Control of Oral Anticoagulant Therapy*

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ABSTRACT

In an effort better to standardize the control of oral anticoagulant therapy, it has recently been recommended that the prothrombin time be reported in the form of an International Normalized Ratio (INR) based upon calibration of the locally employed thromboplastin with an International Reference Preparation. It has been demonstrated in our laboratory that the INR does minimize the differences in results which ensue from variations in the source of thromboplastin and instrumentation and should hopefully allow for better interlaboratory comparisons in the future. Recent studies have also suggested that many American patients tend to be over anticoagulated and at greater risk for hemorrhage. Based upon those findings, over 40 percent of our specimens were above the currently recommended levels.

Introduction

Oral anticoagulants have been employed for many years for the prevention of recurrent thrombosis, and control of dosage has largely been guided by the almost universally available one stage prothrombin time. These agents, divisible into the coumarin and indanedione type compounds, act similarly as inhibitors of vitamin K with major effects on some of the coagulation zymogens, namely factors II, VII, IX, and X, and proteins C and S. Normally before these proteins are released into the circulation and dependent upon the presence of vitamin K, glutamic acid residues are converted to gamma carboxyglutamic acid.20,21 These residues bind calcium, permitting the coagulation proteins to bind phospholipids for activation in the clotting scheme.

In the presence of vitamin K inhibitors, the gamma carboxyglutamic acid residues are not formed, leading to decreased plasma levels of factors II, VII, IX and X, and proteins C and S, and a concomitant rise in proteins induced by vitamin K absence (PIVKA's). With induction of oral anticoagulant therapy, factor VII levels begin to fall within a day followed by factors IX, X, and II reaching a stable anticoagulated state in approximately one week. The rise in PIVKA's may also retard thrombin formation,7 while reduced levels of proteins C and S might possibly have an opposite
thrombogenic effect. Creation of a possible hyper-coagulable state has been suggested during the induction phase when protein C levels fall early, paralleling factor VII, but before the full reduction of the remaining pro-coagulant factors II, IX and X.

Despite broader understanding of their physiologic action and their long history of use, there continue to be many controversies regarding the specific clinical indications as well as the dosage and duration of therapy with oral anticoagulants. In clinical practice, the ideal therapeutic range has been assumed to be just below the hemorrhagic level and has been guided by the one stage prothrombin time. Although not based upon what now would be regarded as solid objective evidence, Wright et al in the early 1950s suggested that optimal therapy was when the patient’s prothrombin time was 2.0 to 2.5 times the control value. By and large this recommendation was accepted and has been adhered to in North America for the past 30 years, as evidenced by similar guidelines in many current textbooks of medicine. An example of these prevailing recommendations is best summarized in Goodman and Gillman which states, “Patients on chronic therapy usually should be maintained at a one stage prothrombin activity of 25 percent, which, expressed in seconds, is about twice the normal baseline of 12 seconds. However, the so called therapeutic range is based more upon the avoidance of bleeding than on the achievement of a proven therapeutic effect.” Although Moschos et al suggested in 1964 that those recommendations resulted in excessive anticoagulation, only more recently have randomized studies evaluating different anticoagulant intensities with clinically related outcomes begun to address objectively the questions posed by Hirsh.

Clearly one portion of the confusion stems from lack of standardization of the prothrombin time which is performed with a wide variety of reagents and instruments. For many years after the prothrombin time was introduced by Quick in 1935, many laboratories prepared their own thromboplastin reagents. These have gradually given way to more uniform, frequently commercially prepared reagents; but great differences continue to exist, namely,—the thromboplastins of rabbit tissue origin commonly used in North America versus those prepared from human tissues such as the British Comparative Thromboplastin (BCT), and bovine brain origin favored in Scandinavia. The rabbit brain thromboplastins in commercial use in North America are generally known to be less sensitive to the reduction in the vitamin K-dependent clotting factors than standardized human brain thromboplastin commonly used in Great Britain. Secondly, and while citing surveys conducted by the College of American Pathologists and the Center for Disease Control, it has been shown that the type of instrumentation used in performing the prothrombin time has almost as much effect as the type of thromboplastin.

In response to these issues, the World Health Organization (WHO) has prepared a primary International Reference Preparation of human brain thromboplastin to promote standardization of oral anticoagulant control. Subsequently, an international committee recommended adopting a uniform calibration system based upon an International Normalized Ratio (INR) derived from the calibration of commercial thromboplastin reagents against the International Reference Preparation. The validity of this system has been confirmed in international collaborative studies.

It has been proposed that manufacturers of thromboplastins used in oral
anticoagulant control should indicate the relationship of each batch of their material to the WHO International Reference Preparation by a number which describes the comparative slope (or sensitivity). This is presently referred to by the WHO as the International Sensitivity Index (ISI). Manufacturers should also provide a table or graph showing the relationship between the conventional terms of expression of results of the prothrombin time test and the INR. The INR is calculated by the equation, INR = \frac{R}{c}, where R is the prothrombin ratio (patient prothrombin time/mean normal prothrombin time) and c is the comparative slope (ISI) of the thromboplastin used.\textsuperscript{13}

Manufacturers of commercial thromboplastins in the United States are now beginning to make available batches of reagents for which the ISI has been determined. It was, therefore, the purpose of this study to evaluate the feasibility of utilizing these reagents and adopting the reporting of the INR along with our currently employed methods and results. Furthermore, it was our wish to evaluate the degree of anticoagulation achieved in our community and compare those results with more recent recommendations and define whether or not the utilization of the INR served to standardize better those results.

Methods

One hundred six blood samples were obtained from 42 patients who had been on oral anticoagulants for one month or longer. The blood was drawn into glass evacuated tubes with Siliconized stoppers\textsuperscript{*} containing 3.8 percent sodium citrate anticoagulant. The blood was centrifuged and transferred into plastic tubes for testing within two hours of phlebotomy. Prothrombin times were performed on the Fibrometer\textsuperscript{†} with rabbit brain thromboplastin\textsuperscript{‡} (Thromboplastin C, batch # TPCD – 399, ISI not available). The remainder of plasma was frozen and transferred to another laboratory where prothrombin time determinations were performed in batches utilizing Thromboplastin C\textsuperscript{†} (ISI values – 2.28 mechanical and 2.53 phototical), and Simplastin\textsuperscript{§} (ISI values – 1.81 mechanical and 1.9 phototical). Each plasma sample was tested in duplicate with both reagents utilizing the Fibrometer\textsuperscript{†} representing a mechanical system, and the MLA 800\textsuperscript{†} a photo-optical method. Normal values had been previously established as follows: Thromboplastin C/Fibrometer – 11.7 seconds; Thromboplastin C/MLA 800 – 12.4 seconds; Simplastin/Fibrometer – 11.9 seconds; and Simplastin/MLA 800 – 12.3 seconds. Results were expressed in seconds, ratio of patient prothrombin time to normal time, and the INR, and compared with the recommendations for antithrombotic agent usage by the American College of Chest Physicians and the National Heart Lung and Blood Institute National Conference on Antithrombotic Therapy.\textsuperscript{2}

Statistical Methods

A two-way, blocked analysis of variance was used to determine whether or not the use of different data reporting methods, (i.e. prothrombin time, ratio of prothrombin time to normal, or INR), would result in significant differences in the stability of the reported results when

\begin{itemize}
  \item\textsuperscript{*} Venoject, Terumo Products.
  \item\textsuperscript{†} Baltimore Biological Laboratory, Cockeysville, MD.
  \item\textsuperscript{‡} American Dade, Inc., Miami, FL.
  \item\textsuperscript{§} General Diagnostics, Morris Plains, NJ.
  \item\textsuperscript{†} Medical Laboratory Automation, Inc., Mount Vernon, NY.
\end{itemize}
the four different reagent-instrument procedures were applied. Each sample was viewed as a separate block, and the four reagent-instrument procedures and three data reporting methods were the two treatment effects. Prothrombin times were used as the dependent variable in this model. A natural logarithm was used as a variance stabilizing transformation before the analysis was conducted. The presence of a statistically significant two-way interaction in this model would indicate that the use of different data reporting methods have resulted in differential stability of the prothrombin results when the different reagent-instrument procedures were applied.3

Three one-way, blocked analysis of variances were used to determine which of the three data reporting methods displayed the least variability when the different reagent-instrument procedures were used. In each analysis, each sample number constituted a block, and the reagent-instrument procedure was the single treatment effect. Prothrombin times were the dependent variable in each of the three models. A natural logarithm was used as a variance stabilizing transformation before the analyses were conducted. Analyses were done separately for the different data reporting methods. The partial F-statistic associated with the single main effect, obtained from each of the three analyses, were compared to each other to determine which of the data reporting methods displayed the greatest stability over the different laboratory methods. Lower F values indicate greater stability.

Results

The patients had been receiving coumadin for an average of 13 months, (range one month to 180 months), and the mean dose of all patients was 4.8 mg per day. The mean prothrombin time obtained by immediate analysis of the 106 unfrozen plasma samples was 18.4 seconds. Analysis after freezing resulted in mean values that were approximately 2.2 seconds longer by comparable methodology.

The mean prothrombin times of the 106 samples analysed by the various reagent-instrument combinations and expressed by the three reporting methodologies, (i.e., prothrombin time in seconds, prothrombin time to normal ratio, and INR), are displayed on table I. The mean prothrombin time expressed in seconds ranged from a low of 20.6 seconds to a high of 27.2 seconds. For the prothrombin time to normal ratio, the range was 1.66 to 2.28, while the INR resulted in a range of 4.02 to 4.73. The results of the two-way, blocked analysis of variance indicates that there is a significant difference (p < 0.001), in the stability of the three different data presentation methods. The results of the three one-way, blocked analyses of variances indicate that the INR reporting

<table>
<thead>
<tr>
<th>Thromboplastin</th>
<th>ISI</th>
<th>Instrument</th>
<th>P.T. (sec)</th>
<th>PT/N</th>
<th>INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>*</td>
<td>Fib</td>
<td>18.4</td>
<td>1.56</td>
<td>*</td>
</tr>
<tr>
<td>D2</td>
<td>2.28</td>
<td>Fib</td>
<td>20.6</td>
<td>1.76</td>
<td>4.15</td>
</tr>
<tr>
<td>D2</td>
<td>2.53</td>
<td>MLA</td>
<td>20.7</td>
<td>1.66</td>
<td>4.02</td>
</tr>
<tr>
<td>G</td>
<td>1.81</td>
<td>Fib</td>
<td>27.2</td>
<td>2.03</td>
<td>4.31</td>
</tr>
<tr>
<td>G</td>
<td>1.90</td>
<td>MLA</td>
<td>25.1</td>
<td>2.28</td>
<td>4.73</td>
</tr>
</tbody>
</table>

D1-Dade-Thromboplastin C, Lot # TPCD-399
D2-Dade-Thromboplastin C, Lot # TPCD-391A
G-General Diagnostics-Simplastin, Excel, Lot # OC201
Kit # OC222
Fib-Fibrometer
MLA-MLA Electra 800
*-ISI not available
PT/N-Patient prothrombin time in seconds/normal time in seconds
INR-International Normalized Ratio
ISI-International Sensitivity Index
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method is the most stable when the four reagent-instrument procedures are compared as follows:

<table>
<thead>
<tr>
<th>Data Reporting Method</th>
<th>F-statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT in seconds</td>
<td>203.60</td>
</tr>
<tr>
<td>PT/N</td>
<td>213.86</td>
</tr>
<tr>
<td>INR</td>
<td>26.99</td>
</tr>
</tbody>
</table>

Each method of reporting prothrombin times was evaluated to determine if a given sample value was within the therapeutic range of anticoagulation, above that range, or below it, (for the prothrombin time/normal ratio, the therapeutic range was defined as between 1.2 to 1.5, and for the INR 2.0 to 3.0). First, individual samples were compared to see how frequently that sample fell into the same therapeutic range when tested by the various reagent-instrument combinations and reported by either of the two methods. As can be seen in table II, the INR led to total agreement of all four reagent-instrument combinations more frequently than the prothrombin time to normal ratio, although the differences were not great.

Lastly, the 424 challenges, (i.e., 106 samples tested by four different reagent-instrument combinations), were evaluated to compare each reporting method in terms of how many samples would be regarded as at, above, or below the recommended therapeutic range. Since patient diagnoses were not obtained as part of this study, it was not known how many of these patients may have had clinical indications for a higher therapeutic range. Therefore, all specimens were simply evaluated according to those recommended criteria which would apply most frequently, i.e., INR = 2.0 to 3.0 or PT/Normal ratio of 1.2 to 1.5. As can be seen in table III, all methods of reporting showed many specimens to be above the newly recommended therapeutic range. Specifically, those tests run on fresh plasma indicated 42 percent of the samples were above the therapeutic range; however, when analysed after freezing, 69 percent were above the therapeutic range if reported by prothrombin time/normal ratios and 56 percent when reported by the INR (table III).

### Discussion

It has been demonstrated that important variables can be expected in prothrombin testing owing to use of different commercial reagents and instruments as reported by others. These differences were seen despite many factors which tended to minimize the differences in our study, – namely the use of thromboplastins of a single animal tissue source and batching of analyses which were performed in a single laboratory by the same technologist. Despite these stabilizing factors, a great variation was observed by us in the various reagent-instrument combinations when the results were expressed in seconds or prothrombin to normal ratios. When expressed by the use of the INR, however, statistically significant stabilization of results between the various reagent-instrument combinations occurred. Clearly in view of the frequent long term use of oral anticoagulants and

<table>
<thead>
<tr>
<th>(\text{PT/N} ) (1.2 - 1.5)</th>
<th>(\text{INR} ) (2.0 - 3.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All 4 Values Agree</td>
<td>76 (72%)</td>
</tr>
<tr>
<td>3 Agree/1 Variance</td>
<td>20 (19%)</td>
</tr>
<tr>
<td>2/2 Split</td>
<td>10 (9%)</td>
</tr>
<tr>
<td>Total Tested</td>
<td>106</td>
</tr>
</tbody>
</table>

\(\text{PT/N} \) - Prothrombin time in seconds/normal time in seconds

\(\text{INR} \) - International Normalized Ratio
TABLE III
Prothrombin Time Comparison of Interpretative Results Between Reporting Methodologies

<table>
<thead>
<tr>
<th></th>
<th>Initial*</th>
<th>PT/N</th>
<th>INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above Therapeutic Range</td>
<td>45 (42%)</td>
<td>292 (69%)</td>
<td>239 (56%)</td>
</tr>
<tr>
<td>Within Therapeutic Range</td>
<td>52 (49%)</td>
<td>114 (27%)</td>
<td>110 (26%)</td>
</tr>
<tr>
<td>Below Therapeutic Range</td>
<td>9 (8%)</td>
<td>18 (4%)</td>
<td>75 (18%)</td>
</tr>
<tr>
<td>Total Specimens Tested</td>
<td>106</td>
<td>424</td>
<td>424</td>
</tr>
</tbody>
</table>

* PT/N run on fresh unfrozen plasma
PT/N-Prothrombin time in seconds/normal in seconds (frozen plasma)
INR-International Normalized Ratio (frozen plasma)

In our increasingly mobile society, there is an urgent need for standardized interlaboratory comparisons. The use of the INR reporting method appears to address many of these needs, although when its ability to classify patients uniformly as over, under, or adequately anticoagulated according to recent recommendations, it did only slightly better than the traditional method of reporting.

Also of interest was the fact that all reporting methods suggested a large number of patients were over anticoagulated when compared to the recommendations of the recent National Conference on Antithrombotic Therapy. Briefly, that report recommended maintaining patients on oral anticoagulants at an INR of 2.0 to 3.0, (corresponding rabbit brain thromboplastin ratio of 1.2 to 1.5), for prophylaxis of venous thromboembolism, treatment of venous thrombosis, and prevention of systemic embolism in patients with atrial fibrillation, valvular heart disease, bioprosthetic heart valves, and acute myocardial infarction. Only in patients with mechanical heart valves and in patients with recurrent systemic embolism was a higher effect recommended, namely, an INR of 3.0 to 4.5, equivalent to a rabbit brain thromboplastin ratio of 1.5 to 2.0. While the specific diagnostic indications for anticoagulation were unknown in our group of patients, it seems likely that the therapeutic goal for the majority of these patients would have been an INR 2.0 to 3.0. Of interest, however, and serving to illustrate the still prevailing huge divergence of opinion and the lack of uniformity in therapeutic goals for these agents is the synopsis for recommended ranges cited by Leoliger et al16 in 1985, extending from an INR of 2.0 to 20.0!

In an international survey of dosage and control of oral anticoagulants, Poller and Taberner19 compared the mean dose prescribed in hospitals in 23 countries. While there was a constancy of overall mean doses, there were wide inter-laboratory differences between the mean doses of Warfarin. The lowest mean dose was 2.45 mg for one Hong Kong center, whereas one United States center prescribed a mean dose almost four times that amount of drug. In general, North American centers which used rabbit reagent thromboplastins tended to prescribe higher doses of Warfarin and advocate more intensive therapeutic ranges.

These views were further emphasized in 1982 by Hull et al. They compared two groups of patients with proximal vein thrombosis which were treated with Warfarin. In the first group where the Warfarin administration was regulated with the Manchester Comparative Reagent, (human brain thromboplastin), a lower dose resulted (average 4.9 mg per day) than in the group where Simplastin (rabbit brain thromboplastin) was used for the regulation of the Warfarin dose, (5.8 mg per day). While both groups of patients had similarly low rates of recurrent thromboembolism, two percent, the group with more intense Warfarin had a much higher rate of hemorrhagic complications, that is, 22 percent versus four percent in the less intensely treated group. Whereas the original therapeutic ranges set at the beginning of their study were 1.5 to two...
times the control for Simplastin and two times the control for the Manchester Comparative Reagent, Hull et al\textsuperscript{11} concluded that those recommendations lead to higher doses when the guideline for rabbit brain thromboplastin was used. Furthermore, the lower dosage was associated with equal clinical effectiveness and a much lower incidence of hemorrhage.

Further support for the effectiveness of lower Warfarin doses was furnished by Francis et al\textsuperscript{5} in 1983. They compared the safety and efficacy of Warfarin sodium with Dextran 40 in the prevention of venous thrombosis in patients at high risk for deep vein thrombosis after elective total hip or knee replacement. Warfarin was given in a two step regimen designed to avoid operative bleeding complications while still preventing venous thrombosis. This was accomplished by a 14 day pre-operative Warfarin sodium dose sufficient to prolong the prothrombin times 1.5 to three seconds more than the control, and then sufficient Warfarin post-operatively to prolong the prothrombin time 1.5 times the control. Compared to Dextran, the Warfarin patients had a much lower incidence of venographically demonstrated thrombosis, (21 percent versus 51 percent) and femoral or popliteal vein thrombosis (two percent versus 16 percent). Furthermore, the incidence of hemorrhage was infrequent and similar in the groups, and thus Francis et al\textsuperscript{4} concluded that the two step Warfarin provided highly effective prophylaxis of post-operative venous thrombosis without excessive risk of peri-operative bleeding.

There is a growing bulk of evidence which acknowledges that the generally accepted prothrombin time ratios of 1.5 to 2.5 with rabbit brain thromboplastin result in a greater intensity of therapy than the British human brain ratios of 2.0 to 4.0, and that rabbit brain pro-

thrombin time ratios over 2.0 can explain the apparently greater number of bleeding complications reported in the United States.\textsuperscript{26} In 1986, the National Conference on Antithrombotic Therapy\textsuperscript{2} combining the talents of an international group of experts examined extensive objective and subjective evidence on this topic and reached similar conclusions recommending that where oral anticoagulants are used, a rabbit thromboplastin prothrombin time ratio of 1.2 to 1.5 (INR 2.0 to 3.0) is appropriate. Only in the case of a mechanical prosthetic heart valve or recurrent systemic embolism was a higher ratio of 1.5 to 2.0 (INR 3.0 to 4.5) recommended.

The question of whether or not the conversion of all patient results to the INR as recommended by the International Committees for Standardization in Haematology and Thrombosis and Hemostasis\textsuperscript{13} warrants attention. Poller\textsuperscript{18} after three years experience asks, "Is the scheme working satisfactorily?" He indicates that some manufacturers are not yet calibrating their reagents correctly and that some coagulometers cause serious deviations from the correct INR. Furthermore, the INR cannot be relied upon during the induction phase of Warfarin treatment because of the varying responses of reagents to depression of individual coumarin clotting factors. Despite these limitations, however, the INR will tend to prevent gross differences in dosage between centers and should allow better comparison of published results. Lastly, with our increasingly mobile society, the availability of uniformly comparable prothrombin time studies at different laboratories should greatly assist individual patients to systematically regulate their oral anticoagulant needs.

Acknowledgments

The author gratefully acknowledges the technical expertise of Ms. Caroline Glenn, B.S, M.B.A., with-
References