from all participant centers from both linear and orthogonal regression on the same calibrant plasmas. Results with the 5 validation plasmas are shown in Table 2. The mean deviation from certified INR with the PT/INR Line using linear regression was 0.4% with human, 2.9% with bovine/combined, and 1.1% with rabbit reagents. The mean $r^2$ value for the results with 5 calibrant plasmas for linear regression was 0.96 ($r^2$ range 0.88–1.00), and for orthogonal regression, 0.96 (0.91–1.00).

**INR CORRECTION WITH THE LOCAL PT/INR LINE**

Percentage deviation from the certified INR plotted against the certified values for human thromboplastins is shown in Fig. 2 alongside INR before correction and local ISI calibration.

With 5 ECAA calibrant plasmas for the PT/INR Line, INR deviation with the validation plasmas was reduced from 11.2% to 0.4% (mean INR 2.40; 95% CI 2.32–2.47) with human reagents (Table 1). The total proportion of validation plasmas with INR within 10% of certified INR significantly improved with the PT/INR Line ($P < 0.001$).

At centers using bovine/combined reagents, a deviation of 4.9% became 2.5% based on the PT/INR Line (mean INR 2.09; 95% CI 1.94–2.25). The total

| Table 2. Mean INR results of each of the 5 independent validation plasmas. |
|-----------------------------|--------|----------|--------|--------|--------|--------|
| Mean INR (SD) of the 5 lyophilized validation plasmas |
| Human reagents | n | Plasma 1 | Plasma 2 | Plasma 3 | Plasma 4 | Plasma 5 | Mean |
| Certified values | 42 | 1.71 | 2.02 | 2.51 | 2.81 | 3.01 | 2.41 |
| Before correction | 1.88 (0.19) | 2.25 (0.24) | 2.74 (0.33) | 3.17 (0.43) | 3.37 (0.44) | 2.68 (0.32) | 2.81 (0.29) |
| Local ISI calibration | 1.71 (0.08) | 2.04 (0.1) | 2.46 (0.13) | 2.83 (0.16) | 3.00 (0.18) | 2.41 (0.11) |
| PT/INR Line with 5 calibrant plasmas |
| Orthogonal regression | 1.67 (0.08) | 2.00 (0.09) | 2.45 (0.12) | 2.83 (0.17) | 3.02 (0.19) | 2.40 (0.11) |
| Linear regression | 1.68 (0.08) | 2.01 (0.09) | 2.45 (0.12) | 2.83 (0.16) | 3.01 (0.18) | 2.40 (0.11) |
| Bovine/combined reagents | 8 | 1.48 | 1.67 | 2.04 | 2.40 | 2.59 | 2.04 |
| Certified values | 1.37 (0.04) | 1.64 (0.08) | 1.97 (0.09) | 2.29 (0.08) | 2.41 (0.18) | 1.94 (0.08) |
| Before correction | 1.40 (0.04) | 1.69 (0.09) | 2.09 (0.08) | 2.48 (0.11) | 2.59 (0.09) | 2.05 (0.08) |
| Local ISI calibration | 1.41 (0.1) | 1.72 (0.07) | 2.13 (0.08) | 2.55 (0.1) | 2.66 (0.11) | 2.09 (0.06) |
| PT/INR Line with 5 calibrant plasmas |
| Orthogonal regression | 1.43 (0.09) | 1.73 (0.07) | 2.14 (0.08) | 2.54 (0.1) | 2.66 (0.1) | 2.09 (0.06) |
| Linear regression | 1.74 (0.2) | 2.36 (0.18) | 2.81 (0.2) | 3.29 (0.25) | 3.91 (0.36) | 2.82 (0.22) |
| Rabbit reagents | 6 | 1.81 | 2.30 | 2.77 | 3.32 | 3.81 | 2.80 |
| Certified values | 1.64 (0.07) | 2.15 (0.15) | 2.51 (0.19) | 2.89 (0.23) | 3.38 (0.43) | 2.51 (0.20) |
| Before correction | 1.65 (0.13) | 2.31 (0.15) | 2.80 (0.19) | 3.34 (0.26) | 4.05 (0.48) | 2.83 (0.20) |
| Local ISI calibration | 1.74 (0.2) | 2.36 (0.18) | 2.81 (0.2) | 3.29 (0.25) | 3.91 (0.36) | 2.82 (0.22) |
| PT/INR Line with 5 calibrant plasmas |
| Orthogonal regression | 1.77 (0.2) | 2.38 (0.18) | 2.82 (0.2) | 3.29 (0.25) | 3.89 (0.36) | 2.83 (0.22) |
| Linear regression | 1.77 (0.2) | 2.38 (0.18) | 2.82 (0.2) | 3.29 (0.25) | 3.89 (0.36) | 2.83 (0.22) |

*a Mean INRs before and after correction with local ISI calibration and with 5 ECAA calibrant plasmas used to derive the PT/INR Line using linear and orthogonal regression.*
proportion of validation plasmas within 10% of certified values was 87.5% which increased to 90.0% but was not significant \((P = 1.0)\).

At centers using rabbit reagents, a deviation of 10.4% was reduced to 0.7% (mean INR 2.82; 95% CI 2.53–3.11). The total proportion of validation plasmas within 10% of certified values was significantly increased \((P = 0.03)\).

Discussion

The WHO system of PT standardization in 1983 \((1)\) was a considerable advance in providing safe and effective dosage of oral anticoagulation. The major limitation of the INR system has been the difficulty of its implementation, requiring reliable ISI calibration of the local test systems. The difficulty has been due to the demands for local manual ISI calibration, because testing with the essential reference manual PT technique is now almost completely superseded.

It is reassuring, therefore, that the use of the PT/INR Line dependent on the small number of ECAA calibrant plasmas certified with the manual PT technique gives INRs as reliable as those obtained with local ISI calibrations. INRs derived with the PT/INR Line also show close agreement with certified INRs obtained with the recommended manual PT technique at experienced certifying centers.

With the PT/INR Line the following are no longer required:

- local ISI and MNPT;
- thromboplastin IRP;
- manual PT testing;
- PT on 60 stabilized anticoagulated patients;
- PT on 20 normal subjects;
In the present study, there was INR correction with only 3 calibrant plasmas with the PT/INR Line, but with 4 and 5 calibrant plasmas, results became as accurate as local ISI calibration. There was no further gain in INR reliability from increasing numbers of calibrant plasmas. The PT/INR Line showed greatest improvement with human reagents, and the reductions in INR dispersion with this method were similar to those achieved with local ISI calibrations.

The PT/INR Line used in the present study has similarities to the “Direct INR” method described previously (20, 21). However, in the present study, the number and types of plasmas are different. Only 2 abnormal plasmas and 1 normal plasma were used by Houbouyan and Goguel, and 3 abnormal plasmas and 1 normal in the SSC Guidelines. The present report shows that the use of normal plasma is not necessary for the PT/INR Line, as an MNPT is not required.

In their wide-ranging study, Adcock and Johnston (32) showed that their PT/INR curve was more successful than local ISI calibration, but their approach was based on deep-frozen plasmas, which are notoriously unstable. Their use of only 5 abnormal frozen plasmas and a single-species IRP (human) were limitations. The use of frozen (or fresh) plasmas on a multicenter basis would introduce variations due to uncontrolled storage instability.

The present study based on the PT/INR Line gave satisfactory results, similar to those with the manual PT at the certifying centers and with local ISI calibration. With this approach, the total proportion of validation plasmas showing <10% deviation from the certified values increased significantly for both human and rabbit reagents. With bovine/combined, overall mean deviation from certified INR was also reduced, although the proportion of validation plasmas <10% from certified values was unchanged.

The recommendation of 5 ECAA plasmas for the PT/INR Line allows for a possible aberrant result with 1 plasma, as INR derivation was shown to be reliably performed with only 4 plasmas.

Ideally, orthogonal regression should be used, but the present study indicates that with the set of 5 ECAA plasmas, both linear and orthogonal regression analyses gave similar results. This is in contrast with conventional local ISI calibrations, which we showed previously gave inferior results with linear regression (30). The number of plasmas used in conventional ISI calibration is much greater than with the PT/INR Line, and any difference in results between orthogonal and linear regression would be increased.
In this study, only 1 set of calibrant plasmas, ranging across the therapeutic interval, was used to derive the PT/INR Line. The demonstration of the success of the PT/INR Line with different sets and numbers of ECAA calibrant plasmas, with INR outside the therapeutic limits and including normal plasmas, would provide further evidence of the reliability and stability of the PT/INR Line. Such a study has been performed and will be reported (Ibrahim S, Poller L, Keown M, Jespersen J, unpublished data).

The present report details a simple, rapid method for reliable INR derivation that can be performed at the local level on only a small number of ECAA-certified lyophilized plasmas without requiring local manual PT testing.

The PT/INR Line gives immediate correction in terms of reference values, and reliable INRs can be obtained with only a small number of calibrant plasmas. That results compare well with local ISI calibrations is reassuring, particularly as similar INRs were obtained with rigorous manual PT testing at the experienced certifying centers.

The PT/INR Line has therefore been shown to simplify PT standardization, achieving reliable INRs without the need for ISI calibration.

Appendix

Worked Example

The principles involved are shown below of how an INR can be determined with a line estimated by using simple linear regression based, for example, on 5 calibrant plasmas. Linear regression can be calculated simply by using a conventional scientific calculator or widely available computer programs.

The PT/INR Line is derived by plotting certified INR (ln x_i) against local PT (ln y_i) of the calibrant plasmas on a natural log scale (ln) which has an intercept (a) of 2.43 and a slope (b) of 0.84. The INR result for a single validation plasma sample with a PT of 25 s can be derived by rearranging the regression equation to derive the INR (e^x) as follows:

\[ y_i = a + bx_i; \]
\[ y_i - bx_i = a; \]
\[ -bx_i = a - y_i; \]
\[ x_i = (a - y_i)/b; \]
\[ x_i = (-a/b) + (1/b)y_i, \]

where \( a \) (intercept) = 2.43, \( b \) (slope) = 0.84;
\[ x_i = (-2.43/0.84) + (1/0.84)y_i; \]
\[ x_i = -2.90 + 1.19y_i \]
\( (x_i \text{ on } y_i \text{ line fitted in Fig. 4}); \]
\[ x_i = -2.90 + 1.19*\ln(25 \text{ s}) = 0.93. \]

The INR of the validation plasma is therefore the exponential (antilog) of \( x_i = \exp(0.93) = 2.54 \).

It is not necessary to devise a graph such as shown in Fig. 4 to obtain the local INR, but the figure gives an illustration.

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As for the source of the ECAA plasmas, the sets of 5 ECAA certified calibrant plasmas are being made available from the European Concerted Action on Anticoagulation via Hart Biologicals Limited. Income received from their sale will be dedicated to continuing research in anticoagulation.

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