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The PFA-100®, A Simple Assay for Platelet Function

Primary Hemostasis: Primary hemostasis is a process whereby platelets interact with elements of the damaged vessel wall, leading to the initial formation of a platelet plug. The platelet/injured vessel wall interaction involves a series of events that includes platelet adhesion to components of the subendothelium, platelet activation and shape change, release of platelet granular contents (dense bodies and α granules) with subsequent formation of fibrin-stabilized platelet aggregates, and clot retraction. Following the platelet plug, coagulation factors are assembled on the activated platelet membrane, leading to generation of thrombin and subsequent fibrin deposition. The platelet plug and fibrin are analogous to the cork in a bottle of champagne that is stabilized by a network of wire mesh. The prothrombin time, activated partial thromboplastin time and platelet count are routinely used to evaluate hemostasis. Now, there is a simple test to evaluate platelet function.

Measuring Platelet Function: The two methods currently popular to evaluate platelet function are aggregation studies using platelet rich plasma and a whole blood method using the PFA-100®. Although the two methods correlate well, they are different. The time honored platelet aggregation studies are usually done in research labs; the results are not easy to interpret; and the studies are done in a low sheer environment unlike the blood vessel. The PFA-100® is an instrument that measures platelet adhesion and aggregation in citrated whole blood. It measures platelet function in a high sheer environment that simulates in vivo vessel blood flow. The PFA-100® results should be viewed as a screening test and if abnormal, further investigation is required.

How the PFA-100® Works and the Results: The PFA-100® test induces platelet activation as blood is made to flow through an aperture cut into a membrane that is coated with collagen fibrils and epinephrine and/or collagen fibrils and adenosine diphosphate (ADP). The time taken for blood to form a platelet plug that occludes the aperture is an indication of platelet function and is referred to as the Closure Time (CT). A normal CT with epinephrine indicates normal platelet function and no additional testing is done. If the CT is abnormal, then a second test is done with the ADP membrane. A prolonged CT with epinephrine but a normal CT with ADP is characteristic of aspirin (ASA) effect. A prolonged CT with both epinephrine and ADP suggests an acquired or congential platelet defect. Platelet dysfunction is seen in von Willebrand's disease, anti-platelet drugs, and acquired (uremia) or inherited platelet abnormalities. Commonly used medications (list available upon request) such as NSAID's, some antibiotics, diuretics, psychiatric and antihypertensives also induce temporary platelet dysfunction. These drugs have long CT with both epinephrine and ADP. The test results should always be evaluated in conjunction with clinical history and other laboratory findings.

Normal Closure Time Ranges
(Blood drawn in 3.2% citrate)

Epinephrine	68 - 172 seconds
ADP	58 - 127 seconds

Limitations of Test: Closure times longer than the reference ranges may be caused by a hematocrit less than 30% or platelet count less than 100,000/ul. The association between low hematocrit and impaired platelet function is well recognized. In this regard the PFA-100® simulates *in vivo* conditions. Blood samples drawn in 3.8% citrate instead of the standard 3.2% citrate have a 12% longer closure time. Hemolysis, microthrombi in the specimen tube, a high sedimentation rate and some dietary fatty acids may interfere with the closure time. The specimen should be evaluated within a 4-hour period from the time drawn. When interpreting results, it is important to keep in mind the hematocrit and platelet count as well as platelet inhibiting agents such as ASA, NASID's, GpIIb/IIIa antagonists and Plavix. Discontinuing the drug for 3 to 5 days can reverse drug effect (including aspirin) on platelets.

Prolonged Closure Times May Be Caused By

1. Hematocrit <30%
2. Platelet counts <100,000/ul
3. High sedimentation rate
4. Sample drawn in 3.8% citrate

When to Measure Platelet Function: The PFA-100® is able to detect abnormal platelet function secondary to von Willebrand's disease (vWD) , inherited and acquired platelet defects, and drug induced abnormalities. A platelet function assay should be considered in the following circumstances:

Platelet Function Assay (PFA-100®)

1. Screening for:
    a. Von Willebrand's disease (diagnosis & monitoring)
    b. Pre-surgical (only if positive history of bleeding)
2. Cardiovascular medicine:
    a. Assess aspirin therapy (resistance vs. compliance)
    b. Evaluation of anti-platelet therapy (Plavix or ReoPro)
    c. Identify need for platelet transfusion post op., i.e. if platelet count and function are normal, then no benefit achieved from platelet transfusion.
3. All patients with a bleeding history
4. Detect surreptitious aspirin use prior to a procedure
    a. e.g. epidural spinal anesthesia

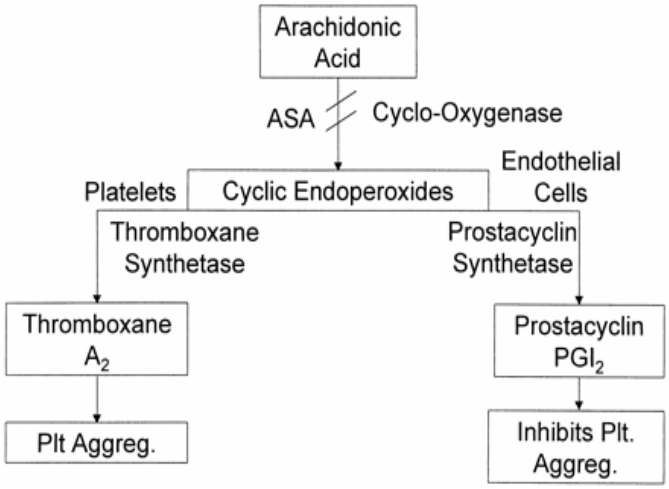
Platelet Receptors and Inhibitors: There are many pathways of platelet activation that can lead to a platelet plug. If one pathway is blocked, another may result in platelet activation and a platelet plug. The various receptors on the platelet membrane serve as targets for agonists of platelet activation. Activation of the GP1b/V/IX receptor is necessary for initial platelet adhesion to the vessel wall. Circulating von Willebrand factor attaches to GP1b/V/IX and tethers the platelet to the endothelium. This adhesion step occurs prior to platelet activation. The pharmaceutical industry has engineered drugs to inhibit platelet activation by blocking certain platelet receptors. The activation of the ADP receptor results in platelet aggregation. When blocked by clopidogrel (Plavix) or ticlopidine (Ticlid), no ADP is generated and platelet aggregation is prevented. The GPIIb/IIIa receptor is inhibited by abciximab (ReoPro), eptifibatide (Integrelin) and tirofiban (Aggrastat). These three are potent inhibitors of platelet aggregation and are used to prevent ischemic complications during percutaneous coronary intervention.

The following is an example of expected closure time patterns observed with the PFA-100®:

Expected Closure Time Patterns

	ASA	vWD	Intrinsic defect/drug induced
Collagen/Epinephrine	abnormal	abnormal	normal or abnormal
Collagen/ADP	normal	abnormal	abnormal

Aspirin Inhibition: Aspirin inhibits cyclo-oxygenase to prevent platelet activation for the life of the platelet (10 days). Platelets and endothelial cells contain pathways for metabolism of Arachidonic Acid (AA). When platelets or endothelial cells are activated, an enzyme, phospholipase A_2, is activated, liberating AA. AA is then converted to thromboxane A_2 by cyclooxygenase and thromboxane synthetase. Thromboxane A_2 is a vasoconstrictor and potent activator of platelets, leading to platelet aggregation. In the endothelial cells, prostacyclin synthetase converts cyclic endoperoxides to prostacyclin (PGI_2). Prostacyclin inhibits platelet aggregation and is a vasodilator. ASA inhibits cyclooxygenase (Cox-1). Low dose (81mg.) ASA is beneficial in primary prevention of heart disease because it believed to inhibit thromboxane A_2 to a greater degree than prostacyclin.



Aspirin Resistance: The PFA-100® is sensitive enough to detect platelet inhibition at a dose of 81 mg. of aspirin in normal subjects. It is estimated that 22% of the normal population will not respond to low dose ASA. Of this group of non-responders, an additional percentage will respond to 325mg. ASA. This may be a result of variable plasma levels of von Willebrand factor (an acute phase reactant) or other factors. In patients with cerebral vascular and cardiovascular disease, the incidence of ASA non-responders has been reported to be as high as 50%. In one study, ASA non-responders were associated with a higher risk of death at 6 months following their first acute coronary syndrome. Those who do not respond to low or high dose ASA would likely respond to a direct platelet inhibitor drug such as Plavix.

Proposed Mechanisms of Aspirin Resistance

1. Exogenous substances simulating platelet activation (e.g. cigarette smoke)
2. Drugs interfering with acetylation of Cox-1 (e.g. NSAID's)
3. Insufficient dose of ASA
4. Increased platelet turnover – overcoming daily aspirin dosing
5. Polymorphism is active site of Cox-1 preventing acetylation
6. Inducible Cox-2 (not inhibited by ASA) thereby allowing for platelet thromboxane A_2 production despite Cox-1 inhibition

Cox-2 Inhibitors (Vioxx and Celebrex): The use of Cox-2 inhibitors has increased primarily because of ease of administration and less GI side effects then other NSAID's. In contrast to aspirin and other NSAID's, the Cox-2 inhibitors have no effect on platelet function (the PFA-100® closure time is normal). The other NSAID's (ibuprofen) differ from aspirin in that they inhibit both Cox-1 and Cox-2 enzymes and temporally block the binding of aspirin to platelets.

Von Willebrand's Disease (vWD): The PFA-100® is highly sensitive (96.5%) and superior to the bleeding time for screening patients with vWD. The degree of prolongation of the closure time correlated with the level of vWF and in one study was able to detect all of the cases of vWD disease except for those with the type 2N subtype (<5% of the cases). The 2N subtype has normal vWF/platelet interaction. Patients with blood group O who normally have lower levels of vWF, also have prolonged closure times. Approximately 10 to 15% of the women with heavy menstrual bleeding have vWD (most undiagnosed). The test is also useful for therapeutic monitoring of vWD patients treated with either desmopressin (DDAVP) or factor VIII/vWF concentrate.

Cardiovascular Surgery and Platelet Function: Many factors contribute to the bleeding risks of cardiopulmonary bypass (CPB): Two examples are the hemodilution effect of crystalloid priming solutions resulting in a decrease concentration of coagulation factors and platelets and the activation of platelets on the synthetic surfaces of the extracorporeal circuit. In a recent study by Slaughter at Duke University using the PFA-100® instrument, closure times with ADP were shown to increase as expected during CABG surgery. Interestingly, after protamine neutralization, the ADP measured closure times returned to baseline values in 90%

of the patients. The authors suggest that shear-mediated platelet dysfunction is largely reversible during CPB following the administration of protamine and post-op prolongation of the ADP measured closure time may warrant use of platelet concentrates in a bleeding CABG patient.

The usefulness of the closure time to identify CABG patients who may not benefit from platelet transfusions was further investigated in a retrospective analysis of ADP closure times measured after the administration of protamine. In this study, normal closure times identified a subgroup of patients whose bleeding could not be controlled by platelet transfusions alone and required other means. This was in sharp contrast to a second group of patients with prolonged closure time whose bleeding was controlled by platelet transfusion only. These studies suggest that closure times may be able to discriminate platelet related bleeding from other causes. Nevertheless, post-op bleeding is multi-factorial in nature and these findings warrant further studies to determine the clinical utility of the closure time in cardiothoracic surgical procedures involving extracorporeal circulation.

The Bleeding Time is Discontinued: The bleeding time (BT) has been widely utilized in the past as a means of accessing the ability of platelets to form a primary hemostatic plug in a vessel. Unfortunately, the BT is relatively insensitive in identifying abnormalities of primary hemostasis. Despite the introduction of the newer devices, there remains substantial variability between individuals performing bleeding times and the possible complication of scar formation at the test site. The BT has never been able to predict surgical bleeding. Effective mid November the BT will no longer be available at Rex Laboratory. If a BT is ordered, the PFA-100® platelet function assay will be done.

Laboratory Orders and Charges: Beginning November 17th, platelet function assays will be done at Rex Lab using the PFA-100®. If the closure time results for collagen/epinephrine and collagen/ADP will be reported with a generic interpretive comment as a footnote. The CPT test code is 85576. The charge is $84.00 for the both test results. The test is available on 1st and 2nd shifts with a turn-around time of one hour. When drawn in the office, the specimen should be drawn in 3.2% sodium citrate (blue top tube), maintained at room temperature and tested within 4 hours from the time it was drawn. The lab will handle all requests as stat.

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