Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Anti-Xa: A Versatile and Quantitative Anticoagulant Drug Assay

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Slide 1:
Hello, my name is Paul Riley. I am a scientific affairs specialist for Diagnostica Stago, Inc. Welcome to this Pearl of Laboratory Medicine titled "Anti-Xa: A Versatile and Quantitative Anticoagulant Drug Assay."

Slide 2:
This presentation will provide an overview of the anti-Xa assay methodology, role of assay additives including antithrombin (AT) and dextran sulfate (DS), major clinical applications, and benefits of use for monitoring heparin and measuring direct oral anti-Xa anticoagulants. In this manner, the versatility of the assay for quantification of many different anticoagulant drugs will be highlighted.

Slide 3:
This slide shows the coagulation cascade from tissue factor mediated activation at the top center to fibrin and thrombus formation at the lower right. Also shown are targets of anticoagulant drugs along with their AT dependence or direct action on their respective target coagulation factors. We can see how inhibition at various critical places within the coagulation cascade can effectively prevent thrombus formation, in this way preventing or treating pathological thrombosis. Thrombosis is also known as venous thromboembolism (VTE), which can start out as a deep vein thrombosis (DVT) and lead to a pulmonary embolism (PE) or stroke. In addition to preventing thrombus formation, anticoagulant therapy can also prevent thrombin mediated activation of platelets, which can also serve to reduce the extent of platelet aggregation in atherosclerosis.

Slide 4:
This slide provides an overview of the anti-Xa assay methodology. These points apply to all anti-Xa assays available regardless of manufacturer. The assay is a calibrated, quantitative method for plasma concentrations of all anticoagulant drugs targeting factor Xa (FXa or Xa). Depending on the calibrator and control set used, anti-Xa assays can be used to monitor and provide concentrations in plasma of unfractionated heparin (UFH; also called heparin) as well as all low molecular weight heparin (LMWH) and fondaparinux, which are all antithrombin dependent indirect anti-Xa anticoagulants related to heparin. In addition, by using the appropriate calibrator and controls, the anti-Xa can also measure rivaroxaban and apixaban, two orally dosed direct anti-Xa drugs approved for DVT/VTE treatment and prevention along with stroke prevention in atrial fibrillation (AF) patients.

As shown in the slide, the benefit of using any anti-Xa assay vs. alternative clotting-based methods is that the anti-Xa is much more specific and not vulnerable to changes in factor concentrations or lupus anticoagulants. In addition, the anti-Xa assay is not vulnerable to analytical variables since the calibrators and controls for the anti-Xa are calibrated against international standard preparations in the case of UFH and LMWH, or liquid chromatography-mass spectrometry (LC-MS) in the case of rivaroxaban and apixaban.

Slide 5:
A schematic of the anti-Xa methodology is shown here. Again, this applies to any anti-Xa assays available regardless of manufacturer or whether it is run in manual or automated mode. It shows how the patient plasma, containing the anticoagulant drug, along with antithrombin (either from the patient or added as a supplementation in the assay), is added to a reaction mixture containing a chromogenic substrate and an excess of FXa. The chromogenic substrate used in the assay resembles the natural substrate of FXa, and so is a specific substrate for FXa. As FXa cleaves the chromogenic substrate, a dye is released, which is then detected by the instrument. The resulting inhibition of FXa results from the competition between the chromogenic substrate and the anticoagulant drug from the assay. This competition results in an inverse relationship between the chromogenic readout of the assay and the anticoagulant drug concentration. In other words, the result is reported in drug concentration, such that low results indicate subtherapeutic concentrations and high results indicate supratherapeutic concentrations. The assay results for heparin related molecules are reported in international units per milliliter (IU/mL) whereas for direct oral anti-Xa drugs, the units reported are gravimetric, or nanograms per mL (ng/mL).

Slide 6:
Some anti-Xa reagents contain added AT. The perceived benefit of AT addition is to supplement low AT concentrations that may be found in patients who have lower concentrations of AT than normal. This happens in certain disease states, or could be found in patients with a genetic AT deficiency, or neonates. However, there is a potential for overestimating heparin concentrations due to measuring the anticoagulant effect of heparin in vitro that is not acting in an anticoagulant manner in vivo. Thus, anti-Xa assays without added AT more closely emulate the in vivo therapeutic heparin effect, since only that heparin bound and actively acting in an anticoagulant fashion in vivo is measured in the assay. However, a disadvantage of anti-Xa assays without added AT includes adverse effects from dilution of patient AT concentrations in samples with high heparin concentrations. If the AT is lower than normal, the already low AT concentration could be further diluted.

Slide 7:
Some anti-Xa reagents also contain added dextran sulfate (DS). As a structurally similar molecule compared to heparin, DS will release heparin bound to off target plasma proteins, in this way measuring heparin that was not participating in the anticoagulant effect. Though all heparin in the plasma is measured, there again is the potential for the in vitro result to not emulate what is happening in vivo.

Slide 8:
Unfractionated heparin (UFH; also referred to as heparin) is a commonly used anticoagulant with a fast acting mechanism of action to treat thrombosis in hospitalized patients. Though monitoring is required

for heparin, monitoring is not required for LMWH in most situations. The activated partial thromboplastin time (aPTT) assay has been traditionally used as an assay to monitor heparin. However, there is a well-known discordance issue between aPTT and anti-Xa in heparin monitoring. This is due to the fact that aPTT is interfered with by several biological, preanalytical, and analytical factors. Thus, the College of American Pathologists (CAP) and American College of Chest Physicians (ACCP) have recommended since 1998 and 2001, respectively, to calibrate the heparin therapeutic range for a given aPTT reagent against an anti-Xa range of 0.3 – 0.7 IU/mL. This is to provide a concentration relationship of the aPTT against heparin, mitigating the reagent/instrument variability of the aPTT assay. The method to calibrate the aPTT reagent in this fashion was described in a paper by Brill-Edwards et al. In addition, the CAP guideline recommends the anti-Xa for patients with lupus anticoagulants, high baseline aPTT, or heparin resistance. Heparin resistance is defined as high doses of heparin needed to achieve the HTR due to an acute phase reaction.

Slide 9:

This graph shows the heparin therapeutic range (HTR) commonly determined by the method of Brill-Edwards et al. The method calls for the use of several dozen patients on heparin to have their aPTT and anti-Xa run, and then graphed in the manner shown. As we can see, a majority of the paired results are concordant, but many are discordant.

If a lab was relying on the aPTT alone and did not have the anti-Xa available for a given patient, there are several situations where the aPTT result is super- or subtherapeutic, leading the clinical staff to change the heparin dose. However, the anti-Xa in these cases may have actually been therapeutic, and the patient in the correct HTR. Thus, the heparin dosage would be increased or decreased unnecessarily, leading to a change in heparin concentrations either above or below the recommended HTR. The result is that the patient does not get into the recommended HTR as quickly, or bounces in and out of the HTR during their therapeutic regimen, and may not ever be in the correct anti-Xa HTR during the hospital stay.

Slide 10:

This slide lists several benefits for laboratories when the anti-Xa assay is used for full time unfractionated heparin monitoring rather than the aPTT.

One benefit is potentially better patient outcomes. A 1994 study by Levine et al showed that patients monitored with the anti-Xa were less likely to bleed due to the fact that they were given less heparin overall compared to patients monitored with the aPTT.

Another benefit stems from a study from 2012 by Guervil et al showing that the time to HTR is faster when the anti-Xa is used to monitor compared to the aPTT. Fewer monitoring tests were performed, fewer dosage changes occurred, hemorrhage rates were lower, mortality was lower, and length of hospital stay (LOS) was lower.

A third benefit is institution wide. Two different articles (Vandiver and Vondracek, 2012, and Rosborough, 1999) cited simplification of dosing nomograms, by use of the anti-Xa assay, resulted in a positive cost benefit due to fewer dosing changes, and fewer laboratory tests run. Thus, as we can see, there are many benefits to using anti-Xa to monitor heparin as compared to aPTT. However, a baseline

aPTT must still be run in order to screen for potential coagulopathies or factor elevations which may require clinical intervention.

Disadvantages of using anti-Xa for full time heparin monitoring include the higher anti-Xa reagent cost. In addition, bridging therapies could be made more complicated due to drug interferences, such as would be the case if bridging a patient from apixaban during outpatient use to heparin when that outpatient is admitted to a clinic.

Slide 11:
This slide discusses the newer oral direct anti-Xa drugs, referred to as direct oral anticoagulants (DOACs). These DOACs include rivaroxaban and apixaban, which specifically target FXa without depending on the action of AT. Though monitoring is not required, there are still situations where there may be an interest in measuring the concentrations of these drugs. These situations are displayed here, along with some current clinical guidelines from the International Society on Thrombosis and Haemostasis (ISTH) regarding choice of tests. Although it should be stated that there are no agreed therapeutic ranges for the DOACs, there are peak and trough concentrations reported in the literature which can be used as a guide for laboratory measurement of the concentrations in situations of DOAC reversal and also assessment of drug concentrations.

Slide 12:
We see here results of a study from 2014 by Francart et al where different prothrombin time (PT) assays, both laboratory and point-of-care, were compared to different anti-Xa measurements of DOACs. This study is different from other comparable studies because the Francart et al study used patient samples rather than spiked plasmas. We can see how, in the left panel, some PT assays are sufficiently sensitive to the effects of rivaroxaban. However, the anti-Xa reagent, as shown in the right panel, is more sensitive to this anticoagulant drug, providing a wide range of concentration linearity over the full range of the max and trough drug concentrations. In addition, there is far less variability for the anti-Xa assays compared to the PT.

Slide 13:
In this slide, we see the results of a 2010 study by Barrett et al focusing on apixaban. The drug concentration displayed on these graphs was validated by liquid chromatography-mass spectrometry (LC-MS). In the left panel, a PT assay used in the study [reflected as the International Normalized Ratio (INR) readout], is not sensitive to the effects of apixaban. No other PT assay evaluated in this particular study was sufficiently sensitive to apixaban, and these findings have been reported in other publications. As shown in the right panel, only an anti-Xa assay is sensitive to the effects of apixaban while also displaying linearity over a wide range of drug concentrations.

Slide 14:
In conclusion, we see that the anti-Xa is a very versatile test that offers many benefits over other tests for measuring both traditional and newer direct oral anticoagulants targeting FXa. In addition, we have

seen that any additives such as AT and DS have an effect on the assay's performance so it is very important that users of these assays understand the role of these additives in order to be able to evaluate the performance of the test being used and how it relates to other labs reporting the assay in proficiency testing programs. Use of the assay for monitoring traditional and newer oral anticoagulants has many benefits, especially with regards to reporting a specific drug concentration. These points shown in this slide are critical takeaway points to keep in mind after viewing this Pearl, which I hope you have found informative and educational.

Slide 15: References

Slide 16: Disclosures

I am a salaried employee of Diagnostica Stago, Inc., where I am a member of the scientific affairs and marketing teams.

Slide 17: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "Anti-Xa: A Versatile and Quantitative Anticoagulant Drug Assay." My name is Paul Riley.