Saint Luke's Regional Laboratories
Clinical Laboratory Letter

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Guidelines for Monitoring and Reversing Anti-platelet Drugs

Antiplatelet medications are prescribed to prevent thrombosis in patients with coronary artery disease, stroke, transient ischemic attacks and peripheral arterial disease. When patients taking these medications develop bleeding or require an emergent invasive procedure, the antiplatelet effect needs to be rapidly reversed. Specific antidotes are not available. Randomized clinical trials have not been conducted and there are no evidence based guidelines. The recent transfusion literature contains some recommendations for platelet transfusion therapy in these situations.

The standard dose of aspirin varies from 81 to 325 mg per day. Aspirin is immediately absorbed after ingestion and irreversibly inhibits platelets within 15 to 30 minutes by binding to cyclooxygenase 1 (COX1). The half life of aspirin is 30 minutes.

Aspirin has a low bleeding risk. Discontinuation of aspirin for 2 days will usually result in production of enough new platelets to provide adequate hemostasis for elective surgery. Aspirin effect on platelets can be assessed with platelet function testing by PFA-100. Aspirin effect includes a prolonged closure time with epinephrine and a normal closure time with ADP. A single dose of apheresis platelets may be warranted for intracerebral hemorrhage or urgent neurosurgery.

Dipyridamole (Persantin) inhibits 2,3 phospho-diesterase enzyme in platelets and inhibits ADP induced platelet aggregation. By itself, dipyridamole is considered to be a weak antiplatelet medication that seldom causes bleeding. Aggrenox is a more effective antiplatelet medication that is often prescribed for patients with a history of stroke. It is a combination of 25 mg aspirin and 200 mg of dipyridamole. Bleeding risk is low. Platelet transfusion is usually not necessary unless a patient needs to undergo urgent neurosurgery or presents with intracranial hemorrhage. The effect of dipyridamole can only be assessed by whole blood platelet aggregation.

Clopidogrel (Plavix) irreversibly inhibits platelet function by preventing binding of ADP to the platelet P2Y12 receptor. Clopidogrel is a prodrug which is metabolized to its active form by the cytochrome P450 enzyme pathway. A loading dose of clopidogrel (300-600 mg) inhibits platelet function within 2 to 4 hours, while a daily dose of 75 mg becomes effective within 24 hours and has maximum effect within 4 to 7 days. Circulating half life is 7 to 8 days.

Daily clopidogrel increases the risk of bleeding. Clopidogrel should be discontinued before elective surgery. At least 3 clinical practice guidelines provide direction regarding the timing of surgery in patients receiving Plavix. Each of these guidelines recommends discontinuing Plavix for at least 3 to 7 days prior to surgery to allow platelet function to return to normal and decrease the risk of bleeding.

All of these recommendations are based on the pharmacokinetics of clopidogrel and do not take into account individualized response to drugs. Approximately 40% of patients have suboptimal antiplatelet response to clopidogrel. Patients who are hyporesponders or nonresponders would be expected to normalize platelet function even sooner than 5 to 7 days after discontinuing clopidogrel. In our experience more than 50% of patients have <30% platelet inhibition within 3 days after discontinuing clopidogrel. An individual's response to clopidogrel can only be determined by performing platelet function testing with the VerifyNow assay. Assessing P2Y12 inhibition with this assay may allow elective surgery to be performed sooner.

One dose of apheresis platelets is recommended for bleeding or urgent surgery. Patients taking a combination of clopidogrel plus aspirin may experience more serious bleeding. Two units of

apheresis platelets may be necessary for intracranial hemorrhage or neurosurgery.

Like clopidogrel, prasugrel (Efient) also irreversibly inhibits binding of ADP to the platelet P2Y12 receptor. It is a prodrug which is metabolized to its active form by the cytochrome P450 enzyme pathway. Prasugrel is given as a loading dose of 60 mg followed by daily doses of 10 mg for patients weighing more than 60 kg. The daily dose is reduced to 5 mg per day for patients weighing less. Onset of action and circulating half life are similar to clopidogrel.

Prasugrel has a higher bleeding risk than clopidogrel. Prasugrel should be discontinued for 5 to 7 days before elective surgery. Platelet function can be assessed with the VerifyNow assay. One dose of apheresis platelets is recommended for bleeding or urgent surgery. Two units of apheresis platelets may be required for neurosurgery.

In summary, no single test can detect the anti-platelet effect of these different drugs. Platelet aggregation, PFA-100 and VerifyNow are available. One to two units of apheresis platelets may be needed to treat bleeding or prior to urgent surgery.

BCR/ABL1 Quantitation

Chronic myeloid leukemia (CML) is one of the myeloproliferative disorders defined by a *BCR/ABL1*gene translocation which encodes a non-regulated tyrosine kinase protein that is the main driver of the disease. Quantitation of BCR/ABL1 transcripts has become the preferred method for monitoring the major molecular response (MMR) of therapy and predicting patient outcome. For decades laboratories have developed their own tests using different techniques for RNA extraction or cDNA synthesis. Lack of established reference material for calculating standard curves has added to variability between laboratories. In order to harmonize minimal residual disease (MRD) measurement, a proposal was made in 2005 at the NIH to develop an international scale (IS) for *BCR/ABL* real-time quantitative polymerase chain reaction (RQ-PCR). This proposal included the development and validation of laboratory specific conversion factors (CFs) so that local values could be converted to IS. A standardized baseline was defined as 100% *BCR/ABL* and MMR as 0.1% *BCR/ABL,* because these values were used in the International Randomized Study of Interferon and ST1571 (IRIS) study. Since then, many laboratories have begun using CFs to report MRD on IS scale. However, a study performed by Branford *et al.* (Blood, 2008:112(8):3330-3338) showed that a bias of plus or minus 1.2 fold exists between different methods used for MRD assessment even after conversion to IS scale.

Saint Luke's Health System recently changed reference laboratories from Mayo Medical Laboratories to the Laboratory Corporation of America. Some patients with CML may have MDR results from both laboratories. Results may differ because the laboratories use different methods for *BCR/ABL* measurement. One of our clinical pathologists recently compared the results from both laboratories for 9 patients with an established diagnosis of chronic myeloid leukemia. Only one of these patients had a bias of 1.4 in MRD measurements between the two reference laboratories. This bias did not impact patient management since the patient had not achieved MMR.

A total of 29 specimens have been sent to LabCorp since October, 2013. Only 9 of these patients had CML. *BCR/ABL* RQ-PCR test should NOT be ordered for diagnosis of chronic myeloid leukemia because of its extreme sensitivity. RQ-PCR detects low-level *BCR/ABL* transcripts in the peripheral blood of 50% of healthy normal individuals. Saint Luke's Regional Laboratories therefore strongly recommends NOT using *BCR/ABL* quantitative test for diagnosis. Fluorescence in situ hybridization (FISH) for *BCR/ABL* is the preferred test for initial diagnosis. Moreover, since MMR has a defined value, quantitation of *BCR/ABL* transcripts can be delayed until after the establishment of diagnosis.

How Long is Rh Immune Globulin Detectable?

Rh immune globulin is routinely given to all pregnant Rh negative women, who have not already developed anti-D, at 28 to 30 weeks of gestation. Most patients have detectable anti-D up to 3 months after administration and a few still have detectable anti-D at 6 months. Anti-D due to Rh immune globulin is usually present in low titer. Persistence of anti-D makes it difficult to distinguish passive administration from active Rh sensitization.