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Multifarious Effects of Direct Factor Xa Inhibitors on Coagulation Tests

Apixiban (Eliquis) and Rivaroxaban (Xarelto) are oral direct Factor Xa inhibitors. The laboratory has noticed an increasing incidence of orders for coagulation and thrombophilia tests, without notification that a patient is receiving one of these anticoagulants. Failure to provide this medication history can result in the reporting of erroneous results that may be misinterpreted. Also, the laboratory spends a lot of time investigating discrepant results and patients may be billed for meaningless tests.

Routine coagulation tests consist of prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen. PT may be prolonged by therapeutic levels of rivaroxaban, but is not affected by apixiban. INR is not a reliable indicator of the anticoagulant effect of Factor Xa inhibitors because the International Sensitivity Index (ISI) has been established for vitamin K antagonist (VKA) therapy and does not reflect sensitivity towards apixiban or rivaroxaban. APTT is elevated by rivaroxaban more than apixiban. Measurement of fibrinogen is not affected by these drugs. Likewise, thrombin time is not affected.

Heparin levels are measured by anti-factor Xa activity. Direct Factor Xa inhibitors prolong these assays and interfere with heparin measurement. If a patient who is taking one of these drugs is started on heparin therapy, anti-factor Xa activity will reflect the combination of heparin and the Factor Xa inhibitor. The reported heparin concentration will be erroneously high.

Thrombophilia testing includes activated Protein C resistance (APC), Factor V Leiden (FVL), prothrombin gene mutation (PGM), protein C activity, protein S activity, antithrombin, and lupus anticoagulant. APC is a screening test for FVL. Both apixiban and rivaroxaban factitiously increase the APC ratio, leading to falsely normal results. In this situation, the specimen would not be reflexed

for FVL mutation analysis and the most common inherited cause of thrombosis would be missed. Genetic tests for FVL and PGM are not affected by FXa inhibitors. Antithrombin and Protein C are chromogenic assays that are not affected, but Protein S activity is a clot based assay that is factitiously overestimated.

Tests for lupus anticoagulant include aPTT with mixing studies, hexagonal phase phospholipid neutralization test, and dilute Russell's viper venom test (DRVVT). As mentioned above, aPTT is variably prolonged by Factor Xa inhibitors. Mixing studies in patients receiving these drugs may not correct, producing a falsely positive mixing study. This falsely positive result leads to a cascade of additional testing for lupus anticoagulant. The hexagonal phase phospholipid neutralization test is based on the aPTT. Therefore, Factor Xa inhibitors may cause false positive results. DRVVT activates Factor X to initiate the common coagulation pathway. Factor Xa inhibitors can produce false positive results. Immunoassays for anticardiolipin antibodies and anti-beta2-glycoprotein1 antibodies are not affected.

This summary pertains to coagulation testing performed in the Saint Luke's Health System using Stago coagulation instruments and reagents. Results obtained in other laboratories using different products may differ.

The effects of direct Factor Xa inhibitors on coagulation and thrombophilia tests are complex. The best practice is to avoid ordering these tests until these drugs have been cleared. Circulating half life is approximately 15 hours for apixiban and 12 hours for rivaroxaban, assuming normal renal function. If immediate testing is necessary, the laboratory should be notified of the patient's anticoagulant therapy.

Cardiac Surgery Transfusion Strategy

The seminal Transfusion Requirements in Critical Care (TRICC) trial, which was published in 1999,

compared clinical outcomes in intensive care patients randomized to a restrictive versus a liberal transfusion strategy (NEJM 1999;340:409-17).. The TRICC trial demonstrated that a more restrictive transfusion strategy was safe in the ICU patient population and that the liberal use of transfusions increased the risk of death.

Since that time, many more randomized controlled trials involving surgical and intensive care unit patient populations in both adult and pediatric patient populations have demonstrated the non-inferiority of a restrictive transfusion strategy. However, previous randomized controlled trials have not focused on cardiac surgery patients.

The Transfusion Indication Threshold Reduction (TITRe2) trial was recently published. It was a multicenter trial in the United Kingdom which recruited 2007 patients older than 16 years of age who were undergoing nonemergent cardiac surgery (NEJM 2015;372:997-1008). Patients with a postoperative hemoglobin level of less than 9 g/dL were randomly assigned to a liberal or a restrictive transfusion strategy. Patients in the liberal group were transfused when the hemoglobin fell below 9.0 g/dL while patients in the restrictive group were transfused at hemoglobin below 7.5 g/dL. Transfusion rates were 92% in the liberal group and 53% in the restrictive group.

The primary outcome was a serious infection or an ischemic event within 3 months after randomization. The primary outcome occurred in 33.0% of patients in the liberal group and 35.1% in the restrictive group (odds ratio 1.11, 95% CI 0.91-1.34). Other serious postoperative complications occurred in 34.2% of patients in the liberal group and 35.7% of patients in the restrictive group. Length of stay and overall cost were not statistically significantly different between the groups.

Most notably, patients in the restrictive group had an increased risk of all-cause mortality at 90 days compared to the liberal group: 4.2% of patients in the restrictive group died compared to 2.6% of patients in the liberal group (hazard ratio 1.64, 95% CI 1.00 to 2.67, P=0.045).

This study concluded that a restrictive transfusion threshold after cardiac surgery was not superior to a liberal threshold in regards to morbidity or health care costs. Most notably, it suggests that a liberal

transfusion threshold might increase longer-term survival.

Calreticulin Gene

CALR gene codes for calreticulin, which is a calcium-binding chaperone protein that is believed to be involved in clearing misfolded proteins. Mutations in the *CALR* gene create an abnormal peptide that promotes abnormal expansion of the megakaryocytes. Detection of a *CALR* gene mutation aids in the diagnosis of a myeloproliferative neoplasm (MPN), and helps to distinguish MPN from benign reactive disease.

CALR mutations are mutually exclusive with *JAK2* or *MPL* mutations. *CALR* mutations occur in approximately 25% of essential thrombocythemia (ET) cases overall and in 70% of cases that are negative for *JAK2* and *MPL*. Less than 10% of patients with myelodysplasia have mutations in the *CALR* gene. *CALR* mutations are rarely detected in patients with de novo acute myeloid leukemia, chronic myelogenous leukemia, or lymphoid leukemia.

Patients with *CALR* mutations tend to be younger and have higher platelet counts and lower hemoglobin than those with *JAK2* mutations. Survival is longer and risk of thrombosis is lower in patients with *CALR* mutations compared to patients with *JAK2* or *MPL* mutation.

CALR mutations are present in 88% of patients with primary myelofibrosis without *JAK2* and *MPL* mutations. Patients with *CALR* mutations have longer overall survival than those with *JAK2* and *MPL* mutations.

CALR mutations have not been detected in patients with polycythemia vera. Therefore, *CALR* mutation testing can be used to distinguish polycythemia vera from essential thrombocytosis or primary myelofibrosis

JAK2 testing should be ordered first. *CALR* testing should only be ordered in patients who test negative for *JAK2* mutation. A negative result does not exclude the presence of a myeloproliferative disorder or other neoplastic process. Specimen requirement is 3 to 5 mL of whole blood or 1 to 2 mL bone marrow collected in a lavender-top (EDTA) tube or green-top (sodium heparin) tube.