Variability of INR in patients on stable long-term treatment with phenprocoumon and acenocoumarol and implications for analytical quality requirements

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Summary
Within each patient treated with vitamin K antagonist (VKA), variation of the international normalised ratio (INR) occurs over the treatment period. The purpose of the present study was to assess INR variation in selected patients on long-term treatment in whom the dose of VKA was not changed. This type of variation is considered as “biological variation” which is caused by many factors but not VKA dose changes or other medication. Four groups of long-term patients were examined: each group with a different VKA (acenocoumarol or phenprocoumon) or a different target intensity (INR 2.0–3.5 or 2.5–4.0). All patients were monitored with the same PT system (Hepato Quick, STA-R Evolution coagulation instrument) by one laboratory. The variation of the INR within each patient was expressed as coefficient of variation (CV, in %). The CV was corrected for the average imprecision of the INR measurement (CV, 2.4%). The mean corrected CV values for the four groups were: 10.9% (acenocoumarol, target INR 2.0–3.5); 10.5% (acenocoumarol, target INR 2.5–4.0); 10.4% (phenprocoumon, target INR 2.0–3.5); 9.1% (phenprocoumon, target INR 2.5–4.0). The analytical performance goal for the INR measurement (imprecision) can be derived from the within-subject biological variation. Desirable INR imprecision goals are <4.9% and <5.3% CV for monitoring of phenprocoumon and acenocoumarol, respectively. These goals were achieved using the aforesaid PT system.

Introduction
Vitamin K antagonists (VKA) have a narrow therapeutic range, and the response to a given dose varies considerably between individuals. Also the intra-individual response may vary, depending on genetic and environmental influences. The quality of the VKA therapy depends on therapy compliance of the patient and also of the competence of the physicians who manage VKA therapy. They should do so in a systematic and coordinated fashion, incorporating education of and communication with patients, reacting properly on interfering medicines and intercurrent diseases, systematic international normalised ratio (INR) testing, tracking and observing the follow-up of results and dose adjustments (1). The dose is adjusted according to INR measurements. To guarantee an appropriate quality of INR test performance, each laboratory performing INR measurements should have an appropriate analytical quality control system. Analytical quality is controlled by internal quality control procedures and external quality assessment. Nowadays, it is widely accepted that analytical performance goals (analytical quality specifications) should be based on the biological variation. Fraser et al. introduced the concept of minimum, desirable and optimum quality goals for imprecision and inaccuracy of laboratory methods (2). Inasmuch as analytical performance goals are derived from the biological variation, knowledge about this is essential for establishing the limits for minimum, desirable and optimum performance.

The primary purpose of the present study was to determine the total variation of the INR within patients in whom the dose of VKA was not changed during the observation period. The total variation is determined by a combination of the biological, analytical and preanalytical variation. In the present study, all INR measurements were performed by one laboratory using a single brand of thromboplastin and a single brand of coagulation instru-
The analytical between-run imprecision of the laboratory was assessed using accepted methods (3). The data were used to estimate the biological variation of the INR, i.e. the within-patient variation due to pharmacokinetic and pharmacodynamic effects of a fixed dose of VKA, under conditions of real life.

As far as we know, within-patient biological variation of the INR in patients treated with phenprocoumon has been investigated in one study only (4). In the present work we report the within-patient biological variation of the INR in patients treated with acenocoumarol or with phenprocoumon, at two different target INR intervals.

The second purpose of our study was to assess the desirable imprecision goals for INR determination as defined by Fraser et al. (2).

### Table 1: Between-day imprecision of INR estimated from EQA.

<table>
<thead>
<tr>
<th>EQA sample</th>
<th>Testing period (months)</th>
<th>Number of tests</th>
<th>Mean INR</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-23</td>
<td>28</td>
<td>10</td>
<td>3.14</td>
<td>4.0</td>
</tr>
<tr>
<td>C-24</td>
<td>18</td>
<td>7</td>
<td>2.93</td>
<td>2.7</td>
</tr>
<tr>
<td>C-25</td>
<td>8</td>
<td>5</td>
<td>4.05</td>
<td>4.1</td>
</tr>
<tr>
<td>C-26</td>
<td>12</td>
<td>6</td>
<td>2.98</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Patients and methods

The present study is a retrospective analysis of INRs determined in out-patients treated with phenprocoumon or acenocoumarol under the supervision of the Thrombosis Service of Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands. Patients were instructed to take the dose once daily at 6 p.m. Blood for INR measurement was collected in the morning.

Two different therapeutic ranges were applied, i.e. INR 2.0–3.5 (low intensity) and INR 2.5–4.0 (high intensity). Four groups of 75 patients each were selected retrospectively, i.e. acenocoumarol/low intensity, acenocoumarol/high intensity, phenprocoumon/low intensity, and phenprocoumon/high intensity. For each patient, the following inclusion criteria were used:

- Treatment period was six months or longer;
- At least six consecutive INRs were within the patient’s therapeutic range;
- The interval between consecutive INR measurements was two weeks or longer;
- The dose of phenprocoumon or acenocoumarol was not changed;
- There were no changes in conditions which may influence the INR such as intercurrent diseases, invasive procedures, starting or stopping drugs interacting with phenprocoumon or acenocoumarol.

Each patient participated once. If there were several stable periods, the most recent period was included.

Patients’ files were viewed in alphabetical order of their surnames and patients meeting the above-mentioned criteria were included until the required number of 75 was achieved.

Venous blood was collected using Vacutainer tubes containing 0.105 M buffered sodium citrate. Prothrombin times were measured in citrated plasma using the reagent Hepato Quick (Roche Diagnostics, Almere, The Netherlands) and a STA-R Evolution coagulation instrument (Diagnostica Stago). Prothrombin times were converted to INRs using the International Sensitivity Index (ISI) provided by the manufacturer. Analytical imprecision was determined from daily internal control samples, i.e. Preciclot levels 2 and 3 (Roche Diagnostics, Almere, The Netherlands).

The laboratory participated in a national external quality assessment (EQA) scheme organised by the Netherlands Foundation for Quality Control of Medical Laboratory Diagnostics (SKML). In this scheme, lyophilised plasmas obtained from patients on VKA-therapy were provided to the participants for INR determination. Since each external plasma was analysed in multiple surveys, we could calculate the between-day imprecision for the laboratory.

The biological variation of the INR (CVB) was calculated from the total variation of the INR (CVT) and the between-day imprecision (CVi) according to the formula given by Lassen et al. (4):

\[
CV_B = \sqrt{CV_T^2 - CV_i^2}
\]

Desirable and optimal imprecision goals were calculated according to Fraser et al. (2).

### Results

Between-day imprecision of the INR was estimated from daily internal control samples during 30 successive days. The CVi was 1.5% for both Preciclot levels 2 and 3. Between-day imprecision of the INR was also estimated for four different test samples provided in the national EQA scheme in which our laboratory participated (Table 1). The mean imprecision (CVi) for the external samples ranged from 2.4% to 4.1% with an average value of 3.3%. Since there was a difference between CVi calculated from

### Table 2: Basic patient characteristics.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic range (INR)</th>
<th>Men/total</th>
<th>Mean age ± SD (years)</th>
<th>Mean dose ± SD (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenprocoumon</td>
<td>2.0 – 3.5</td>
<td>43/75</td>
<td>63.8 ± 13</td>
<td>2.71 ± 1.0</td>
</tr>
<tr>
<td>Phenprocoumon</td>
<td>2.5 – 4.0</td>
<td>54/75</td>
<td>66.1 ± 11</td>
<td>2.46 ± 0.8</td>
</tr>
<tr>
<td>Aacenocoumarol</td>
<td>2.0 – 3.5</td>
<td>44/75</td>
<td>74.2 ± 11</td>
<td>2.33 ± 0.9</td>
</tr>
<tr>
<td>Aacenocoumarol</td>
<td>2.5 – 4.0</td>
<td>48/75</td>
<td>72.0 ± 10</td>
<td>2.58 ± 1.1</td>
</tr>
</tbody>
</table>
internal and from external control samples, we decided to use the average of these values (i.e. 2.4%) for the calculation of CV_B from CV_T and CV_I.

Patients’ files were viewed by one of the authors aiming to select a limited number of 75 patients for each group. The total numbers of viewed files were 255 (acenocoumarol/low intensity group), 309 (acenocoumarol/high intensity group), 156 (phenprocoumon/low intensity group), and 143 (phenprocoumon/high intensity group). The proportions of included patients were 29.4%, 24.3%, 48.1% and 52.4%, respectively. Basic patient characteristics are given in Table 2. The average age of the patients treated with phenprocoumon was lower than that of the patients treated with acenocoumarol. The proportion of male patients was greater than that of the females. Interestingly, the mean dose of phenprocoumon for the patients at high intensity (i.e. INR 2.5 – 4.0) was lower than for the phenprocoumon patients at low intensity (i.e. INR 2.0 – 3.5). We cannot provide an obvious explanation for the anomaly.

The number of INR measurements of the stable period in each included patient ranged from 6 to 24, and the mean numbers are shown in Table 3. For each individual patient CV_T was calculated. The results are shown in histograms (Fig. 1). The mean CV_T for each group are shown in Table 3. The calculated mean CV_B for each patient group are shown in Table 3. The mean CV_B is only slightly lower than the corresponding mean CV_T.

Table 3: Variation of INR in four selected patient groups each consisting of 75 individuals.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic range (INR)</th>
<th>Mean number of observations per patient</th>
<th>Mean INR</th>
<th>Mean CV_T (%)</th>
<th>Mean CV_B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenprocoumon 2.0 – 3.5</td>
<td>8.5</td>
<td>2.90</td>
<td>10.68</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td>Phenprocoumon 2.5 – 4.0</td>
<td>8.2</td>
<td>3.32</td>
<td>9.37</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>Acenocoumarol 2.0 – 3.5</td>
<td>7.7</td>
<td>2.91</td>
<td>11.16</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>Acenocoumarol 2.5 – 4.0</td>
<td>7.4</td>
<td>3.33</td>
<td>10.77</td>
<td>10.5</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Histograms of individual patients’ total variation of INR (CV_T, in percent). Panels A, B, C, and D refer to patients treated with acenocoumarol (target INR: 2.0–3.5), phenprocoumon (target INR: 2.5–4.0), acenocoumarol (target 2.0–3.5), and phenprocoumon (target 2.0–3.5), respectively.
According to Fraser et al., the ratio of optimum analytical imprecision to within-subject biological variation is 0.25 or less (2). The ratio of desirable analytical imprecision is 0.5 or less, and the ratio of minimum imprecision is 0.75 or less. For acenocoumarol, the mean CV$_B$ is 10.7% and the optimum and desirable imprecision goals according to Fraser et al. are <2.6% and <5.3%, respectively. For phenprocoumon, the mean CV$_B$ is 9.75% and the optimum and desirable imprecision goals are <2.4% and <4.9%, respectively.

**Discussion**

Before we could assess the within-patient biological variation, it was necessary to determine the between-day imprecision of the INR measurements by our laboratory.

The between-day imprecision of the INR calculated from 30 daily internal quality control samples was approximately 1.5% CV which compares favourably with values reported in previous studies (3).

In addition, the between-day imprecision of the INR measurements was assessed using EQA samples (Table 1). The number of tests on each sample was limited, i.e. ranging between 5 and 10. These CVs were higher than those of the internal control samples. The difference may be explained by different conditions of internal and external control. The internal controls were performed daily during one month, whereas the frequency of the EQA testing was once per two months or less, and the total testing period for each sample ranged from eight to 28 months. The longer period of the EQA testing may accumulate additional sources of variation such as differences between batches of the thromboplastin reagent. The frequency of patient testing was less than that of internal quality but higher than that of EQA. Therefore we decided to use the average CV of internal and external control (2.4%) as the most appropriate value for CV$_B$.

The patients for the present study were selected retrospectively from the total population of the anticoagulant clinic. We assumed that 75 patients for each group was a sufficient number to obtain representative results. Our groups were more than two times larger than the group of 32 patients studied by Lassen et al. (4).

The histograms of the total variation of the INR per patient (Fig. 1) showed peak values. It should be realised that the average total CV and the average biological variation depend on the therapeutic range. Some patients have a small biological variation of the INR, but others have a high CV. The upper limit of the CV depends on the width of the therapeutic range. Our patients had therapeutic ranges of 2.0–3.5 and 2.5–4.0, respectively. These ranges are slightly wider than the 2.0–3.0 and the 2.5–3.5 INR ranges recommended by the American College of Chest Physicians Guidelines (1). If the therapeutic range would be 2.0–3.0 instead of 2.0–3.5, the upper limit of the CV would be smaller and hence the average CV as well.

We used Hepato Quick reagent for INR determination. This reagent is sensitive to the vitamin K-dependent clotting factors II, VII, and X, but is not sensitive to variation in factor V and fibrinogen. In contrast to Hepato Quick, plain thromboplastin reagents are more or less sensitive to factor V (5) and fibrinogen. The within-patient variation of factor V and fibrinogen may be small when compared to the variation of the vitamin K-dependent clotting factors (6). It is unlikely that within-patient variation of factor V and fibrinogen contributes significantly to the total variation of the INR.

The total variation of the INR in patients on acenocoumarol was slightly greater than in patients on phenprocoumon (Table 3). Our observations are in agreement with better therapeutic quality control for phenprocoumon than for acenocoumarol (7). Greater biological variation of the INR with acenocoumarol may be explained by the shorter biological half-life of this drug leading to greater variation of the concentration and greater variation of factor VII activity (8–9). Likewise, the greater number of records needed to be reviewed for the acenocoumarol patients vs the phenprocoumon patients is a reflection of the shorter half-life of acenocoumarol resulting in more fluctuations of the INR.

Our results have important implications for the analytical performance goals of the INR determination. For acenocoumarol, the optimum and desirable imprecision goals according to Fraser et al. are <2.6% and <5.3%, respectively. For phenprocoumon, optimum and desirable imprecision goals are <2.4% and <4.9%, respectively. The average between-day imprecision of the INR in our laboratory was 2.4%. In conclusion, optimum and desirable imprecision goals were achieved in our laboratory.

Many studies have shown that a good correlation exists between INRs obtained with traditional laboratory PT methods and whole blood point-of-care (POC) monitors (10). It is tempting to speculate that the biological variation of the INR determined with Hepato Quick is also valid for other PT systems and POC monitors. Further studies are needed to assess the biological variation of the INR in patients monitored with other PT sys-
tems. In a recent study, the CV of INR imprecision for a POC whole blood coagulometer ranged from 2.9% to 4.0% in capillary blood testing (11). The imprecision CV for this type of POC monitor is within the desirable goal determined in the present study. Additional studies are required to assess the imprecision of other types of POC monitors with regard to the above-mentioned desirable analytical goals.

The present study is limited to treatment with acenocoumarol and phenprocoumon. Further studies are needed to assess the biological variation of the INR for treatment with warfarin.

References