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The Prothrombin Time and the International Normalized Ratio: What Ontario laboratories should consider

The prothrombin time (PT) is one of the most common tests performed by clinical laboratories.

The PT is often used for monitoring patients who are receiving vitamin K antagonist (VKA) therapy, such as warfarin. The PT assesses the function of factor VII, factor X, factor V, prothrombin and fibrinogen after the addition of thromboplastin and calcium.¹ The factor VIIa/tissue factor complex activates factor X to factor Xa and through the action of the prothrombinase complex, prothrombin is converted to thrombin.¹ The time in seconds for the conversion of fibrinogen to soluble (non-cross linked) fibrin by thrombin is reported as the prothrombin time.

Most commercial thromboplastins neutralize up to approximately 1 IU/mL of heparin activity, and as a result the PT is insensitive to therapeutic doses of heparin.^{3,8} The concentration of heparin that is neutralized, specific for each thromboplastin, can be obtained from the manufacturer. Each laboratory should validate the concentration and type of heparin neutralized by the reagent in use.

The PT can be performed on a wide variety of automated coagulometers. External quality assurance performance administered through the Quality Management Program – Laboratory Services (QMP-LS) has confirmed that the PT is well performed by clinical laboratories in Ontario.

International Normalized Ratio

In an effort to standardize the reporting of the PT for VKA therapy monitoring, the international normalized ratio (INR) is used. The INR is a mathematically derived value based on the equation: $INR = \frac{\text{patient PT/MNPT}}{ISI}$ where the MNPT = mean normal prothrombin time and the ISI = international sensitivity index.^{2,5,13} Current recommendations indicate that a minimum of 20 individual healthy control volunteers should be used to determine the MNPT.^{2,3,5} Laboratories need to recognize that different

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thromboplastins have variable sensitivities to the vitamin K-dependent coagulation factors assessed in the PT (specifically factors VII, X and prothrombin). This variability in sensitivity between thromboplastins is reflected in the ISI that is assigned to the reagent.^{1,13} To minimize clinically significant variability in the INR, the ISI for the thromboplastin used by the laboratory needs to be verified locally and be specific for the lot number of reagent and the coagulometer used for testing.^{2-5,9}

Generally, clinical laboratories have standardized the reporting of the PT to physicians as an INR. This has resulted in the adoption of the INR for the grading of the severity of various disease states, such as liver disease.² In Ontario, the reporting of the PT is variable. Some laboratories report only the INR, while others report both the PT in seconds as well as the INR. Laboratories need to recognize that the ISI used in the calculation of the INR is determined from plasma obtained from patients on stable VKA therapy, not from samples obtained from patients with other conditions. This difference on how the ISI is determined may result in an imprecise INR calculation and impact the clinical interpretation of the INR (for instance, in the staging and/or grading of liver disease).^{2,13,14} As noted by Kovacs et al., however, the PT is also not a reliable measure of the status of the coagulation cascade in patients with liver disease as it also varies between machine-reagent combinations.¹³ Further study of the effect of determining the ISI using plasma from patients on stable VKA therapy for other disease states is required.^{2,13} This may also provide an opportunity for the development of new approaches to standardization of the PT.⁹

Pre-analytical Variables

There are numerous pre-analytical variables that influence the PT/INR, as well as other tests of hemostasis and thrombosis. These include, but are not limited to, appropriate patient identification, sample collection, transport, processing and storage issues.^{5,11} It is recommended that laboratories standardize to one concentration of sodium citrate anticoagulant (either

0.105/0.109 molar (commonly referred to as 3.2%) or 0.129 molar (3.8%)) as the reference intervals for these two concentrations of citrate are not interchangeable. Samples collected into 3.8% sodium citrate may have a prolonged PT when compared to the 3.2% reference interval.⁵ It is recommended that 3.2% sodium citrate be used.^{4,11}

Monitoring of Vitamin K Antagonists

Vitamin K antagonists (VKA) interfere with the function of the vitamin K-dependent factors, specifically factor II, factor VII, factor IX and factor X, as well as the naturally occurring anticoagulants protein C and

with improvement in diagnosis.¹⁰ QMP-LS provide two PT EQA challenges with two samples in each challenge annually. With each challenge, laboratories are required to provide QMP-LS with the PT in seconds, the ISI and MNPT of the thromboplastin and the calculated INR. This EQA program meets Ontario Laboratory Accreditation (OLA) requirements. The thromboplastins in use in Ontario have been shown to provide consistent INR results. As expected, the PT in seconds is variable, which reflects differences in the sensitivity of each thromboplastin. A recent QMP-LS EQA survey confirmed the PT/INR to be well performed in Ontario. There was only one flag of 187 participant laboratories.¹² Nine

Warfarin dosing nomograms improve laboratory test utilization and enable the physician to maintain the patient's INR within the therapeutic range more often, resulting in improved patient safety.

protein S. VKA inhibit the carboxylation of glutamate residues to γ -carboxyglutamate at the N-terminal of the vitamin K-dependent factors.³ The therapeutic functional effect of the VKA is assessed by the PT. The sensitivity of the reagent to the VKA is reflected by the ISI. The closer the ISI is to 1.00, the more sensitive the reagent is to the vitamin K-dependent factors as compared to the World Health Organization (WHO) reference thromboplastins.^{1,13,14}

Monitoring of the PT/INR for VKA therapy is required and physicians should use regular laboratory monitoring in conjunction with a validated warfarin dosing nomogram.⁶ Warfarin dosing nomograms improve laboratory test utilization and enable the physician to maintain the patient's INR within the therapeutic range more often, resulting in improved patient safety.⁶

External Quality Assessment of the PT/INR by Ontario Laboratories

External quality assessment (EQA) schemes for hemostasis tests are associated

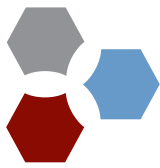
different thromboplastins were used to determine the PT. Twenty-nine coagulometers from five manufacturers were used in the province.¹²

Biological Variation

Consideration of the biological variation and total allowable error for laboratory tests is recommended by accreditation bodies, including OLA. There are few studies available on the biological variation for coagulation parameters and of these, the studies are based on outdated methodologies.⁷ Of the literature available, consensus is that the biological variation for the PT is considered to be low. One of the more recent studies (1992) showed that the intraindividual biological variation of the PT, calculated as the mean coefficient of variation (CV) was 2.3% and the interindividual biological variation as 6.8%.⁷

The measurement of uncertainty in diagnostic laboratory tests should be considered and is required to meet current laboratory accreditation requirements. The measurement of uncertainty for the PT has

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been considered by the Australian group and is the measure of an assay's variability based on the imprecision and the bias of the assay.⁹ Specific to the PT, laboratories need to recognize that different approaches for the determination of the ISI and MNPT will influence the INR that is calculated, resulting in variability that will impact the measurement of uncertainty of the test.⁹ Validation and further study of the measurement of uncertainty for all diagnostic laboratory tests needs to be undertaken.

Laboratories need to be cognizant of the effect and impact of pre-analytical variables on laboratory test results.

Conclusion

The PT is commonly used to monitor VKA therapy and is generally reported as an INR. Laboratories need to ensure that the ISI and the MNPT used to calculate the INR is specific for the reagent lot number and the coagulometer used. Generally, therapeutic doses of heparin (up to approximately 1 IU/mL of heparin activity) do not cause prolongation of the PT when commercial thromboplastins are used.

Laboratories need to be cognizant of the effect and impact of pre-analytical variables on laboratory test results. Development and adherence to appropriate standard operating procedures may lessen these influences and minimize the risk to patients of resultant errors. In addition, an effective quality management system needs to be implemented and maintained by laboratories.

In Ontario, the PT is performed on a variety of coagulometers. External quality assessment through QMP-LS confirms that the PT and reported INR are performed

well by clinical laboratories. Continued recognition by laboratories of the impact of the many variables in the performance of the PT will provide opportunity for diagnostic laboratories to continue to provide quality laboratory results for improved patient care. ♦

References:

1. Harmening, DM. Bethel M. Coagulation Methods, In: Clinical Hematology and Fundamentals of Hemostasis (5th Ed). F.A. Davis Co.; 2009. p. 849–881.
2. Favalaro EJ, Adcock DM. Standardization of the INR: How good is your laboratory's INR and can it be improved? *Semin Thromb Hemost* 2008 34(7): 593-603.
3. Tripodi A. Monitoring oral anticoagulant therapy. In: Kitchen S, Olson JD and Preston FE, editors. *Quality in laboratory hemostasis and thrombosis*. Wiley-Blackwell: 2009. p. 179-189.
4. Olson JD, Brandt JT, Chandler WL, Van Cott EM, Cunningham MT, Hayes TE, et al. Laboratory reporting of the international normalized ratio—Progress and problems. *Arch Pathol Lab Med*. 2007;131(11):1641-1647.
5. Favalaro EJ, Lippi G, Adcock DM. Preanalytical and postanalytical variables: The leading causes of diagnostic error in hemostasis? *Semin Thromb Hemost*. 2008;34(7): 612-634.
6. Crowther MA. Oral anticoagulant initiation: rationale for the use of warfarin dosing nomograms. *Semin Vasc Med*. 2003;3(3):255–260.

7. Banfi G, Del Fabbro M. Biological variation in tests of hemostasis. *Semin Thromb Hemost*. 2008;34(7): 635–641.
8. Alban S, Lühn S. Prothrombin time for detection of contaminated heparins, *N Engl J Med* 2008;359(25):2732–2734.
9. Favalaro EJ, Hamdam S, McDonald J, McVicker W, Ule V. Time to think outside the box? Prothrombin time, international normalized ratio, international sensitivity index, mean normal prothrombin time and measurement of uncertainty: a novel approach to standardisation. *Pathology*. 2008;40(3): 277–287.
10. Jennings I, Kitchen S, Woods TA, Preston FE. Multilaboratory testing in thrombophilia through the United Kingdom National External Quality Assessment Scheme (Blood Coagulation) quality assurance program. *Semin Thromb Hemost*. 2005;31(1): 66–72.
11. Clinical and Laboratory Standards Institute (2008). Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; Approved guideline – Fifth Edition. CLSI: H21–A5.
12. Quality Management Program – Laboratory Services. Committee Comments - COAG-0902. Routine. 2009-05-05.
13. Kovacs MJ, Wong A, MacKinnon K, Weir K, Keeney M, Boyle E, et al. Assessment of the validity of the INR system for patients with liver impairment. *Thromb Haemost*. 1994;71(6): 727-730.
14. Denson DW, Reed SV, Haddon ME. Validity of the INR system for patients with liver impairment. *Thromb Haemost*. 1995;73(1): 162.