EDUCATIONAL COMMENTARY – PROTHROMBIN TIME AND INTERNATIONAL NORMALIZED RATIO

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Learning Outcomes

Upon completion of this exercise participants will be able to:

- explain the uses and interpretations of the International Normalized Ratio (INR) and prothrombin time (PT) testing results.
- identify variables and limitations of the INR.
- describe the best way to utilize the results of the PT/INR.

The most widely performed coagulation test is the prothrombin time (PT). There are many instrument / reagent combinations in the laboratory and thus a wide variability in patient PT results. Understanding the mechanism of the test and the process to standardize the PT can optimize utilization and patient outcomes.

Prothrombin Time

The function of the PT is to measure factors that are in the extrinsic pathway (factor VII) and those in the common pathway (factors I, II, V, and X). The PT is also used to monitor oral anticoagulation, or warfarin therapy, which is used to treat and prevent blood clots. This test monitors the control of the dosage. The optimal dosage will prevent a clot without putting the patient at a risk for hemorrhage.[1] Warfarin works by inhibiting the y-carboxylation step of clotting and rendering the vitamin K-dependent factors (II, VII, IX, X, protein C and S) nonfunctional, resulting in impaired fibrin formation. Dosing must be individualized and the patient must be evaluated for hepatic and cardiac function, age, nutrition, concurrent therapy, and the patient's clinical situation. The response to oral anticoagulants may be markedly enhanced in obstructive jaundice, hepatitis, and cirrhosis due to reduced vitamin K absorption.

Warfarin is used for long-term anticoagulation. Because it is affected by so many drugs, food intake, and comorbidities, it takes 2 weeks for a patient's results to become stabilized.[2] Loss of activity is half-life dependent; factor VII has the shortest half life (4 to 6 hours) and factor II has the longest half-life (2 to 3 days).[3] Warfarin has a half life of between 20 and 60 hours, which varies in individuals. When oral anticoagulants are discontinued, the PT will require 2 to 4 days to return to normal. If oral vitamin K is given, the PT returns to normal within ≈24 hours. The over-anticoagulated state can be quickly reversed by giving vitamin K by subcutaneous injection or by slow intravenous infusion.

International Normalized Ratio

In the early 1970s, North American physicians were reporting a 20% incidence of bleeding in patients on

warfarin in comparison with European physicians, who reported a 5% incidence of bleeding.[4] The reason for this appeared to be that European laboratories had not made the switch to commercial thromboplastins. These reagents were determined to be less sensitive to oral anticoagulant effects. The variability in sensitivity of thromboplastin reagents and instruments resulted in a lack of comparability in results of PT testing. This provided a stimulus for standardization. In 1983, the international normalized ratio (INR) system was adopted by the International Committee for Standardization in Haematology, International Committee on Thrombosis and Haemostasis (ISCH/ICTH). The system is centered around the concept of the international sensitivity index (ISI), which represents the responsiveness of a thromboplastin reagent. It is based on the first World Health Organization (WHO) primary international reference preparation of thromboplastin. The original reagent was called the Manchester reagent, and it was given an ISI of 1. The ISI of a manufacturer's reagent is derived by calibrating its thromboplastin reagent with the WHO reference preparation and comparing it with the specific instrument/reagent combination used in the laboratory.

The INR was developed to help standardize warfarin therapy so patients would be able to obtain an INR from any institution regardless of a laboratory's reagent and instrument combination. The seconds of the PT may be different; however, the INR should remain the same. If the INR is different, the patient may require a change in dose of warfarin.

The formula for the INR is:

$$\left(\frac{\text{Patient PT}}{\text{Geometric Mean*}}\right)^{\text{ISI}}$$
Normal Range

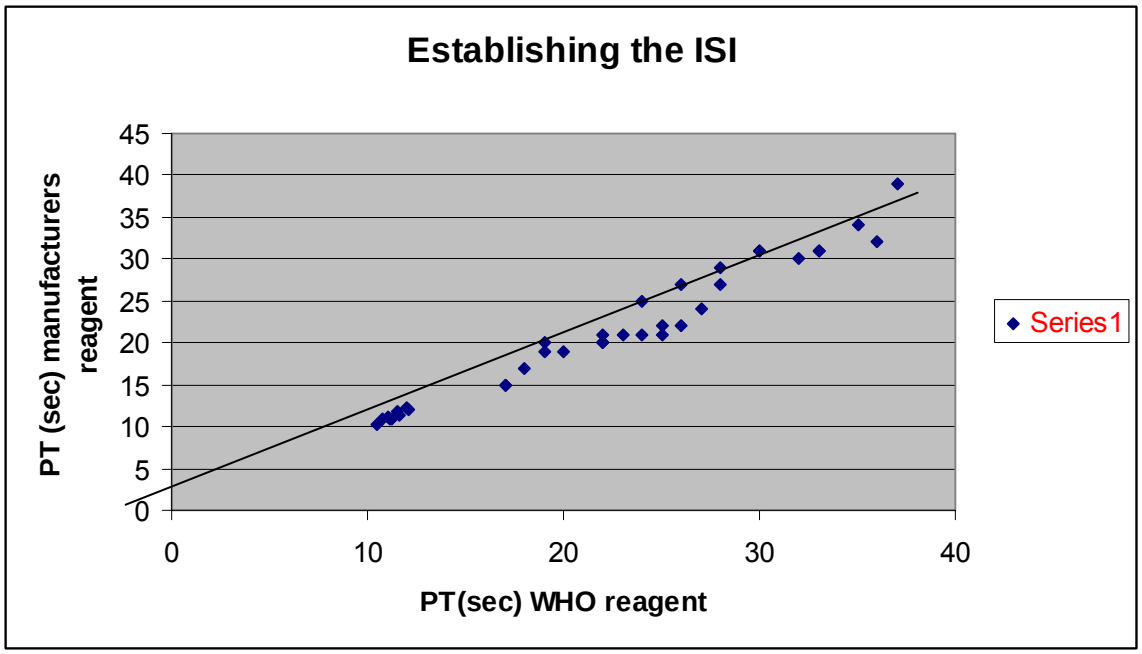
INR = International Normalized Ratio
Patient PT = Patient PT in seconds
ISI = Value of the International Sensitivity Index given by the manufacturer.
*Geometric mean of the laboratory's normal range established for that reagent.

Determining the ISI

The ISI is a number provided by a manufacturer representing how sensitive its reagent is in relationship to the most sensitive reagent, human brain thromboplastin, considered the gold standard. The ISI is the most important variable of the INR equation; because the ISI is exponential, an incorrect ISI will have the greatest impact on the INR value. The procedure for a manufacturer to calculate the ISI of a reagent is:

> 20 normal healthy controls and 60 patients on a stable dose of warfarin (i.e., on warfarin for 2 weeks) have a PT performed with the reference human brain thromboplastin (ISI=1) by a manual tilt-tube method versus the manufacturer's reagent/instrument combination. The results are graphed, and the slope of the line (y = mx +b) is the calculated ISI for the manufacturer's reagent.

This "corrects" the manufacturer's reagent (*y* axis) against the "gold standard" (*x*- axis) thromboplastin.[5]



The more sensitive the reagent, the closer the ISI is to 1, and the longer the PT is in seconds. An insensitive reagent is the opposite, with a higher ISI and shorter PT in seconds. The College of American Pathologists recommends using a reagent with an ISI of between 0.9 and 1.7.

Clinical Use of the INR

The American College of Chest Physicians (ACCP) recommends use of the INR for monitoring oral anticoagulation. An INR of 2.0 to 3.0 is recommended for all conditions with the exception of patients who are anticoagulated for thrombotic complications of mechanical heart valves, for whom an INR of 2.5 to 3.5 is recommended.[6]

Warfarin has a narrow therapeutic index and many variables, including competing medications and patient compliance. Patients who were monitored every 24 days were out of range about 52% of the time. More frequent testing produced more patients who were therapeutic (24% out of range when tested every 8 days and 10% when tested every 2 days). The culmination of these efforts resulted in a 7% incidence in bleeding and a 2.5% occurrence of thrombosis.[7]

No critical values are published for the INR. These need to be determined by the laboratory because the seconds obtained by the result are method-, instrument-, and reagent-dependent. Instruments are linear up to the manufacturer's predetermined ranges, and the PT result that exceeds those seconds will not be

linear and therefore is inaccurate when placed in the formula for the INR. This may help institutions determine at what uppermost limit they can provide a correct INR. (For example, if PT values are not linear past 30 seconds and the result is 45, the result is extrapolated and will not provide an accurate INR result.) Regardless, if a patient has an INR >6, he or she needs to be treated. The response to oral anticoagulation is variable and unpredictable. If the level is inadequate it increases the risk of thrombosis; if it is excessive there is an increased risk of bleeding. The goal is to maintain a narrow therapeutic range.[8]

Variables of the INR

Problems with the INR result can be preanalytical, analytical, and postanalytical. When the ISI is being determined, all samples are collected in 3.2% sodium citrate. If a laboratory uses 3.8% sodium citrate collection tubes, there will be a discrepancy in the calculation of the INR. The INR of critically ill patients may vary more than the INR of patients who are stable. Results are affected by body mass, vitamin K ingestion, diet, and liver function because coagulation proteins are produced in the liver. Patients whose livers are compromised should be monitored by means other than the INR. Pediatric patients may have INR ranges that are lower than those of adults, and should be monitored more frequently. In addition, about 80 drugs interfere with warfarin.

Some common sources of error causing variations in the INR process are:

1. ***The value of the PT in seconds:*** As long as controls are in, and you have validated that your instrument performs well, this result would present with the least amount of variation.

2. ***The normal range:*** Good normal ranges should reflect your patient population including equal amounts of male/female samples, collected over a period of time, and excluding patients in pre-op or the emergency department. Their values may be falsely shorted due to the presence of acute-phase reactants. If a factor is elevated, the result will be shortened. This will greatly impact the results of the normal range, making it appear falsely shortened. As a result the mean of the normal range may be shortened, affecting the INR.

3. ***Use the geometric mean:*** This is less affected by random errors or outliers in normal plasma samples. It is a better representation when using exponential data.

4. ***The value of the ISI:*** Make sure that you use the instrument/reagent ISI specified for your particular combination. The ISI is an exponential number, and will have the greatest impact on your outcome.

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An additional variable is that a third generation of international reference plasma (IRP) is being used. Each generation is calibrated off the previous generation, and each calibration allows for an inherent 3% to 5% coefficient of variation (CV). So for the first generation of IRP there is an allowable 3% CV, plus an additional 3% on the second generation (now a total of 6% CV from the original); therefore the third generation allows for almost a 9% CV.[9]

Other INR Conundrums

Occasionally patients demonstrate unresponsiveness to warfarin; their INR does not change as dosage is increased. A hepatic cytochrome P450 has been identified to be central in metabolizing drug molecules resulting in clinical implications. It has been noted that the crystal structure of human CYP450, CYP2C9 defines unique interaction with warfarin and may undergo an allosteric mechanism during its function giving a structural basis for a drug-drug interaction. Polymorphant variants have also been reported. Understanding a drug's P450 metabolism, along with knowledge of the patient's phenotype, can assist in choice, dosage, and predicted toxicity of the drug.[10]

Many hospitals are beginning to use direct thrombin inhibitors. These new anticoagulants inhibit thrombin directly and are approved for use in patients with heparin-induced thrombocytopenia (HIT). A linear relationship has been observed with the INR in patients on argatroban (ARG). When placing patients on concurrent therapy, the INR may be overestimated. This could lead to premature termination of ARG, resulting in a subtherapeutic state. A study has determined that ARG doses up to 2 µL/kg/min can be discontinued at an INR of 4.0, which corresponds to an INR of 2.2 to 3.7, placing the patient in the therapeutic range.[11]

Local Calibrations

The Clinical Laboratory Standards Institute (CLSI) published guidelines in July 2005 titled, "Procedures for Verification of INR and Local Calibration of PT/INR Systems: Approved Guideline H54-A." These guidelines describe a system of calibration using a set of calibrated plasmas that span the normal and therapeutic range. The assigned values from the plasmas can help to determine local ISI values, or generate a calibration curve from which the INR can be derived. The plasmas are run for 3 days in duplicate. Briefly, the procedures recommend:

To calculate a local ISI: The PT obtained locally is plotted on the *y*-axis against the value-assigned PT on the *x*-axis. The ISI is determined from the slope of the line.

To prepare a direct INR calibration line: The PT obtained locally is plotted on the *y*-axis against the value-assigned PT on the *x*-axis. The best-fit line is determined using orthogonal regression

analysis. The direct INR is independent of the ISI and the geometric mean of the normal range. The INR is read off the calibration line using the patient's PT.[12]

Conclusion

Many variables can alter the INR including the normal range, the calculated value of the INR, and the value of the ISI. In addition, using an insensitive reagent further complicates the accuracy and reproducibility of the result. Using certified plasmas may help to improve standardization of the INR and improve the amount of time patients are therapeutic. It is important for all laboratory personnel to have a good understanding of the INR and its impact on coagulation results.

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