INTERNATIONAL NORMALIZED RATIO (INR)

Clinical Significance and Applications

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Fifty-four year old Dean Garrison is one of over 500,000 patients in the United States on continuous oral anticoagulant therapy. To assure that he is receiving optimal warfarin therapy for his condition, Dean has a prothrombin time test performed bi-monthly. Since this test has always been performed at the same suburban Philadelphia laboratory, his physician has been able to manage Dean’s therapy with a dosage that has yielded prothrombin time results of ~18 seconds, within the laboratory’s established therapeutic range for patients on warfarin therapy. Today, however, Dean is vacationing in Phoenix, Arizona and has just had his prothrombin time test performed by an area laboratory. His dosage has not been altered, but his prothrombin time results from this laboratory place him above the therapeutic range established by the Philadelphia laboratory. What would cause this to happen? What course of action is the clinician to take? What are the possible implications for this patient?

The management of patients on oral anticoagulant therapy has long proven a challenge for clinicians. Compounding this problem is the lack of uniformity between laboratories in their methods for monitoring these patients. Over recent years, the standardization of the prothrombin time test and its reporting for patients receiving oral anticoagulant therapy has gained more interest and attention. Many of the past attempts to standardize this test have failed to consider all of the variables affecting the outcome of prothrombin time results. The International Normalized Ratio (INR) system for reporting prothrombin time results is the latest attempt to standardize these variables on a universal scale. However, the INR system has also been met with controversy, due primarily to a lack of understanding of the system and its applications by clinicians and laboratorians.

Bio/Data Corporation is grateful to Mrs. Harlene Palkuti, B.S., MT(ASCP), for this excellent presentation of the INR. She has held numerous positions in the United States as both a laboratorian and an educator. She served as Clinical Instructor and Student Advisor at The Ohio State University Hospitals, Columbus, Ohio; Fairfax Hospital, Falls Church, Virginia; University of California at Berkeley, Berkeley, California; and San Francisco State University, San Francisco, California. Currently, Mrs. Palkuti is an educator with the American Society of Clinical Pathologists. She has presented over one hundred and fifty workshops and seminars around the country and has published extensively.
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INTRODUCTION

Since the introduction of the one-stage prothrombin time by Dr. Armand Quick in 1935, there have been many attempts to standardize the methodology and reporting of results to reflect its sensitivity to the coagulation defects induced by oral anticoagulants. Variations in the prothrombin time test can be introduced by the type of thromboplastin reagent used, the assay methodology, and the type of instrumentation employed to measure the formation of fibrin. The thromboplastin reagents manufactured in North America from combinations of rabbit brain and/or lung vary significantly in their sensitivities to deficiencies of the vitamin K-dependent coagulation factors. These thromboplastin reagents are less sensitive than the human brain thromboplastins that were used in the United Kingdom and the new thromboplastin reagents prepared from recombinant and non-recombinant human tissue factor. In addition to these testing variables, the lack of standardization in reporting prothrombin time results has led to confusion among clinicians in determining an adequate therapeutic response to oral anticoagulation while minimizing the risk of bleeding.

In 1983, the World Health Organization (WHO) introduced a method exclusively for monitoring patients stabilized on oral anticoagulant therapy using the prothrombin time test for reporting patient values as an International Normalized Ratio (INR). The INR normalizes the PT ratio to an international standard thromboplastin. Therapeutic ranges in terms of INR values for common clinical conditions have been determined by double-blind, randomized clinical trials.

PHARMACOLOGY OF ORAL ANTICOAGULANTS

Oral anticoagulant therapy with vitamin K antagonists (e.g., warfarin) is commonly used for the treatment and prevention of thromboembolic disorders. Ingestion of derivatives of 4-hydroxycoumarin result in the inability of the liver to carboxylate the glutamyl residues of the vitamin K-dependent coagulation factors II, VII, IX, and X, and coagulation inhibitors protein C and protein S. The resultant non-functional proteins are responsible for the impaired formation of fibrin. With the decay of vitamin K-dependent clotting factors, non-carboxylated or insufficiently carboxylated (functionally defective) precursor molecules enter into the circulation. These products, called protein induced by vitamin K antagonists (PIVKA), can interfere with normal clotting factors and with certain thromboplastin reagents in the prothrombin time test.1 The rate of depression of the individual clotting factors is determined by their biological half-life. Factor VII and protein C fall the most rapidly, followed by factors IX, X, and II. These factors return to normal levels in the reverse order (see Figure 1).

Effects of Oral Anticoagulants on Vitamin K-Dependent Coagulation Factors

Figure 1

Reversing the effects of over-anticoagulation with oral anticoagulants is more complicated than with heparin. There is a delay of 6-24 hours in the clotting factors’ return to normal functioning levels with the administration of vitamin K, either intravenously or orally. If the risk of patient bleeding is high, immediate reversal of the effects of over-anticoagulation can be obtained by infusion of fresh frozen plasma or prothrombin-complex concentrates.2,3
Clinical Laboratory Monitoring of Patients Receiving Oral Anticoagulants

Most physicians prefer monitoring oral anticoagulant therapy using the prothrombin time test. The prothrombin time is very sensitive to variations in the concentration of factors II, VII, and X whose activities are depressed when these anticoagulants are administered. Factor V and fibrinogen are also measured by the prothrombin time test; however, these factors are not affected by oral anticoagulants. The historical method of reporting prothrombin time results as a percentage of coagulation activity is almost phased out in the United States, but this reporting method is still frequently used in western Europe and Japan. As indicated in the 1992 College of American Pathologists (CAP) coagulation survey, none of the 1054 participating laboratories responded that they report patient results solely in percent activity, while 1.4% report both the percent activity and patient seconds.4 One reason for relinquishing this method of reporting test results is its lack of usefulness as a universal scale for evaluating the intensity of anticoagulation, due to the variations in sensitivity of thromboplastins to the PIVKAs. Since dilutions of normal plasma do not contain PIVKAs, the accuracy of a “normal dilution curve” to convert patient clot time into percent activity is challenged.1

In an attempt to standardize test results, other methods were introduced in the United States and the United Kingdom (e.g., reporting the prothrombin time ratio and reporting the patient prothrombin time along with the normal range). Because the sensitivity of each of these methods is still dependent upon the thromboplastin used, comparison of test results between laboratories is extremely difficult. The prothrombin time ratio, as first introduced, was derived by dividing the patient’s prothrombin time by the clotting time of the normal control plasma, rather than the mean of the normal range. The clotting time of normal control plasma varies daily and from shift-to-shift, while the mean of the normal range remains constant throughout the period of time that a particular system of reagents and instruments is used. According to a recent CAP survey, less than 1% of the 882 participating laboratories still report using the prothrombin time ratio method. The popularity of reporting results in seconds has decreased from 44% in 1992 to 15% in 1994.5 The latest method introduced for standardizing prothrombin time results, the INR, allows the physician to compare prothrombin time results between laboratories and to relate the INR to suggested “therapeutic ranges” for monitoring oral anticoagulant therapy. According to the CAP survey questionnaires, popularity of the INR method of reporting prothrombin time results on patients receiving oral anticoagulants in the United States increased from approximately 20% in 1991, to 50% in 1992, 79% in 1993, and 91.8% in 1994.45 A recent cross-Canada survey revealed that 51% of both hospital and commercial laboratories report the prothrombin time as an INR result.6

What is an INR and how it is derived?

By definition, the INR represents the prothrombin time ratio which would be obtained for a particular patient if the primary WHO reference thromboplastin had been used in the determination.3 The first international reference plasma (lot 67/40) selected by the WHO was prepared from lyophilized human brain thromboplastin and assigned an International Sensitivity Index (ISI) value of 1.0. The ISI indicates the sensitivity of a particular thromboplastin to an international reference plasma. Because of the demand for this reference plasma, secondary or working thromboplastin preparations with comparable sensitivities have now been introduced for the comparison of thromboplastins to a known standard. A prothrombin time ratio is obtained by dividing the patient’s prothrombin time in seconds by the mean of the normal range. This ratio is then normalized by an ISI. The INR is calculated as follows:

\[
\text{INR} = \frac{\text{PATIENT PROTHROMBIN TIME IN SECONDS}}{\text{MEAN OF THE NORMAL RANGE}} \times \text{ISI}
\]
Calibration of a Working Thromboplastin Against the Primary WHO Standard

Currently, many coagulation instruments can perform this exponential calculation when the ISI value is entered into the software. In addition, a table is provided for deriving an INR from the patient prothrombin time ratio for various ISI values (see Table 4).

The standard procedure for calibrating a new thromboplastin requires performing prothrombin times on 60 patients stabilized on oral anticoagulants and 20 normal control healthy individuals with both the test thromboplastin and the international reference plasma. These prothrombin time results are then plotted on log-log paper. An example of this calibration is provided in Figure 2.1 The test thromboplastin prothrombin times are plotted along the horizontal axis and the WHO standard prothrombin times along the vertical axis. The calibration line is calculated by regression analysis. The slope of the line is the designated ISI for the test thromboplastin. Since the imprecision of the prothrombin time ratio will be magnified by a higher ISI, the use of a thromboplastin with a low ISI value will minimize the variability in the calculated INR. The most precise determinations of the INR would involve the use of a thromboplastin reagent whose value approaches 1.0. A very sensitive thromboplastin reagent (low ISI value) will generate a greater prolongation of the clot time on a patient receiving oral anticoagulants (i.e., prothrombin time ~29.0 seconds) than a less sensitive thromboplastin reagent whose prothrombin time on the same patient may be ~18.0 seconds. Studies have demonstrated that inter-laboratory variations of the INR in groups using thromboplastins with an ISI equivalent to 1.0 yielded a low 6-8% CV.1 However, most thromboplastins available in the United States today have an ISI between 1.8 - 2.99. With these higher ISI thromboplastins, inter-laboratory variations of 15-26% are not unusual.1,4

A critical requirement in the derivation of the INR is the use of the prothrombin time mean of the normal range - not the prothrombin time value of the control plasma. The mean of the normal range is derived from prothrombin time results on fresh plasma samples from 30 or more normal patients. Laboratory normal control plasmas are not manufactured to be a “100% standard”, but are useful as data points in the internal laboratory quality assurance programs for verifying accuracy and possible technical problems with the test procedure. Unfortunately, current publications still mistakenly perpetuate using the normal control plasma value for deriving prothrombin time ratios, which can result in problems in the evaluation of a patient’s level of anticoagulation.5 If the control time is greater than the mean normal range, the ratio for any patient prothrombin time will be smaller, potentially leading to over-anticoagulation. Whereas, if the control time is less than the mean normal range, the ratio for any patient prothrombin time will be greater, potentially leading to under-anticoagulation.6 Table 1 demonstrates the variation in INR values that may occur when the daily fluctuation in normal control values are used to calculate the prothrombin time ratio, placing a patient with a target INR of 2.5 in or out of the therapeutic range. The proper use of the INR was designed and validated for use exclusively on patients who have been stabilized on oral anticoagulants. By the WHO definition, a
The patient is generally considered stabilized on oral anticoagulants after a period of at least three weeks of reproducible prothrombin time results reported in seconds.\(^9\)

**Recommended Therapeutic Ranges for Patients Receiving Oral Anticoagulants When Using the INR**

The optimal range for oral anticoagulation continues to be controversial. However, many experts, including the British Society for Haematology, the American College of Chest Physicians, the National Heart, Lung and Blood Institute, and the Dutch Federation of Thrombosis Centers, have proposed recommendations for prophylaxis and treatment for venous and arterial thrombosis using the INR since 1985.\(^8\) Table 2 lists the most recent recommendations that were published in 1992.\(^10\) When evaluating an unexpected INR result, it is important to keep in mind that the presence of heparin, when given concurrently or as a contaminant, may prolong clot times and lead to the misrepresentation of the patient's state of anticoagulation.

**ADVANTAGES OF THE INR SYSTEM**

One major advantage of the INR system is that it helps to alleviate confusion in the interpretation of prothrombin time results that may occur when a laboratory changes thromboplastin and/or instrumentation. These laboratory changes could go relatively unnoticed by the attending physician. As demonstrated in Table 3, the INR remains constant when the laboratory changes to a more sensitive thromboplastin.

Another major advantage of the INR system is that it affords a reliable comparison of prothrombin time results between laboratories. Because the prothrombin time ratio is standardized by the ISI, this system adjusts for the variety of thromboplastin reagents used in clinical practice.

<table>
<thead>
<tr>
<th>Test</th>
<th>Test</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Patient PT (10.0 - 13.0 sec)</td>
<td>16.1 sec</td>
<td>16.1 sec</td>
</tr>
<tr>
<td>Normal Control (10.6 - 12.6 sec)</td>
<td>11.6</td>
<td>10.6</td>
</tr>
<tr>
<td>Formula (ISI = 2.77)</td>
<td>16.1</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>11.6</td>
<td>10.6</td>
</tr>
<tr>
<td>INR</td>
<td>2.5</td>
<td>3.18</td>
</tr>
</tbody>
</table>

*Table 1*

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**Recommended Therapeutic Ranges for Oral Anticoagulants**

<table>
<thead>
<tr>
<th>Target INR</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>High dose</td>
<td>2.5 - 3.5</td>
</tr>
</tbody>
</table>

*Table 2*
laboratories today. With the introduction of new reagents with ISI values approaching 1.0, markedly different clot times will be generated on all patients. This in turn will certainly perpetuate the need for standardization in reporting prothrombin time results.\(^{11}\)

### Effects of Changing Thromboplastin

<table>
<thead>
<tr>
<th></th>
<th>Old Thromboplastin</th>
<th>New Thromboplastin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT in Seconds</td>
<td>15.7 sec</td>
<td>23.0 sec</td>
</tr>
<tr>
<td>Mean Normal PT</td>
<td>11.0 sec</td>
<td>12.5 sec</td>
</tr>
<tr>
<td>PT ratio</td>
<td>1.43</td>
<td>1.84</td>
</tr>
<tr>
<td>ISI</td>
<td>2.6</td>
<td>1.5</td>
</tr>
<tr>
<td>INR</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**Table 3**

The INR reporting system will also provide a means for more accurate monitoring of patients who travel extensively. Additionally, the standardization of test results for the prothrombin time will allow accurate statistical comparisons of research studies among various centers in the United States and in certain other countries.\(^{3}\)

INR therapeutic ranges for different clinical conditions are based on international collaborative studies. Hospitalization time may be reduced due to the development of therapeutic ranges that maximize oral anticoagulant therapy for a particular type of thromboembolic disease, thus preventing additional thrombotic episodes or incidences of secondary bleeding (which may be as high as 10%).\(^{3}\) Accordingly, a potential for significant cost savings exists for patients who are monitored by the INR system.

### Limitations of the INR System

Of major concern is the validity of the INR assignment. The ISI calibration procedure itself may produce a CV as high as 5% from the true value for a particular source of thromboplastin. Although the INR adjusts for differences in thromboplastin sensitivity, the coagulation instrument also affects the measurement.\(^{5,11-14}\) The original INR work was performed using tilt tube techniques, thus making correlation with current methodologies such as photometric clot detection instruments difficult. A recent study concluded that the instrumentation effect may be clinically meaningful, so coagulation instruments, as well as thromboplastin, should be calibrated.\(^{13,15}\) Others have pointed out that it may be advantageous to determine a local normalized ratio based on a calculated local sensitivity index to more accurately monitor patients on oral anticoagulants between affiliated hospitals.\(^{12}\) Laboratories should also require the ISI value be posted for their particular thromboplastin for every type of clot time instrument used in their laboratory.

Problems may also arise because bedside testing methods may yield significantly different results from laboratory results. Portable monitors frequently used for “off-site” testing have produced discrepant INR results large enough to effect therapy, with a tendency to underestimate the prothrombin time and the INR at the high range.\(^{13,16}\) Additionally, recent surveys have indicated that clinicians as well as laboratorians have difficulty in understanding the significance of the INR and the ISI.\(^{4,9,17}\) Errors in calculating the INR often arise because the prothrombin time ratio is multiplied by the ISI, whereas the correct calculation requires that the prothrombin time ratio be raised to the ISI power.\(^{4,17}\)

Another limitation of the INR system is that the laboratory is often placed at a disadvantage by not having knowledge of which patients are receiving oral anticoagulants. The prothrombin time test is frequently used to evaluate other hemostatic disorders, such as liver disease, DIC, lupus anticoagulants, hereditary factor...
deficiencies, and acquired vitamin-K deficiency. Additionally, it is always included in routine pre-operative screening panels. Since these hemostatic disorders have been excluded from the derivation of the ISI, the INR has diagnostic value only for patients stabilized on oral anticoagulants. Accordingly, most laboratories presently prefer to report both the INR and the patient’s prothrombin time in seconds. 3, 6, 11, 17

**SUMMARY**

The number of laboratories in the United States using the INR method of reporting prothrombin time results has increased significantly in recent years. Use of this system is facilitated by the availability of conversion tables and coagulation instruments with software capabilities for calculating the INR.

The number of variables that affect the prothrombin time test has necessitated standardization in reporting results and monitoring patients on oral anticoagulants. These variables include: the thromboplastin reagent used, the assay methodology, and the type of instrumentation employed. The INR system of reporting prothrombin time results represents the latest effort to correct the effects of these variables, especially those presented by the variety of thromboplastin reagents available throughout the United States. The various compositions and sensitivities of these reagents may yield significant differences in results between laboratories for a given patient, potentially compromising optimal anticoagulant therapy for that patient. Using the ISI to express the sensitivity of the thromboplastin reagent, the INR reporting system attempts to standardize this variance, yielding results that correspond laboratory to laboratory, regardless of the sensitivity of the thromboplastin reagent.

The INR offers many benefits to the clinician, laboratorian, and patient; however, its effectiveness is dependent upon a number of critical factors. These factors include:

- the calibration of the coagulation instrument as well as the thromboplastin reagent used
- the use of a mean normal range derived from fresh plasma samples from 30 or more normal patients
- the correct use of the calculation and the appropriate inputs
- an understanding of the system by clinicians and laboratorians
- the laboratory’s knowledge of those patients to whom the system should be applied

The standard of care for patients on oral anticoagulant therapy will improve with the proper understanding and application of the INR system of reporting prothrombin time results.

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