PEARLS OF LABORATORY MEDICINE

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TITLE: Direct Oral Anticoagulants: Laboratory Methods for Assessing Dabigatran

PRESENTER: Dr Dot Adcock

Slide 1:

Hello, my name is Dr Dot Adcock. I am currently the Chief Medical Officer and senior vice president of LabCorp. Welcome to this Pearl of Laboratory Medicine on "**DOACs: Laboratory Methods for Assessing Dabigatran**." This program was created with Bob Gosselin from the Thrombosis & Hemostasis Center at the University of California, Davis Health System.

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This session is a joint effort between the American Association for Clinical Chemistry (AACC) and the North American Specialized Coagulation Laboratory Association (NASCOLA)

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I would like to review the definition of a number of terms that will be used in this and following presentations. Venous thromboembolism or VTE: clots within the veins most commonly deep vein thrombosis (DVT) and pulmonary embolism (PE)
Pharmacokinetics (PK): drug concentration after administration
Pharmacodynamics (PD): the drug effect after administration
Peak levels: the maximum drug concentration after administration
Trough levels: the drug level just before the next drug dose

On-therapy range which is commonly used to address DOACs as this class of drugs do not have "therapeutic ranges". An on-therapy range reflects the expected drug concentration from lowest trough to highest peak value for a given dose and indication.

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Dabigatran which is administered as Dabigatran etexilate, brand name Pradaxa® is an oral direct thrombin inhibitor which is immediate acting with peak concentration 1.5 – 3 hours after administration. It inhibits free and bound thrombin (also known as activated factor II). It is primarily excreted by the kidneys and has a half-life of about 13 hours.

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This table provides the expected mean peak and trough drug concentrations depending on the dose of dabigatran administered. Note that the 25th-75th percentile ranges are quite broad with overlap between the peak and trough ranges.

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This cartoon of the coagulation cascade demonstrates the various targets for anticoagulant agents and depicts the laboratory testing pathways and assay targets. The currency in the common pathway is a simple trick to remember those factors in this pathway and their order of reactions, 10, 5, 2 and 1 (also known as fibrinogen). Both direct Xa inhibitors and direct thrombin inhibitors can potentially cause prolongation of the aPTT, PT and RVVT as they inhibit factors within these pathways. As dabigatran is a direct thrombin inhibitor, the tests that may be affected by its presence include the PT, APTT, thrombin time, ecarin methods, and other tests that utilize a thrombin substrate.

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These data are from a study we performed soon after dabigatran was FDA approved. Healthy volunteers were administered therapeutic doses and peak levels were obtained. Plasma samples were evaluated with 7 different commonly used APTT reagents and 6 PT reagents. The red horizontal line depicts the upper limit of normal for most reagents and the vertical lines demonstrate the drug concentrations as measured by mass spectrometry. As you can see, response is reagent dependent with significantly more variability in PT reagents than aPTT reagents. I also want to point out that time in seconds for the aPTT reagents plateaus as drug concentration increases. Finally, it is important to note that both PT and APTT results can be normal when individuals have therapeutic concentrations of dabigatran in their blood. Normal PT and/or APTT results therefore are not a reliable indicator of dabigatran presence.

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The thrombin time is a simple test by combining an undiluted or slightly diluted plasma sample with a low concentration thrombin reagent and measuring the time to clot formation. As dabigatran is a direct inhibitor of thrombin, a standard thrombin time is exquisitely sensitive to the presence of dabigatran such that very low drug concentrations, far below an on-therapy range, cause prolongation of the thrombin time outside the upper limit or normal represented by green line. With on therapy concentrations of dabigatran, the thrombin clotting time is undetectable (no clot

detected) and therefore this assay cannot be used to quantify dabigatran concentration. The thrombin time is able to determine drug presence although prolongation of the thrombin time is not specific for dabigatran. Thrombin time prolongation can also be secondary to heparin, parenteral direct thrombin inhibitors such as argatroban, and low or dysfunctional fibrinogen levels.

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To recap, dabigatran in a patient's plasma can be determined using the thrombin time although remember that prolongation of the thrombin time is not specific for dabigatran. A normal PT and aPTT cannot exclude the presence of dabigatran. We will now review the laboratory methods to quantify dabigatran.

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The gold standard method to quantity dabigatran is liquid chromatography tandem mass spectrometry (LS-MS/MS). This is the only method to quantitate direct oral anticoagulant agents (DOACs) that is specific to the drug being measured. For example, if a patient is on dabigatran and heparin, this assay will measure only the dabigatran that is preset. If the patient were on a different direct thrombin inhibitor such as argatraban, it would not be measured in a dabigatran MS assay. When using mass spectrometry active metabolites or conjugates of the drug must be considered as these may contribute to overall anticoagulation but would not be measured in a dabigatran mass spectrometry assay. A conjugate of dabigatran, dabigatran glucuronide adds about 20% anticoagulant activity but an alkaline hydrolysis sample pretreatment splits the conjugate allowing measurement of total dabigatran. This assay has an excellent lower limit of detection and measures drug over a broad range with good discrimination. While LC-MS/MS is the gold standard method to measure dabigatran, the lack of an international reference for assay calibration can lead to variability between testing locations. Accuracy and precision within a single testing location is quite good.

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The dilute thrombin time can be used to quantitate dabigatran if the assay is calibrated using a dabigatran calibrator. Results compare well to a mass spectrometry method. Currently, there are no dabigatran calibrators that are FDA cleared for IVD use, all are research use only (RUO). An adequate lower limit of detection can be achieved if the sample is properly diluted.

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Ecarin, is a thrombin-like metalloprotease from the venom of Echis carinatus (saw-scaled viper), and converts prothrombin to meizothrombin. Meizothrombin is a potent thrombin intermediate that can be inhibited by dabigatran or other direct thrombin inhibitors (e.g. bivalirudin), but not by heparin. The classic ecarin method is a clot based

assay called the ecarin clotting time (ECT). A chromogenic ecarin assay (ECA) is now available and it has advantages over the clot-based method in that the ECA is not affected by low prothrombin or fibrinogen levels, both of which will falsely prolong the ECT. The chromogenic method demonstrates good accuracy and reproducibility.

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This slide depicts the ecarin clotting time assay without (left graph) and with calibration (right graph) with a dabigatran calibrator as compared to a mass spectrometry method. The assay demonstrates a linear response to drug over a broad therapeutic range. Both the ecarin reagent and the dabigatran calibrator are labelled RUO. One disadvantage of this assay is the lot-to-lot variability that can occur with the ecarin reagent.

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These are data from the ecarin chromogenic assay calibrated with a dabigatran calibrator performed in three different laboratories as compared to a mass spectrometry method. There is good agreement between testing locations with a linear response to drug over a broad drug concentration. This assay is labelled RUO as well.

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The chromogenic anti-IIa method is exactly like the heparin anti-Xa assay except the reagent is thrombin and not activated FX. The kit is labeled RUO. The assay is easily automatable. A dual curve is needed to measure drug concentration adequately over the on-therapy range. The assay performance regarding precision and accuracy appears quite good although there is limited published data on the method.

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In summary, screening tests such as the PT and APTT are insufficient for assessing dabigatran anticoagulation and cannot be used to exclude the presence of drug. A normal thrombin time virtually excludes dabigatran presence. Mass spectrometry methods are considered the gold standard for measuring dabigatran although there is no international reference for calibration. Alternative quantitative methods have been demonstrated to be equivalent to mass spectrometry including the drug calibrated dilute thrombin time, ecarin clotting time, and ecarin chromogenic assays. More data is required for chromogenic anti-IIa methods but they appear adequate.
Alternative methods for quantifying dabigatran can be adapted to open-system (that is programmable) automated coagulation analyzer, however, there are no FDA approved methods

Slide 17: References

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Slide 18: Disclosures

Dorothy Adcock has received honoraria from Siemens Healthcare Diagnostics and serves as a consultant to Instrumentation Laboratory.
Robert Gosselin has provided expert testimony for dabigatran and rivaroxaban testing, has received honoraria from Siemens Healthcare Diagnostics, Machaon Laboratories, Diagnostica Stago and serves as a consultant for Diagnostic Grifols and UniQure, and

advisory board member for BioMarin

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Thank you for joining me on this Pearl of Laboratory Medicine on "**DOACs; Laboratory Methods for Assessing Dabigatran**."