Discrepant sensitivity of thromboplastin reagents to clotting factor levels explored by the prothrombin time in patients on stable oral anticoagulant treatment: impact on the international normalized ratio system

Background and Objectives. We tested the principle of local International Normalized Ratio (INR) calibration using INR calibrator plasmas (PT Calibration Plasma Kit, Behring), two thromboplastin reagents (Neoplastin plus, rabbit brain, Stago, and Recombiplastin, recombinant human tissue factor, Ortho Diagnostics) and the same coagulometer (STA, Stago) on 92 patients on stable oral anticoagulant treatment.

Design and Methods. A four-point calibration was obtained with each reagent by linear regression (sec/INR) on a log-log scale ($r \geq 0.999$). The bias between the two reagents (Recombiplastin - Neoplastin Plus) was reduced from 31.7% to 17.5% and 7.5% ($p=0.001$) when results were expressed, respectively, as PT ratio (using the mean prothrombin time as denominator term), INR (using instrument-specific ISI supplied by the manufacturers) and calibrated INR, but there was a consistently significant regression of the differences over the average values even after log transformation ($r \geq 0.566$). The bias between the reagents was reduced to 1% ($p=ns$) when assuming Recombiplastin as the reference thromboplastin and applying Tomenson’s correction, but limits of agreements were as large as ±20%. Factor VII, X, V and II activity was measured with the two thromboplastin reagents in all plasma samples using immunodepleted plasmas (Stago).

Results. Statistically significant biases were observed for all clotting factors with the two reagents (Recombiplastin - Neoplastin Plus) and ranged from 3.5 % (FII) to −37.2% (FVII). In addition, for FVII and FV there was a significant regression of the difference over the average value (after log-transformation, $r \geq 0.282$). The patients were divided into 3 groups according to their degree of anticoagulation (INR <2.0; INR between 2.0 and 3.5; INR >3.5). Factor levels differed significantly with the two reagents throughout the 3 groups of patients. In addition, the relative distributions of the 3 vitamin K-dependent factors also differed in the 3 groups with the two thromboplastin reagents.

Interpretation and Conclusions. The discrepant sensitivity to factor VII, X and V levels of the two thromboplastin reagents explored in this study predicts INR calibration

B

because of its dependency on the vitamin K-dependent factors VII, X and II, the prothrombin time (PT) is used to monitor oral anticoagulant treatment. To account for the different sensitivity of thromboplastin reagents to reduced vitamin K-dependent factors, an International Sensitivity Index (ISI) is assigned to thromboplastin reagents or INR calibrator plasmas are used. The latter approach, first introduced in 1978, has recently gained large acceptance, because of the variation in ISI values resulting from the use of the same thromboplastin reagent with different coagulometers, and the relative feasibility of local INR calibration.

Although introduction of the INR system has undoubtedly improved the laboratory monitoring of patients on oral anticoagulant therapy, it is recognized that for an individual patient’s plasma the INR will not always be identical with different thromboplastins and methods, mainly because of the variation in the responsiveness of different thromboplastins to individual vitamin K-dependent clotting factors and to factor V. The INR system attempts to overcome this by using long-term stabilized patients in ISI calibration when the individual clotting factors should have achieved a stable level. It has, however, been shown that the decrease in the activity of vitamin K-dependent clotting factors induced by vitamin K antagonist is not the same for the different factors even in the stable
phase of anticoagulation. Thus, the issue of discrepant sensitivity of thromboplastin reagents to individual clotting factors is relevant not only to INR standardization, but also to the efficacy of oral anticoagulant treatment, because the roles of factors VII, X and II may not be the same with respect to bleeding and thrombotic risk of patients on oral anticoagulation.

In a study conducted in patients on stable anticoagulant treatment, we evaluated the improvement in the agreement of PT results obtained with two commercial thromboplastin reagents brought about by local INR calibration over the expression of the INR by instrument-specific ISI supplied by the manufacturers. In addition, we explored whether residual variability in calibrated INR values may depend on discrepant sensitivity of the reagents to the clotting factors explored by the prothrombin time.

Design and Methods
Reagents
All tests were performed using one fully automated coagulometer (STA, Stago, France). The two thromboplastin reagents evaluated in this study were from rabbit brain (Neoplastin Plus, Stago, France, lot 694262) and from recombinant human tissue factor (Recombiplastin, IL, Spain, lot rtf193). Coagulometer-specific ISI values provided by the thromboplastin manufacturers were 1.33 and 1.04 for Neoplastin Plus and Recombiplastin. Factor VII, X, II and V coagulant activities were measured with the two reagents using immunodepleted plasmas obtained from Stago and a 6-point calibration curve (100%-3.1%). Lyophilized INR calibrator plasmas from normal subjects and from patients on oral anticoagulant treatment were obtained from Behring (PT Calibration Plasma Kit). Consensus INR values attributed from the Austrian external quality control program were 0.99 for plasma A, 1.99 for plasma B, 2.96 for plasma C and 3.67 for plasma D. On each analytical session, plasmas were reconstituted according to the manufacturer’s instructions, kept at room temperature for 30 min, and tested with the two reagents within 60 min. Normal pooled plasma was obtained from 30 healthy subjects (15 men, 15 women) off medication, processed, frozen and stored as previously described. An arbitrary value of 100% for all clotting factors was assigned to this plasma pool.

Patients and controls
The study was conducted on plasma from patients on stable oral anticoagulant treatment attending the anticoagulation clinic in Cremona. Blood (4.5 mL) was drawn from an antecubital vein using a 19 gauge needle into tubes containing 0.5 mL of 0.129 M tri-sodium citrate (3.9%). Patients were selected to span a large INR interval based on determinations carried out with the thromboplastin routinely used in the clinical laboratory (Neoplastin Plus). On each of four days, 23 plasma samples were selected. Aliquots of plasma (0.3 mL) were dispensed in Nalgene tubes, snap-frozen with methanol and dry ice and stored at -80°C. Fresh plasma aliquots were then re-tested with the two PT reagents. The entire procedure was completed within 3 hours from blood drawing. On each day citrated plasma was also obtained from 6 apparently healthy volunteers (5 on day 3) off medication. Overall, 92 patients on oral anticoagulant treatment because of venous thromboembolism (n=28), arterial thromboembolism (n=12), heart valve prostheses (n=34) or atrial fibrillation (n=18), 34 women and 58 men with a mean age 64.3±12.5 yrs, were included in the study. Forty-eight patients were receiving warfarin and 44 patients were receiving acenocoumarol. The 23 healthy volunteers (12 women and 11 men) had a mean age of 35±12.5 years. Patients and healthy volunteers gave their informed consent to the study.

Plan of the study
Prothrombin time determinations were performed on fresh plasma in four analytical sessions. INR calibrator plasmas, and plasmas from patients (n=23) and healthy volunteers (n=6) were sequentially tested with the two reagents. Plasma samples were first tested with Recombiplastin on days 1 and 3 and with Neoplastin Plus on days 2 and 4. Prothrombin times obtained with Neoplastin Plus at the patients’ selection and at re-testing were not significantly different (p > 0.45, paired Student’s t test). Factor levels were determined in frozen plasma samples (0.25 mL aliquots) with the two thromboplastin reagents in four different analytical sessions. A new aliquot of plasma was thawed for the determination of each clotting factor and tested within 20 to 60 min. Factor V, factor VII, factor X and factor II and the corresponding calibration curves were always tested in this order, first with Neoplastin Plus on days 1 and 3 and with Recombi- plastin on days 2 and 4. All measurements were performed in duplicate and the analytical session was completed within 5 hours.

Prothrombin time results were expressed as (a) PT ratio using the geometric mean of the 23 normal subjects as denominator term (mean normal pro-
thrombin time), (b) INR using the instrument-specific ISI values supplied by the manufacturers (M-INR) and (c) locally calibrated INR by transformation of prothrombin times into INR with the use of INR calibrator plasmas (C-INR). This was accomplished by linear regression on a log-log scale with INR values on the abscissa and prothrombin time (in seconds) on the y axis. All the results of four calibrations with each reagent were plotted, obtaining r values of 0.999 with Neoplastin Plus and 1.000 with Recombiplastin. Finally, optimized INR (O-INR) (d) values with Neoplastin Plus were also obtained by attributing to Recombiplastin a sensitivity index of 1.00. Mean values of prothrombin times (sec) of patients and controls were plotted with Recombiplastin on the y axis and Neoplastin Plus on the x axis (log-log scale). Because the slopes of the orthogonal regression obtained for controls and for patients were significantly different (p=0.005) we applied Tomenson’s correction for non-linearity17 to the O-INR data calculated for Neoplastin Plus using the slope obtained for the patients only (1.499±0.037, standard error) and introducing the scale parameter d=0.102 (correction factor =e^{-0.102}).

Statistical analysis

Repeatability coefficients and method comparison analysis were carried out according to Bland and Altman.18 Continuous variables were analyzed by ANOVA after log-transformation. Concordance (Cohen’s k) and symmetry (McNemar) were calculated after assignment of patients to 3 groups according to their degree of anticoagulation. Dependency of PT results on clotting factor levels was estimated by linear regression analysis after log-transformation of the reciprocal of factor levels and corresponding PT values expressed according to the different modalities (PT ratio, C-INR, O-INR). Independent predictors of optimized INR values (O-INR) were analyzed in a generalized linear model, including reciprocal of factor levels, reagents and the interactions of factor levels with the two reagents as predictors. All the statistical analyses were carried out using a statistical software program (Systat).

Results

Repeatability coefficients obtained from duplicate measurements of prothrombin time and clotting factors are reported in Table 1. Because replicate determinations of all clotting factor levels with the two reagents were not available for 7 patients, data from 85 patients were included in this analysis. Coefficients lower than 1% were observed with both reagents for the prothrombin time, while they ranged from 1.86% to 5.34% for the clotting factor levels and were generally higher with Recombiplastin than with Neoplastin Plus. Method comparison analysis of PT ratio, M-INR, C-INR and O-INR values with the two thromboplastin reagents is shown in Figure 1 and Table 2. Only for O-INR was there no statistically significant regression of the differences over the average O-INR values after log transformation (r = 0.112, p > 0.2, Figure 1). The bias between the reagents (Recombiplastin-Neoplastin Plus) was reduced from 31.1% to 17.5% to 7.5% and 1.0% according the different expression of PT results, but only the bias with optimized INR values was not statistically significant (Table 2).

The results of method-comparison analysis for C-INR were not substantially different when including only the 73 patients with average calibrated INR values ≤3.67, the consensus value of calibrator plasma D (bias of C-INR = 5.4%, r of differences/average = 0.425).

The patients were divided into 3 groups according to their degree of anticoagulation. Irrespective of the modality of expression of PT results, patients with values lower than 2.0 were considered poorly anticoagulated and subjects with values greater than 3.5 excessively anticoagulated. This permitted analysis of symmetry and concordance of the results obtained with the two reagents according to the different modalities of expression of PT results (Table 2). Absence of significant symmetry and best concordance were obtained with C-INR, with 12% of patients showing discordant results potentially involving changes in the dosage regimens with the two reagents. With O-INR values, discordant results were observed for 16% of the patients. Thus, even forcing the model to obtain no bias in INR results obtained with the two reagents, there was a considerable degree of variation, with limits of agreement extending by around 20% in both directions (Table 2).

<table>
<thead>
<tr>
<th>Table 1. Repeatibility coefficients (%) for prothrombin time and clotting factors with the two thromboplastin reagents.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoplastin Plus</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Prothrombin time</td>
</tr>
<tr>
<td>Factor V</td>
</tr>
<tr>
<td>Factor VII</td>
</tr>
<tr>
<td>Factor X</td>
</tr>
<tr>
<td>Factor II</td>
</tr>
</tbody>
</table>
In an attempt to explain the source of such variability, factor levels explored by the prothrombin time were measured with both reagents. Factor V levels did not, as expected, show any relationship with the degree of anticoagulation, but they were consistently lower, both in patients and controls, when determined with Recombiplastin than with Neoplastin Plus (100% ± 23% vs. 112% ± 28%, p = 0.0001). Figure 2 shows the three vitamin K-dependent clotting factors measured with Neoplastin Plus (left panel) and Recombiplastin (right panel) in controls and in patients subdivided according to their degree of anticoagulation determined by the respective O-INR values. With the exception of patients with O-INR > 3.5 with Neoplastin Plus, the levels of the 3 factors differed significantly (Figure 2). In addition, at variance from factor VII and II levels, which showed a progressive decrease with both reagents with increasing degree of anticoagulation, factor X levels were similar with both reagents in patients with O-INR between 2.0 and 3.5 and in those with O-INR greater than 3.5.

Similarly, discrepancies in factor levels measured with the two reagents were also observed in the INR calibrator plasmas (Table 3). With the exception

Table 2. Method-comparison analysis of prothrombin time results (Recombiplastin-Neoplastin Plus) in the entire series of patients on stable oral anticoagulant treatment.

<table>
<thead>
<tr>
<th>PT Ratio</th>
<th>M-INR*</th>
<th>C-INR°</th>
<th>O-INR †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias (%)</td>
<td>31.7</td>
<td>17.5</td>
<td>7.5</td>
</tr>
<tr>
<td>p</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lower limit of agreement (%)</td>
<td>-38.9</td>
<td>-50.5</td>
<td>-20.5</td>
</tr>
<tr>
<td>Upper limit of agreement (%)</td>
<td>+55.2</td>
<td>+86.6</td>
<td>+25.4</td>
</tr>
<tr>
<td>r (Differences/Average)</td>
<td>0.896</td>
<td>0.643</td>
<td>0.628</td>
</tr>
<tr>
<td>p</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Symmetry (McNemar)</td>
<td>0.0001</td>
<td>0.009</td>
<td>ns</td>
</tr>
<tr>
<td>Concordant/ Discordant</td>
<td>54/38</td>
<td>71/21</td>
<td>81/11</td>
</tr>
<tr>
<td>Concorance (Cohen κ)</td>
<td>0.368 ± 0.074</td>
<td>0.654 ± 0.068</td>
<td>0.819 ± 0.054</td>
</tr>
</tbody>
</table>

* M-INR = INR calculated using the instrument-specific ISI values supplied by the manufacturers; ° C-INR = INR obtained with the calibrator plasmas; † O-INR = INR values calculated assuming a sensitivity index of 1.00 for Recombiplastin and applying Tomenson's correction (see text for explanations).
of factor II levels measured in the calibrator plasma with the highest INR value, all the remaining comparisons showed statistically significant differences.

Method comparison analysis of the two reagents (Recombiplastin – Neoplastin Plus) - restricted to patients on oral anticoagulant treatment - showed a significant bias for all the clotting factors, which was minor for factor X and factor II levels, but important for factor V and factor VII levels (Table 4). In addition, for factor V and VII, there was also a highly significant regression of the differences over the average factor levels (Table 4).

The differences observed with the two thromboplastin reagents in the measurement of clotting factor levels both in patients’ plasmas and INR calibrator plasmas strongly suggested that discrepant thromboplastin sensitivity to clotting factors explored by the PT might be a major determinant of the variability observed in PT results whatever the method of expression chosen. Figure 3 shows the relationship between PT ratio, C-INR and O-INR values and the vitamin K-dependent clotting factors measured with the two thromboplastin reagents in patients on oral anticoagulation. Whatever the modality of expression, PT results were strongly correlated with vitamin K-dependent factor levels with r values ranging from 0.67 to 0.94. However, the regression curves obtained with the two thromboplastin reagents differed significantly according to the modality of expression of PT results. For PT ratios, dependency on factor VII levels was similar with the two reagents; however, factor VII levels lower than 10% - not detected in any patient with Neoplastin Plus - were observed in 16 patients with Recombiplastin, in association with greater PT ratios. The regression curves obtained for factor X and II levels diverged significantly (p = 0.0001), as a result of an apparently higher sensitivity of the recombinant reagent. INR calibration with calibrator plasmas resulted in significant divergence of the

<table>
<thead>
<tr>
<th>INR calibrator plasma</th>
<th>A (0.99)</th>
<th>B (1.99)</th>
<th>C (2.96)</th>
<th>D (3.67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoplastin Plus (sec)</td>
<td>13.0±0.2</td>
<td>22.6±0.5</td>
<td>31.5±0.4</td>
<td>40.5±2.0</td>
</tr>
<tr>
<td>Recombiplastin (sec)</td>
<td>11.4±0.2</td>
<td>22.8±0.3</td>
<td>35.6±0.4</td>
<td>49.2±2.7</td>
</tr>
<tr>
<td>p</td>
<td>0.001</td>
<td>ns</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>FVII, Neoplastin Plus (%)</td>
<td>109±4</td>
<td>40±2</td>
<td>24±1</td>
<td>15±1</td>
</tr>
<tr>
<td>FVII, Recombiplastin (%)</td>
<td>92±2</td>
<td>28±1</td>
<td>15±1</td>
<td>8±1</td>
</tr>
<tr>
<td>p</td>
<td>ns</td>
<td>0.002</td>
<td>0.001</td>
<td>0.0001</td>
</tr>
<tr>
<td>FX, Neoplastin Plus (%)</td>
<td>92±2</td>
<td>22±1</td>
<td>11±2</td>
<td>11±2</td>
</tr>
<tr>
<td>FX, Recombiplastin (%)</td>
<td>81±2</td>
<td>16±1</td>
<td>9±1</td>
<td>8±1</td>
</tr>
<tr>
<td>p</td>
<td>0.009</td>
<td>0.001</td>
<td>0.023</td>
<td>0.002</td>
</tr>
<tr>
<td>FV, Neoplastin Plus (%)</td>
<td>88±4</td>
<td>38±1</td>
<td>22±1</td>
<td>13±1</td>
</tr>
<tr>
<td>FV, Recombiplastin (%)</td>
<td>76±2</td>
<td>32±1</td>
<td>21±1</td>
<td>13±1</td>
</tr>
<tr>
<td>p</td>
<td>0.0001</td>
<td>0.009</td>
<td>0.006</td>
<td>ns</td>
</tr>
<tr>
<td>FV, Neoplastin Plus (%)</td>
<td>103±4</td>
<td>84±6</td>
<td>80±4</td>
<td>94±7</td>
</tr>
<tr>
<td>FV, Recombiplastin (%)</td>
<td>83±4</td>
<td>69±3</td>
<td>66±2</td>
<td>72±2</td>
</tr>
<tr>
<td>p</td>
<td>0.0001</td>
<td>0.01</td>
<td>0.003</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Correlation coefficient (r) of the regression of the difference in log over average log values.

Table 3. Factor levels in calibrator plasmas with the two thromboplastin reagents (mean±SD of 4 replicate determinations).

Figure 2. Box plots of vitamin K-dependent clotting factor levels determined in controls (C) and in patients with the two thromboplastin reagents (left panel, Neoplastin Plus; right panel, Recombiplastin). Patients are divided into 3 groups (n = 24, n = 47; n = 21) according to optimized INR values calculated assuming a sensitivity index of 1.00 for Recombiplastin and applying Tomenson’s correction (see text for explanations). p values referring to the significance of the differences between the levels of the 3 clotting factors in each group are reported. Open box = factor VII; dashed box = factor X; cross-hatched box = factor II.
curves for factor VII, identity of the curves for factor X and incomplete coincidence of the curves related to factor II. Local calibration carried out assuming Recombiplastin as the reference thromboplastin led to full coincidence of the curves related to factor II, but increased divergence of those for factor VII and X (Figure 3).

The independent contribution of the clotting factors in the resultant O-INR value was explored using the generalized linear model, including first only the factor levels as predictors and then also including reagents and the interaction of reagents with factor levels as additional predictors. The two models explained, respectively, 79% and 87% of the total variation in O-INR values. As expected, factor VII (F value 111.8, p = 0.0001), factor X (F value 123.7, p = 0.0001) and factor II (F value 46.4, p = 0.0001) explained most of the changes in O-INR, but reagents (F value 11.5, p = 0.0009), and the interactions of reagents with factor VII (F value 12.7, p = 0.0005), factor X (F value 10.4, p = 0.0016) and factor V levels (F value = 3.9, p = 0.05) explained a significant part of the variability in O-INR values observed with the two PT systems.

Discussion
In this study we tested the principle of INR calibrator plasmas in improving commutability of PT results in patients on stable oral anticoagulant treatment using two thromboplastin reagents of human and rabbit origin and the same fully automated coagulometer. We used calibrator plasmas tested in Austrian quality control programs, which had been assigned consensus INR values ranging

Figure 3. Relationship between PT results (expressed according to different modalities) and vitamin K-dependent clotting factor levels as determined with the two thromboplastin reagents (open circles: Neoplastin Plus; closed circles: Recombiplastin). Regression lines of log-transformed data refer to reciprocal values (1:PT results/1:clotting factor levels) with only denominator terms shown for the convenience of the reader. p values indicate significance of the difference in regression curves obtained with the two reagents (continuous line: Neoplastin Plus; dashed line: Recombiplastin). Correlation coefficients (r values) ranged from 0.67 to 0.94.
from 0.99 to 3.67. The two reagents exhibited optimum repeatability coefficients of less than 1%.

We compared, by Bland and Altman analysis the agreement in the results expressed as PT ratios (using the mean normal prothrombin time of 23 healthy subjects), as INR values (using the instrument-specific ISI supplied by the manufacturers), and as INR values using INR calibrator plasmas. The latter process of calibration was accomplished on a log-log scale, yielding r values greater than 0.999. In comparison with the expression as PT ratios, the two above principles of INR calibration adopted in this study yielded a better agreement between the two thromboplastin reagents, but the bias observed in Bland Altman plots could not be abolished by log-transformation of the differences detected, resulting in a reduction from 32% to 17% to 7%. The bias observed with the ISI values supplied by the manufacturers may be partially explained by the higher than recommended citrate concentration in our blood collection tubes (0.129 M vs. 0.109 M) because the ISI of several, but not all, thromboplastin reagents was found to be approximately 10% lower when determined with samples collected into 0.129 M citrate than with samples collected into 0.109 M citrate.

However, in the same study, variations in citrate concentration did not affect linearity of the orthogonal regressions in PT values between the different thromboplastin reagents explored. Thus, the bias observed with the INR plasma calibrators can hardly be explained by the citrate concentration, which might have influenced the accuracy of INR values attributed to the calibrators, but not the relationship between the two thromboplastin reagents. The latter bias was not introduced by average INR values exceeding the upper value of the calibrator plasmas, because it was only minimally affected by removing from the analysis plasma samples with average INR values greater than 3.67. In addition, the bias was also virtually unchanged when excluding the normal plasma calibrator (plasma A) from regression analysis (6.9% vs. 7.5%, not shown).

Because the bias observed with M-INR and C-INR may be slightly underestimated - not accounting for the improvement in precision resulting from duplicate measurements, these data indicate an intrinsic failure in the process of INR calibration using the two reagents and the INR calibrator plasmas evaluated in this study. When optimizing INR calibration assuming one of the two reagents (Recombiplastin) as the reference thromboplastin, the bias between reagents was no longer statistically significant, but the variability in resultant INR values was high, with limits of agreements extending to 20% around the average INR values.

We looked for a possible explanation of these findings resulting from a discrepant sensitivity of the two thromboplastin reagents to the clotting factor levels screened by the prothrombin time. Again repeatability coefficients, determined for the different factors using the same factor deficient plasmas were acceptable with both reagents, although the rabbit reagent performed slightly better than the human recombinant reagent.

In both patients and healthy subjects we observed different mean factor levels detected by the two reagents. In addition, after dividing patients according to optimized INR values of less than 2, between 2 and 3.5 and greater than 3.5, there was also a significant difference in the levels of the different vitamin K-dependent factors, an observation confirming data previously reported in the literature.

Method comparison analysis on log-transformed data confirmed the bias between the two thromboplastin reagents for all clotting factors, indicating a higher sensitivity of the recombinant human reagent for all clotting factors but factor II. As previously noted for the recombinant thromboplastin evaluated in this study, with factor VII the bias was greatest (-37%) and we looked at the possibility that discrepant sensitivity to the reduction in clotting factors levels induced by vitamin K antagonists might be responsible for the variability in optimized INR values observed in our series of patients. By analysis of regression curves of PT values (according to the different modalities of expression) over clotting factor levels it appeared that the process of calibration had apparently led to identical sensitivity of the two reagents to factor II levels, but to discrepant sensitivity to factor VII and X levels.

The independent contributions of clotting factor levels, reagents and the interaction of reagents with clotting factor levels as determinants of O-INR values were evaluated in a generalized linear model. As expected, vitamin K-dependent clotting factor levels explained a major part of O-INR values, but reagents and differences in the sensitivity of the two reagents to factor VII, X and V contributed a significant portion of the variability in O-INR values. A correlation between factor V levels and the difference in INR with different recombinant and plain thromboplastin reagents in patients stabilized on oral anticoagulants has been recently reported.

The overall error of the INR has been attributed to lack of specificity of the PT tests and to calibration errors. Our data point to the intrinsic limitations of
the principle of INR calibration, which is dependent on the discrepant sensitivity of the two PT reagents explored in this study to the reduction in vitamin K-dependent clotting factors and to factor V levels. We could not evaluate the clinical significance of these findings. However, even optimizing the INR calibration process 16% of our patients would have been prescribed a change in the dosage of oral anticoagulant drug with one reagent but not with the other or vice versa. Because INR values are attributed by consensus to the calibrator plasmas investigated, it is expected that, dependent on the characteristics of sensitivity of the most widely used thromboplastin reagents, results such as those found in our study may be valid for some, but not all reagent comparisons. Interestingly, a discrepancy in the sensitivity of thromboplastin reagents could be expected from the measurement of clotting factors in INR calibrator plasmas. This might be particularly helpful in the evaluation of new thromboplastin reagents in routine laboratories involved in the monitoring of oral anticoagulant treatment.

In a multicenter study evaluating the response of different thromboplastin reagents/instruments combinations to a lyophilized normal plasma, we observed statistically significant differences between combined, plain and recombinant reagents. Future attempts to standardize laboratory monitoring of oral anticoagulant treatment should probably be directed at single factor measurements. Instead of the INR, the monitoring of factor II levels has been suggested to reflect the antithrombotic potential of oral anticoagulant treatment better.

In this study, the bias observed in method comparison analysis for factor X and factor II, albeit statistically significant, did not exceed 4% and was not influenced by a statistically significant regression of the differences over the average. Our results clearly indicate that the process of INR calibration aims to obtain a comparable sensitivity to factor II levels. We have shown a closer agreement of prothrombin fragment 1+2 levels with factor II than with INR values both during the early and the steady phase of oral anticoagulant treatment.

Direct measurement of this factor in plasma should be preferred as an indicator of the in vivo degree of anticoagulation.

Contributions and Acknowledgments
ST, LG AF and ADA contributed to the design of the study, analyzed the data and prepared the manuscript. GM and ND performed the clotting assays reported in this study.

Disclosures
Conflict of interest: none.
Redundant publications: none.

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References
Discrepant thromboplastin sensitivity


Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received April 12, 2002; accepted October 15, 2002.

What is already known on this topic

The prothrombin time is used to monitor oral anticoagulant treatment in clinical practice. The INR system has considerably improved this approach but has not completely removed laboratory variability.

What this study adds

This study shows that residual variability in calibrated INR values may depend on discrepant sensitivity of the reagents to the clotting factors explored by the prothrombin time.

Potential implications for clinical practice

Future attempts in the standardization of the laboratory monitoring of oral anticoagulant treatment should probably be directed at single factor measurements.

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