Biological variation of International Normalized Ratio for prothrombin times, and consequences in monitoring oral anticoagulant therapy: computer simulation of serial measurements with goal-setting for analytical quality

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Oral anticoagulant therapy (OAT) has a well-established efficacy in prophylaxis and treatment of thromboembolic disorders. Because complications are related to intensity of OAT, optimal control of treatment is mandatory. In studies of OAT, as many as 30% of International Normalized Ratio (INR) measurements for prothrombin times fall outside the therapeutic interval. Preanalytical, analytical, and biological variation all contribute to this. Computer simulations of serial INR measurements were performed for various assumed in-treatment setpoints within the therapeutic interval INR 2.0–3.0 and for an “in-treatment within-subject variation” (CV) of 10.1%. Results are presented in difference plots with therapeutic intervals and critical differences. If the in-treatment setpoint is mid-interval (INR = 2.5), only 5% of simulated INR values fall outside the therapeutic interval. Setpoints deviating from the mid-interval and increases in the in-treatment within-subject variation considerably increase the number of observations outside the therapeutic interval and the critical differences. In conclusion, random variation, biological or analytical, and setpoints (targets) deviating from mid-interval explain a substantial number of the INR values outside therapeutic intervals observed in clinical studies. Analytical imprecision should be kept <5% and analytical bias <±0.2 INR.

Oral anticoagulants (vitamin K antagonists) are well-established in the treatment and prophylaxis of various thromboembolic disorders [1]. However, monitoring and management of oral anticoagulant therapy (OAT) is still a complex task. The performance of different treatment regimens during clinical trials is assessed by the frequency of side effects, e.g., thromboembolic and hemorrhagic episodes occurring during the treatment period. The risk of side effects is closely related to the intensity of OAT and correlates with the time the patient spends with values outside the therapeutic interval [2]. The International Normalized Ratio (INR) for prothrombin times has improved laboratory monitoring. However, in controlled clinical trials oral anticoagulants as well as in centers where computer-assisted decision-support systems are applied, optimal treatment, defined as INR values within the therapeutic interval, is achieved only 60–70% of the time [3, 4]. In part this may be related to a highly variable response to OAT in individual patients and to the fact that vitamin K antagonists lack a simple relationship between a given dose and therapeutic effect. Interactions with factors such as dietary intake of vitamin K, ethanol, and intercurrent infections may also be important [1, 5]. Furthermore, analytical and preanalytical factors, e.g., variability in procedures for blood drawing, handling, and processing also add to the total variation in prothrombin time determinations.

It is therefore reasonable to assume that INR values, even from patients on stable OAT, fluctuate for a given intensity of anticoagulation. Extremes of such fluctuation occurring on the day of venipuncture could give values that interfere with adjustment of coumarin therapy. We recently estimated that the intraindividual variation of INR during treatment (the “in-treatment within-subject variation”) was 10.1% [6]. No studies so far have considered the impact of this intraindividual variation, which

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reflects both biological and analytical components, on measured INR and on therapeutic control in patients in OAT.

In daily clinical practice it is difficult to obtain a sufficient number of INR measurements from individual patients within a reasonable timespan to address the problem. We therefore decided to illustrate the problem by computer simulations, as has been done elsewhere to address various problems in clinical situations [7–10]. The aims of our study were (a) to develop an educational tool with the scatterplot, which would allow the effect of the in-treatment within-subject variation on consecutive INR measurements to be visualized, and (b) to investigate to what extent in-treatment within-subject variation alone, or with assumed in-treatment setpoints selected above and below the mid-interval setpoint, could explain values outside the intended therapeutic intervals and critical differences in patients in stable OAT. Further, the effects of analytical bias and imprecision were investigated. The results are presented in relation to a graphical model with difference plots that incorporate the therapeutic interval and limits for critical differences [11].

Materials and Methods

Computer Simulation

The simulations performed were based on the assumptions that:

1) INR values for a patient on continuous, constant OAT would be gaussian-distributed around an assumed in-treatment setpoint, \( \mu \), with an assumed in-treatment standard deviation, \( \sigma \).

2) The simulations reflected steady-state conditions, e.g., a patient receiving a constant dose of vitamin K antagonist.

3) Consecutive measured INR values were independent of each other.

To mimic already established therapeutic intervals and targets for in-treatment setpoints for OAT, we chose the assumed in-treatment setpoints as being within the interval INR 2.0–3.0 [5]. The total CV—the in-treatment within-subject variation—of 10.1%, was based on our previous study in patients in stable OAT [6]. Given the assumed in-treatment setpoint, \( \mu \), and the in-treatment within-subject variation, CV, we calculated \( \sigma \) as follows:

\[
\sigma = CV\% \times \frac{\mu}{100}
\]

The critical difference for the 95% range of differences was calculated as

\[
1.9 \times \sqrt{\frac{2}{n}} \times \sigma = 2.77 \times \sigma,
\]

which for \( CV = 10.1\% \) gives 28%

Arrays of gaussian-simulated INR values were generated with the computer software, SPIDA [12]. One thousand random values for INR were generated for the following assumed in-treatment setpoints within the therapeutic interval 2.0–3.0: \( \mu = 2.1, 2.5, \) and \( 2.9 \). Corresponding assumed in-treatment \( \sigma \) values were calculated with the above equation for in-treatment within-subject variations of 10.1% and 15%. The arrays of generated INR values were regarded as consecutive measurements within single patients and the differences between consecutive measurements were calculated. To control the simulation procedure, we calculated the estimated in-treatment setpoint, \( \bar{x} \), and the estimated in-treatment standard deviation, \( s \), from the values produced. To incorporate information about the degree of anticoagulation in relation to the therapeutic interval and in relation to the last measured value, we recently proposed the use of difference plots. According to this model, the difference between the last measured INR value and the previous value, \( \text{INR}_i - \text{INR}_{i-1} \), should be plotted against INR [11].

Calculation of Number of Observations Exceeding Therapeutic Intervals

The fraction of INR observations observed outside the therapeutic interval was calculated from the gaussian-distributed simulated data for the assumed in-treatment setpoints \( \mu = 2.1, 2.3, 2.5, 2.7, \) and \( 2.9 \), according to different assumed in-treatment within-subject CVs (7.5%, 10%, 15%, and 20%). For this purpose we used the gaussian distribution as available in the computer software Statistix 4.0 [13].

Introduction of Analytical Bias

Analytical bias will impose a systematic error on the measured INR values. We investigated the effect of analytical bias, i.e., from \(-0.4 \) to \( 0.4 \), on the number of INR measurements falling outside the therapeutic interval INR 2.0–3.0 when the clinician’s target is INR 2.5. The percentage of true INR values falling outside the therapeutic interval was estimated by the use of the gaussian-distributed data. In the model of analytical bias, the “true” patient value is \( X_i = \mu + w_i \), where \( \mu \) signifies the steady-state value and \( w_i \) the within-subject variation (analytical and biological variation). The measured value will therefore be \( Y_i = \mu + w_i + B \), where B is the analytical bias.

Results

Assumed In-treatment Setpoint \( \mu = 2.5 \) INR

The plots of INR against INR revealed an ellipsoid cluster centered around the estimated in-treatment setpoint (Fig. 1, top). For the assumed in-treatment setpoint \( \mu = 2.5 \) within the therapeutic intervals 2.0–3.0 and the assumed in-treatment within-subject variations CV = 10.1%, \( \sim 5\% \) of the simulated results were below or above the vertical lines that represent the therapeutic interval. Because the random array of INR values generated and the lines
Fig. 1. Simulation of 1000 INR values during OAT; the vertical lines represent the therapeutic interval for INR 2.0–3.0, and the diagonal lines represent the limits for the upper and lower critical differences for a two-sided test at 95% significance when CV is 10.1%.

(Top) Combined presentation of paired observation of INRi and ΔINR for assumed in-treatment setpoint, μ = 2.5, and assumed in-treatment standard deviation, σ = 0.25 (CV = 10.1%). The distribution of the simulated data showed a mean value for INR (estimated in-treatment setpoint), x̄, of 2.5 and an estimated in-treatment standard deviation, s, of 0.25. The effect of “regression toward the mean” is evident from the simulated points. (Middle) For μ = 2.1 and σ = 0.21, the simulated distribution showed x̄ = 2.1 and s = 0.21. (Bottom) For μ = 2.9 and σ = 0.29, the simulated distribution showed x̄ = 2.9 and s = 0.29.
EFFECT OF OTHER ASSUMED IN-TREATMENT SETPOINTS
If different assumed in-treatment setpoints for INR were applied within the therapeutic interval 2.0–3.0, the ellipsoid cluster would be centered according to the assumed in-treatment setpoints. For μ = 2.1 and 2.9, e.g., this resulted in a considerable number of observations below or above the therapeutic interval, respectively, as shown in Fig. 1 (middle and bottom). However, the total number exceeding the critical differences will be unchanged, for this is calculated according to the setpoint. Table 1 summarizes the number of INR measurements that fall outside the therapeutic interval for different assumed in-treatment setpoints, μ, calculated by the use of the gaussian distribution.

EFFECT OF INCREASING VALUES FOR THE IN-TREATMENT WITHIN-SUBJECT CV
Too intense or inadequate anticoagulation poses considerable risks for complications in patients receiving OAT. The effect of increasing the assumed in-treatment within-subject CV to 15% is illustrated in Fig. 2. The percentage of observed INR values above or below the therapeutic interval was calculated according to the gaussian-distributed data, and is presented separately and combined with the total percentage exceeding the limits for the therapeutic interval for various assumed in-treatment setpoints in Fig. 3 (top). In Fig. 3 (bottom), the corresponding calculations for various assumed in-treatment setpoints and increasing in-treatment within-subject variation are presented.

Differences in serial measurements of INR (ΔINR) that fall outside the lines for critical difference reflect a considerable change between serial INR measurements, and changes in dose of anticoagulants should be considered. If the in-treatment within-subject variation is lower or higher than 10.1%, then theoretically the 95% coverage intervals should be changed accordingly. However, the coverage intervals in individual patients will in most instances not be known. If the coverage interval based on our previous estimate of the in-treatment within-subject variation of 10.1% is used with various assumed values for within-subject variation, the number of INR results determined by the use of computer simulation to be outside the coverage interval will be distributed according to Fig. 4.

CONSEQUENCE OF ANALYTICAL BIAS
The size of the assumed in-treatment setpoint and the in-treatment within-subject variation will determine the number of INR values outside therapeutic intervals. Clinical decisions, such as a change in OAT dose, based on INR values accidentally outside the therapeutic interval will lead to fluctuating INR measurements (i.e., a “ping-pong” effect: too intensive anticoagulation alternating with insufficient anticoagulation [11]). Analytical bias giving rise to a shift in the setpoint (and often not apparent to the clinician) also poses a risk for misinterpretation of INR values obtained and may lead to too little or too much anticoagulation.

The effect of increased CV is easy to grasp but the effect of analytical bias may need further explanation. If a laboratory has an analytical bias of +0.4 INR, then all measured values are 0.4 INR too high. This will lead the clinician to compensate by changing the target to a measured 2.5 INR, the mid-point in the interval 2.0–3.0—which, however, in the patient corresponds to 2.1 INR. If the patient’s CV is ~7.5%, then the clinician will expect virtually no measured values (Y) to be outside the interval; but the patient will be monitored at a lower INR, with the result that ~25% of the “true” values (X) will be <2.0.

### Table 1. Percentage of INR measurements <2.0 and >3.0, with different assumed in-treatment setpoints for INR and 10% CV.

<table>
<thead>
<tr>
<th>Clinical setpoint</th>
<th>μ</th>
<th>INR values &lt;2.0 (%)</th>
<th>INR values &gt;3.0 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INR</td>
<td>σ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>0.21</td>
<td>31.7</td>
<td>0.0</td>
</tr>
<tr>
<td>2.3</td>
<td>0.23</td>
<td>9.6</td>
<td>0.1</td>
</tr>
<tr>
<td>2.5</td>
<td>0.25</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>2.7</td>
<td>0.27</td>
<td>0.5</td>
<td>13.3</td>
</tr>
<tr>
<td>2.9</td>
<td>0.29</td>
<td>0.1</td>
<td>36.5</td>
</tr>
</tbody>
</table>

* Calculated from gaussian distribution of data.
Most likely, this will not be observed by the clinician. Consequently, an analytical bias will lead to mistreatment of all patients as long as it persists. The effect may be acceptable for bias between $-0.2$ and $+0.2$ INR, but greater bias may have significant consequences for the patients. The effect of introducing analytical bias and increasing CVs is presented as function of analytical bias in Fig. 5, which shows the number of “true” values, $X_i$, outside the therapeutic interval.

**Discussion**

Effective OAT requires an intensity of anticoagulation sufficient to guard against thromboembolism without unnecessary hazards to patients in the form of major bleeding complications. To solve the problem of variability of different thromboplastins, the INR system was proposed in 1983. The INR system improved the laboratory control through improved trueness (accuracy), and opened up for universal applicability guidelines for intensity of anticoagulation for different indications.

When treatment with OAT is initiated in a patient, a therapeutic interval and a target for in-treatment setpoint are selected according to the indication for treatment and individual risk factors. During initiation of therapy and in steady-state, all INR values will be compared with this setpoint or therapeutic interval and doses adjusted accordingly. The intensity of anticoagulation actually achieved, the steady-state in-treatment setpoint, in terms of the INR value, is, however, affected by several factors that may lead to poor therapeutic control. As is obvious from controlled trials, where therapeutic control must be assumed to be more optimal than in daily clinical practice, a substantial number of INR measurements fall outside the intended therapeutic interval (poor therapeutic control). The reason for this is unclear, but spontaneous variation, in part due to biological variation of the prothrombin time and thus INR, could contribute. This
variation might lead to frequent changes in the dosing and introduce “ping-pong” effects with regard to dosing with vitamin K antagonist [11].

To address some of the problems when monitoring OAT patients, we performed this computer simulation study as an extension of our previous study. Thus, the computer simulations were based on the assumption of a gaussian distribution of consecutive INR values around assumed in-treatment setpoints, and on our previous estimate of the in-treatment within-subject variation of INR measurements in patients in stable OAT [6]. Computer simulation is not necessary to obtain information about the number of INR measurements that fall outside the therapeutic interval; this information may also be obtained with software that provides a cumulative gaussian distribution or with use of statistical tables. Regarding the number of observed differences falling outside the critical differences, however, the theoretical calculations are much more complicated (with simulation of $\sigma$/ but calculation of critical differences based on constant CVs), so here the computer simulations provide a simple solution.

With a mean in-treatment setpoint of 2.5 and in-treatment within-subject variation of 10.1%, only 5% of INR measurements will be $<2.0$ or $>3.0$, a generally accepted therapeutic interval for the treatment of deep venous thromboembolism and pulmonary embolism [1]. However, even a small change in the assumed in-treatment setpoints substantially influences the fraction of INR values observed outside the therapeutic interval.

The fact that as many as 30% of INR measurements are outside therapeutic intervals in major studies of anticoagulation could reflect a combination of higher CVs and deviating setpoints. The simulation study suggests that the intrindividual variation is actually $>10.1\%$, so for the optimal setpoint of 2.5 an intrindividual variation as great as 18% is likely (Fig. 3, bottom). In a prospective study of patients receiving OAT, we found a total within-subject CV (analytical and biological variation) of 14.1% [14].

We have previously suggested a graphical method that uses difference plots for presenting consecutive INR measurements [6, 11]. This model incorporates lines that reflect the critical differences (expressed as 95% coverage intervals of difference) and the therapeutic interval and is a clinically relevant approach. This method, however, gives rise to regression towards the mean, given the mathematical relationship between the difference $\text{INR}_i - \text{INR}_{i-1}$ and $\text{INR}_i$, contributing to both of the variables that are plotted. Plotting $\text{INR}_i$ against $1/2(\text{INR}_i + \text{INR}_{i-1})$ would correct these findings but would be less relevant.

![Percentage of real patient values outside the therapeutic interval](image)

**Percentage of real patient values outside the therapeutic interval**

- **CV=20%**
- **CV=15%**
- **CV=10%**
- **CV=7.5%**

Calculations are based on gaussian distribution for different assumed in-treatment within-subject variations (7.5%, 10%, 15%, and 20%) and for increasing analytical bias. The effect of bias is inverse to the effect of wrong target/setpoint but unknown to the clinician.
within-subject variation may have lead to an underestimation of the in-treatment therapeutic control, whatever the reason, were excluded. This neous factors. All patients with unstable or poor therapeu analytical, biological factors, and interactions with extraneous factors. All patients with unstable or poor therapeutic control, whatever the reason, were excluded. This may have lead to an underestimation of the in-treatment within-subject variation.

Selection of the patients that were used for calculation of the in-treatment within-subject variation may have influenced the estimated CV. The CV value we used was based on retrospective data from patients in whom sufficient consecutive INR measurements were available and where no change in OAT had taken place during the study period—thereby lumping together preanalytical, analytical, biological factors, and interactions with extraneous factors. All patients with unstable or poor therapeutic control, whatever the reason, were excluded. This may have lead to an underestimation of the in-treatment within-subject variation.

Use of biological variation obtained from healthy individuals would have been meaningless for this computer simulation because the quantity in question, the prothrombin time (INR), is dependent on the dosage of vitamin K antagonist. Furthermore, the intraindividual biological CV is generally accepted as being higher in the diseased state than in health. In consequence, intraindividual variation of INR must be assessed in patients receiving vitamin K antagonists when they are in steady-state of OAT; that evaluation is probably a realistic estimate of the minimal variation that can be expected during OAT.

Estimating the in-treatment within-subject variation and the use of consecutive INR values in the computer simulation studies provides information about the probability, under standardized conditions, of obtaining INR values outside a therapeutic interval. Knowledge regarding the size of this variation could perhaps lead to improved dose adjustments in patients receiving vitamin K antagonists; this information also would allow the clinician, in selected cases, to perform optimal treatment with oral anticoagulants for other targets and intervals. The differences in results of simulation performed with setpoints deviating from mid-interval (Fig. 1, middle and bottom) stress that the therapeutic interval must be selected accordingly. Estimating the total CV for individual patients in whom anticoagulation is in a steady-state may be worthwhile, if lifelong treatment is planned. Using difference plots with therapeutic interval and the critical differences could perhaps reduce the risk of changing the dose of anticoagulant when no significant change has actually taken place, even though the value for INR may be outside the therapeutic interval [11]. Such an approach can reduce the risk of a “ping-pong” effect.

In the majority of laboratories the imprecision is well below 5% and will have only negligible effect on the biological in-treatment CV, which usually exceeds 10% [16]. The influence of analytical bias, unknown to the clinician, should also be considered in assessment of quality of treatment with oral anticoagulants. The present study suggests that analytical bias will lead to mistreatment of patients if it exceeds ±0.2 INR (Fig. 5). Estimation of the analytical bias for individual institutions requires analysis of externally provided samples. This was illustrated in a Nordic external assessment of analytical quality, where an analytical bias accounted for a between-laboratory CV of 10.5% without use of a common ISI-calibrator. This between-laboratory imprecision was reduced to 3.9% by use of a common ISI-calibrator, emphasizing the need for proper international calibration [17].

Simulation studies of clinical situations, as performed in this study, are inexpensive and do not entail health risks. They allow ethical evaluation of hypotheses according to various assumed conditions—optimal as well as extreme (unacceptable to patients and others). Thus, computer simulation may be an important tool for performance testing of clinical strategies before their introduction. The simulation procedure allows as many measurements as required, but caution is required when positive results are obtained and when the results are transferred to the clinical situations that initiated the simulation. Nevertheless, a simulation study may disclose the limits for what is possible. Data from the present study seem to suggest that some of the INR values measured outside therapeutic intervals in patients during stable OAT may be attributable to spontaneous fluctuations. Given that the in-treatment within-subject variation probably is higher than suggested in the previous retrospective study, this spontaneous variation may be even more important. The impact of higher than expected within-subject variation may be aggravated by a tendency in controlled clinical trials to aim at a lower target for in-treatment setpoints than the central value within the therapeutic interval [3] (reflecting a concern over severe hemorrhagic complications [5]). Systematic analytical bias, a laboratory problem that usually is not apparent to the clinician who is treating the patient and who is reacting to the INR measurement, will substantially increase the number of INR values that fall outside the therapeutic interval but without the clinician’s knowledge. Therefore, analytical bias will probably be reflected only in the number of patients experiencing side-effects to the treatment, e.g., thromboembolic complications and hemorrhagic events.

In conclusion, the simulations suggest that therapeutic intervals can be chosen to be unrealistically narrow when related to the steady-state in-treatment within-subject variation. Attempts to decrease the total variation by better education of the patients could perhaps help solve this problem. Many of the factors affecting the effectiveness of anticoagulation, however, cannot be corrected. The therapeutic interval should be considered more as a target for optimal treatment than a standard for rigorous decision limits for changing dosage. Otherwise, the ping-
pong effect will dominate the clinical performance \cite{11}. If a setpoint of 2.5 mean INR is chosen and the total CV is 10.1%, it will be impossible to obtain 100% of INR measurements within the INR interval 2.0–3.0, even if the patient is in steady-state during the total period of observation. Either one has to be satisfied with a quality of anticoagulation in which \(~80\)% of the INR measurements are within the therapeutic interval, or the therapeutic interval should encompass INR 2.5 \(\pm\) 3 s (target \(\pm\) 3 times the standard deviation). The effect of analytical imprecision may be negligible because it is usually <5\%, which is less than one-half of the CV of in-treatment variation, but the effect of analytical bias may be considerable if it exceeds \(\pm\)0.2 INR.

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\textbf{Appendix: Glossary}

Target for in-treatment setpoint: clinical goal for the mean INR values in OAT.

In-treatment within-subject variation: the CV of INR measurements for patients in steady-state OAT.

Assumed in-treatment setpoint: the setpoint applied in computer simulation.

Assumed in-treatment variation/standard deviation: the CV and \(\sigma\) assumed for a patient in the computer simulations performed.

Estimated in-treatment setpoint: the calculated setpoint achieved by performing the computer simulations.

Estimated in-treatment variation/standard deviation: the calculated CV and \(\sigma\) achieved by computer simulation.

\textbf{References}