CCTC Clinical Chemistry Trainee Council | AACC Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

www.traineecouncil.org

TITLE: Direct Oral Anticoagulants: Laboratory Methods for Assessing anti-Xa DOACs

PRESENTER: Dot Adcock M.D.

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 on "**DOACs: Laboratory Methods for Assessing anti-Xa DOACs**." This program was created with Bob Gosselin from the Thrombosis & Hemostasis Center at the University of California, Davis Health System. Slide 2:

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

Slide 3:

I would like to review the definition of a number of terms that will be used in this and following presentations. Venous thromboembolism: 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
Therapeutic range: the recommended target drug effect, usually either a concentration or test effect (e.g. INR and warfarin therapy).

Slide 4:

There are currently 4 FDA approved anti-factor Xa DOAC: rivaroxaban, apixaban, edoxaban and betrixaban. These are commonly called anti-Xa drugs. While all of these DOACs are direct reversible inhibitors of free and bound factor Xa, there is variability in other characteristics, most importantly for laboratory considerations are the time to achieve peak levels (Tmax) and drug half-life.

Slide 5:

This table details the expected peak or Tmax concentrations based on prescribed dose for each indication for each anti-Xa DOAC. These values and ranges do not account for any adjustment due to renal insufficiency. Note the wide variability both within and between DOACs for each indication. Additionally, note the use of percentiles, such that a range that represents the 10^{th} - 90^{th} percentile would indicate that 80% of the treated patients would have these expected drug levels. Conversely, 1 out of 5 treated patients would fall outside (either lower or higher) these expected ranges.

Slide 6:

This table details the expected trough concentrations based on prescribed dose for each indication for each anti-Xa DOAC. These values and ranges do not account for any adjustment due to renal insufficiency. Similar to the previously described peak DOAC table, there is wide variability both within and between DOACs for each indication.

Slide 7:

As there are no therapeutic ranges provided by DOAC manufacturers, the term “on-therapy” has been introduced which represents the expected drug levels from lowest trough to high peak drug concentration based on observations of patients taking these drugs at prescribed doses. Note the wide range of expected drug levels and the variability between DOACs, as well as overlap between trough and peak concentrations.

Slide 8:

Based on clinical and laboratory literature, there are a number of presumptions that have been made regarding the laboratory assessment of anti-Xa DOACs. Included is the notion that 1) the prothrombin time can reliably assess anti-Xa DOAC concentration, 2) a normal prothrombin time excludes significant anti-Xa DOAC levels, 3) the use of heparin drug calibrated anti-Xa tests to estimate anti-Xa DOAC concentration or presence, and finally that drug calibrated anti-Xa tests are substantially equivalent to mass spectrometry measurements. This presentation will explore whether these presumptions are true.

Slide 9:

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 inhibitor anticoagulants and direct thrombin inhibitor anticoagulants can potentially cause prolongation of the aPTT, PT and RVVT as they inhibit factors within these pathways. Direct Xa inhibitors however will not affect the thrombin time, dilute thrombin time or ecarin methods.

Slide 10:

The table represents the “on-therapy” range for each anti-Xa DOAC. The figures demonstrate the response of two commonly used PT reagents in the US, Recombiplastin 2G and Innovin to various concentrations of DOAC enriched normal plasma. The red line represents the upper limit of normal for the reagent/instrument platform used. Note the drug concentration for each DOAC that intercepts the upper limit of normal. These data suggest the unreliability of the PT to adequately rule out drug presence or estimate levels.

Slide 11:

This cartoon represents the chromogenic anti-factorXa test that is commonly used in the US for measuring unfractionated heparin, low molecular weight heparin or the pentasaccharide (or Fondiparinux) anti-Xa activity. With this method, excess activated factor X is added to patient sample. The activated FXis bound by the anti-Xa drug, which can be either a heparin or anti-Xa DOAC (or both). The second step is the addition of a specific substrate that, is cleaved by residual activated FX releasing p-nitroaniline which is yellow in color. The amount of yellow color is inversely proportional to anti-Xa drug activity. It should be emphasized that this method cannot differentiate between the anti-Xa drugs heparins or anti-Xa DOACs, and furthermore there will be additive effect in this assay if both drugs are present in the sample. Anti-Xa assays may have added antithrombin, but this two-step method is not recommended for DOAC testing, as it has been demonstrated to overestimate DOAC concentration.

Slide 12:

These figures represent data we generated from contrived DOAC samples and tested on two different anti-Xa reagent platforms, the Stago Liquid Heparin assay calibrated to low molecular weight heparin and the COAMATIC low molecular weight heparin assay. As you can see, there is a linear relationship between anti-Xa DOACs and LMWH calibrated anti-Xa tests. These data would suggest a higher sensitivity to anti-Xa DOACs than the PT as shown in slide 9. However, this approach should only be used as an estimation of anti-Xa DOAC concentration such as may be necessary in an emergency situation and local verification is necessary.

Slide 13:

This table compares low concentrations of apixaban, rivaroxaban and edoxaban to unfractionated heparin or low molecular weight heparin calibrated anti-Xa assays from 3 different reagent manufacturers. Except for the noted exoxaban exception, these low anti-Xa DOAC levels had measurable anti-Xa activity, all well within the lower limit of quantitation of 0.05U/mL. With the lower table representing the “on-therapy” range for the anti-X DOACs, these data would suggest that heparin calibrated anti-Xa methods can reliably detect low levels of most anti-Xa DOACs.

Slide 14:

These data demonstrate rivaroxaban (left) and apixaban (right) calibrated anti-Xa activity assay as compared to mass spectrometry. The lower limit of quantitation for both methods was determined to be <10ng/mL. This unpublished LLOQ data was derived from linearity studies, whereas the apixaban figure would suggest a higher LLOQ.

Slide 15:

In summary, the prothrombin time has a highly variable, reagent dependent response to DOACs. As such, it is not a reliable indicator of DOAC concentration. A normal PT does not exclude significant levels of anti-Xa DOAC, especially when using Innnovin as the PT reagent, and for essentially all PT reagents in the presence of apixaban. It has been demonstrated that the UFH and LMWH calibrated anti-Xa assays can be used to estimate anti-XaDOAC concentration and can potentially be used to exclude significant DOAC presence, with edoxaban being more reagent variable. This estimation of DOAC concentration or presence should be locally verified emphasizing that these results are only an estimation of DOAC exposure. It has been demonstrated that DOAC calibrated anti-Xa tests are substantially equivalent to mass spectrometry.

Slide 16: References

1. Food and Drug Administration. Bevyxxa—Prescribing Information. Available at: https://www.bevyxxa.com/wp-content/uploads/2017/11/bevyxxa-betrixaban-capsules-prescribing-information-pdf.pdf. Last accessed Sep 3, 2019.
2. Gosselin RC, Adcock DM, Bates SM, Douxfils J, et al. International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. Thromb Haemost. 2018;118(3):437-450. doi:10.1055/s-0038-1627480.
3. Douxfils J, Gosselin RC. Laboratory Assessment of Direct Oral Anticoagulants. Semin Thromb Hemost. 2017;43(3):277-290. doi: 10.1055/s-0036-1597296.
4. Gosselin RC, Adcock DM, Douxfils J. An update on laboratory assessment for direct oral anticoagulants (DOACs). Int J Lab Hematol. 2019;41 Suppl 1:33-39. doi: 10.1111/ijlh.12992.
5. Adcock DM, Gosselin RC. The danger of relying on the APTT and PT in patients on DOAC therapy, a potential patient safety issue. Int J Lab Hematol. 2017;39 Suppl 1:37-40. doi: 10.1111/ijlh.12658.

6. Gosselin R, Grant RP, Adcock DM. Comparison of the effect of the anti-Xa direct oral anticoagulants apixaban, edoxaban, and rivaroxaban on coagulation assays. Int J Lab Hematol. 2016;38(5):505-13. doi: 10.1111/ijlh.12528.
7. Gosselin RC, Adcock DM. The laboratory's 2015 perspective on direct oral anticoagulant testing. J Thromb Haemost. 2016;14(5):886-93. doi:10.1111/jth.13266. Epub 2016 Feb 19. Review. Erratum in: J Thromb Haemost. 2019;17(4):698.
8. Gosselin RC, Francart SJ, Hawes EM, Moll S, Dager WE, Adcock DM. Heparin-Calibrated Chromogenic Anti-Xa Activity Measurements in Patients Receiving Rivaroxaban: Can This Test Be Used to Quantify Drug Level? Ann Pharmacother. 2015;49(7):777-83. doi: 10.1177/1060028015578451.

Slide 17: 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
.

Slide 18: Thank You from www.TraineeCouncil.org

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