

## Comparison Between International Normalized Ratio Using a Portable Device and Conventional Methodology

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**Objective:** To compare the international normalized ratio (INR) measured by a point-of-care (POC) testing device with that measured by the conventional method in patients undergoing anticoagulation therapy with warfarin sodium.

**Methods:** The INR of 383 warfarin-treated patients (mean age: 56.5 years; 207 female) was measured in capillary blood using the Hemochron Jr. device and compared with that of venous plasma samples determined by the conventional method performed in a Coag-A-Mate analyzer. Results were evaluated globally and for the following subgroups: INR < 2.0, from 2.0 to 3.5, and > 3.5.

**Results:** Using both methods, the comparison between INR values yielded a correlation coefficient ( $r$ ) of 0.86. However, mean differences in INR in both tests, considering the three subgroups, proved to be statistically significant ( $p < 0.001$ ):  $0.14 \pm 0.21$  (INR < 2.0);  $0.54 \pm 0.31$  ( $2.0 \leq \text{INR} \leq 3.5$ ), and  $1.64 \pm 1.10$  (INR > 3.5). Paired Student's  $t$ -test analysis revealed a  $p$  value < 0.001 for the three subgroups studied.

**Conclusion:** The use of point-of-care testing for monitoring oral anticoagulation has some limitations. Anticoagulation intensity was underestimated by this method in the three subgroups studied.

**Key words:** Warfarin, blood coagulation tests/methods.

Vitamin K antagonists, used in oral anticoagulant therapy, have been proven highly effective in the prevention and treatment of thromboembolic events in a variety of clinical situations<sup>1-3</sup>.

Some patients require long-term oral anticoagulant therapy, such as those with recurrent venous thromboembolism, chronic atrial fibrillation, mechanical prosthetic valves, and family history of thrombophilia. Warfarin sodium is the oral anticoagulant most widely used nowadays. This drug acts by inhibiting vitamin K-dependent coagulation factors (factors II, V, VII, IX e X)<sup>1,3</sup>.

The optimal dosage regimen to provide effective and safe anticoagulation should be established for each patient. Bleeding is the major complication of this therapy<sup>4,5</sup>. Therefore, treatment and prevention of thromboembolic disease require periodical laboratory control and the use of standard tests<sup>1,3</sup>. It is currently agreed that the best examination for monitoring oral anticoagulation is the international normalized ratio (INR) of prothrombin time (PT).

The INR may be determined daily until an adequate level of anticoagulation is achieved, after which it may be performed at periodic intervals<sup>1-3</sup>. This periodicity, however, changes patients' routine, thus affecting their quality of life.

In order to facilitate anticoagulation monitoring and reduce the waiting time from blood collection to test results,

devices have been developed that analyze samples obtained by fingerstick and allow rapid INR measurement (point-of-care testing)<sup>6-9</sup>. It has been debated, however, as to whether results from point-of-care testing and standard methods are equivalent.

This study aims, therefore, at evaluating the effectiveness of a point-of-care testing device, Hemochron Jr., in monitoring oral anticoagulant therapy, compared with the conventional method.

### Methods

**Case series** - Patients who came to the Heart Institute Clinical Laboratory for oral anticoagulation monitoring and who had been taking warfarin sodium for at least three months were invited to participate in the study.

Three hundred and eighty-three patients ranging in age from 13 to 86 years ( $56.5 \pm 13.9$ ), 54.0% of whom were female, were selected and distributed as follows: 36%, with heart valve prosthesis; 24% with arrhythmia; 7% after a stroke episode; 6% with venous thromboembolism; 5% with a pacemaker; and 22% associated with other causes.

At the time of blood sampling, all patients completed a questionnaire covering the following: name, hospital ID, age, gender, main diagnosis, reason for anticoagulation, weekly

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dosage of the anticoagulant drug, use of other medications and their respective dosages, and bleeding events within the last 10 days.

Patients that experienced problems during sampling were eliminated from the study.

**Conventional prothrombin time measurement** - Venous blood samples were collected into tubes containing 3.8% sodium citrate solution. Plasma samples were obtained by centrifugation at 2,500 rpm for 10 minutes and tested using the Coag-A-Mate MTX II automated analyzer, Organon Teknika, Biomerieux, (Marcy-L'Etoile, France), and a specific kit containing rabbit-brain thromboplastin with an ISI (International Sensitivity Index) of 1.2.

A plasma pool prepared with samples collected from normal subjects was assayed daily. External quality controls (international and national) were used to assure test reliability. Results were divided into three groups, according to the INR obtained.

- INR < 2.0: inadequately anticoagulated patients
- $2.0 \leq \text{INR} \leq 3.5$ : adequately anticoagulated patients
- INR > 3.5: overanticoagulated patients

**Point-of-care prothrombin time measurement** - Capillary blood samples were obtained by fingerstick, and one blood drop was dispensed into the Hemochron Jr. II portable device, ITC, NJ, USA (Fig. 1). Rabbit-brain thromboplastin (ISI = 1.0) was used.

The device has a cuvette containing lyophilized thromboplastin, stabilizers, and buffers. Clot detection is performed by a series of optical detectors aligned with the channel of the test cuvette. The speed at which the blood sample moves between both detectors is measured. As clot formation begins, blood flow is blocked and the movement slows. The electronic optical detection of a fibrin clot in the blood sample automatically terminates the test, causing the instrument's timer to display the clotting time in seconds. PT, already corrected for plasma value, and INR results appear alternately on the display.



Fig. 1 - Capillary blood sample obtained by fingerstick, using the Hemochron Jr. portable device.

Calibration was carried out daily, as well as internal control tests provided by same manufacturer. A training course for proper device operation was provided for all laboratory personnel that performed measurements.

**Statistical analysis** - INR values for both devices were analyzed in the whole group and in the three subgroups, namely, < 2.0; from 2.0 to 3.5, and > 3.5, using the paired t test, the Bland-Altman test, and Pearson's correlation to evaluate result differences. A p-value < 0,05 was considered statistically significant.

## Results

The 383 study participants were distributed as follows: 101 (26%) patients with INR < 2.0 (inadequately anticoagulated), 237 (62%) with INR between 2.0 and 3.5 (adequately anticoagulated) and 45 (12%) with INR > 3.5 (overanticoagulated).

Pearson's correlation coefficient (r) obtained by comparing global performance of the two methods was 0.86 (Fig. 2). When INR values were divided into the subgroups, coefficients dropped to 0.65 (< 2.0), 0.72 (between 2.0 and 3.5), and 0.55 (> 3.5). The Bland-Altman analysis demonstrated significant differences for both the whole group and subgroups. In addition, an increase in mean INR was accompanied by a significant increase in INR differences (Tab. 1). Frequency distribution of values measured in the three categories showed an agreement of only 66% between both methodologies (Tab. 2), with a high percentage of patients adequately anticoagulated by the conventional method yet inadequately anticoagulated by the POC test.

In the subgroup with INR > 3.5, two aberrant results, that is, showing more than 50% deviation from the true INR, were detected after the point-of-care test: 7.2 and 9.4 by the conventional method versus 3.4 and 2.4, respectively, by the point-of-care test.

Statistical analysis using the paired Student's t test showed significant differences between both methodologies for the three subgroups analyzed (p<0.001).

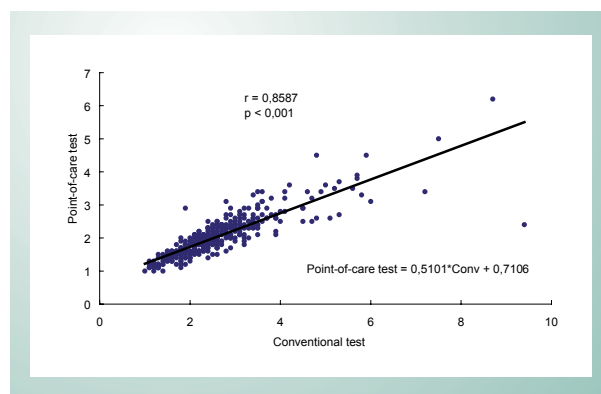


Fig. 2 - Linear regression line and Pearson's correlation coefficient for INR results obtained by point-of-care and conventional tests (Conv.)

	Conventional test	Point-of-care test	Difference (mean ± SD)
TOTAL	2.6 ± 1.09	2.03 ± 0.65	0.56 ± 0.63*
INR < 2.0	1.88 ± 0.37	1.63 ± 0.32	0.14 ± 0.21*
2.0 ≤ INR ≤ 3.5	2.92 ± 0.28	2.26 ± 0.29	0.54 ± 0.31*
INR > 3.5	4.80 ± 1.32	3.16 ± 0.77	1.64 ± 1.10*

Mean, standard deviation (SD) and mean difference between both methodologies by the Bland-Altman test, \*p < 0.001.

Table 1 - Comparison between INR values for the whole group and subgroups

Conventional test	Point-of-care test		
	INR > 2.0	2.0 ≤ INR ≤ 3.5	INR > 3.5
INR > 2.0	99 (25.8%)	2 (0.5%)	0 (0%)
2.0 ≤ INR ≤ 3.5	92 (24.0%)	145 (37.9%)	0 (0%)
INR > 3.5	0 (0%)	36 (9.4%)	9 (2.3%)

Number of patients and total percentages.

Table 2 - Frequency distribution of values measured by both tests (n = 383)

## Discussion

The number of patients undergoing oral anticoagulant therapy has been increasing sharply in recent years, overloading monitoring centers. Hence, point-of-care testing devices have been used as a means of surpassing this problem. These tests are primarily intended to improve the patient's quality of life, in that they provide greater flexibility, higher frequency and less discomfort<sup>10</sup>.

In a number of studies carried out to validate different portable devices, accuracy levels of POC tests were considered clinically acceptable<sup>6-9</sup>. On the other hand, some authors reported problems regarding the reliability of test results, pointing to the need of better calibration and use of external quality controls<sup>11-14</sup>.

In the present study, we evaluated the performance of the Hemochron Jr. device by comparing its results with those obtained with the standard method. In spite of the good correlation found in global performance ( $r = 0.86$ ), paired Student's t-test and Bland Altman test (Tab. 1) revealed significant differences ( $p < 0.001$ ) between both methodologies.

For a significant number of patients within the therapeutic

range (Tab. 2), POC values were lower than 2.0 (24%). These results are clinically relevant, because these patients would be prescribed higher doses of warfarin, thereby becoming prone to bleeding. In addition, overanticoagulated patients had their values underestimated by point-of-care testing. In this overanticoagulated group, two aberrant results were detected, in which patients at high risk of bleeding would be left untreated if the values taken into account were those of the point-of-care testing.

This study was carried out by trained professionals, to reduce the likelihood of errors during the preanalytical phase. Thus, we believe that the differences found should not be entirely related to inadequate sample collection or even management of the sample in the device. One of the possible explanations is related to differences between the matrixes in both methods. Also, the presence of platelet proteins with modulating activities of coagulation in whole blood samples may have contributed to the deviations observed.

Data presented in this study indicate lack of accuracy of point-of-care testing, thereby restricting its use for monitoring oral anticoagulant therapy.

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