Assessment of Platelet Function With the Platelet Function Analyzer (PFA-100®)

Tests of platelet function are used to screen patients with defects in primary hemostasis or to test asymptomatic relatives of a patient with a heritable platelet function disorder. One of the oldest tests of platelet function is the template bleeding time test which has been used to measure in vivo platelet function and vascular integrity. Under controlled conditions, an incision is made in the skin and the duration of bleeding is measured using a modified template device. However, the template bleeding time is a highly operator-dependent test that is affected by numerous technical factors such as location of the incision, pressure applied, operator experience, and patient factors such as age, gender, diet, skin laxity and medications.

While assessment of platelet function was previously a part of preoperative screening, strong evidence shows that bleeding times do not correlate with bleeding tendencies nor predict the likelihood of surgical bleeding. Thus, the bleeding time as a preoperative screening test is not warranted. An accurate bleeding history is considered to be a more valuable screening test.

The bleeding time has been replaced by the Platelet Function Analyzer (PFA-100®, Dade Behring) at many medical centers. The PFA-100 rapidly assesses whole blood platelet function in vitro and measures both platelet activation and aggregation.

How does the PFA-100 work?
The PFA-100 uses a disposable cartridge (Figure 1) that has a biochemically active membrane with a central aperture. Citrated whole blood is aspirated under vacuum pressure through a capillary tube that activates the platelets via the shear force generated. The shear rate (5000-6000 seconds⁻¹) corresponds to flow conditions present in small arteries. The blood then passes through an aperture in a membrane coated with combinations of platelet agonists, either with collagen and ADP (CADP) or collagen and epinephrine (CEPI).

As platelets adhere to the collagen, they become activated by the agonists and release their granules, resulting in platelet aggregation. The PFA-100 instrument determines the number of seconds it takes the platelet plug to form and stop blood flow through the aperture. This is reported as the closing time (CT). A prolonged CT is the laboratory equivalent of a prolonged clinical template bleeding time.

<table>
<thead>
<tr>
<th>Table 1 PFA-100 Reference Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonists</td>
</tr>
<tr>
<td>Collagen and epinephrine (CEPI)</td>
</tr>
<tr>
<td>Collagen and ADP (CADP)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2 Comparison of the Sensitivity of the PFA-100 with the Template Bleeding Time?</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWD=von Willebrand Disease, GT=Glanzmann Thrombasthenia, BSS=Bernard Soulier Syndrome, HPS/SPD=Hermansky-Pudlak Syndrome/Storage Pool Defect, PSD=Primary Secretory Defects</td>
</tr>
<tr>
<td>PFA-100</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>84-100%</td>
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<tr>
<td>48-65%</td>
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DIAGNOSIS OF BLEEDING DISORDERS: Interpretation of PFA results.

**CEPI normal; CADP normal – Normal Results:**
Normal CT results virtually exclude severe thrombocytopenia, severe von Willebrand disease (VWD), and severe platelet dysfunction. While the test has high sensitivity for detecting most types of VWD, it may miss mild forms as vWF levels can fluctuate over time, especially related to estrogen secretion in women over the course of a menstrual cycle. If initial results are normal but clinical suspicion is high, repeating the test with a new sample at a different time is recommended. Additionally, VWD type 2N (Normandy) will not be identified by the PFA-100, but this entity clinically resembles hemophilia A and will have low factor VIII activity.

**CEPI > 180 s; CADP normal – “Aspirin Effect”**
Aspirin-induced platelet dysfunction will generally result in a CEPI CT >180 sec and normal CADP. Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDS) inhibit platelet function by blocking cyclooxygenase 1 (COX-1) function. This COX-1 inhibition prevents thromboxane generation and the resulting platelet aggregation, resulting in a prolonged CEPI CT and a normal CADP CT. Studies have shown that when NSAIDS are discontinued, the CT abnormalities return to normal by 6 days with aspirin and by 24 hours with ibuprofen.

There have been numerous studies on the potential utility of the PFA-100 in the setting of aspirin therapy; the PFA-100 has excellent sensitivity in identifying patients on aspirin (Table 2). An additional focus was the role of the PFA-100 in identification of patients in whom there is a potential for “aspirin failure” or “aspirin resistance”. The current recommendations suggest that there is not enough clinical evidence to establish diagnostic parameters or therapeutic guidelines for this second application, and that prospective clinical trials should be undertaken.

There is also evidence that platelet storage pool defects and release defects can give similar results on the PFA-100. Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disease that causes albinism, storage of a protein-fat substance in tissues, and absence of dense bodies in platelets on electron microscopy. One study of confirmed HPS showed that all 5 patients had normal CADP results and 4 of 5 had significantly prolonged CEPI results. Other small studies have confirmed a similar sensitivity of the PFA-100 for detecting rare platelet release disorders (see Table 2). These storage pool defects can be distinguished from aspirin by formal platelet aggregation testing.

**CEPI > 180 s; CADP > 110 s – Abnormal Platelet or vWF Function**
Patients with increased CT in both sets of agonists have abnormal platelet function but thrombocytopenia and anemia should be excluded as causes.

**Von Willebrand Disease**
VWD is the most common hereditary abnormality of coagulation. It is caused by either a qualitative or a quantitative deficiency in von Willebrand factor. Von Willebrand factor binds to exposed collagen and adheres platelets via their glycoprotein Ib receptor. Clinical signs of VWD include epistaxis, menorrhagia, and easy bruising.

Patients with severe type 1 or type 3 VWD usually have abnormal CEPI and CADP values. However, patients with mild type 1 VWD and some who have type 2 VWD (particularly type 2N) may not have abnormal results.(Table 3)

For VWD overall, the CEPI measurement has higher sensitivity, 93%, than the CADP, 86%. Von.Willebrand factor antigen levels, ristocetin cofactor activity, and multimeric analysis are recommended for definitive diagnosis and classification of VWD. A detailed discussion of these tests is beyond the scope of this article.

**Table 3. Summary Of Multiple Studies Regarding PFA-100 Sensitivity To VWD By Subtype**

<table>
<thead>
<tr>
<th>VWD Subtype</th>
<th>1 CADP</th>
<th>1 CEPI</th>
<th>2A either</th>
<th>2B either</th>
<th>2N either</th>
<th>2M either</th>
<th>3 either</th>
</tr>
</thead>
<tbody>
<tr>
<td># of patients</td>
<td>221/278</td>
<td>230/268</td>
<td>42/42</td>
<td>40/43</td>
<td>0/3</td>
<td>40/41</td>
<td>44/44</td>
</tr>
<tr>
<td>Sensitivity(%)</td>
<td>79.5</td>
<td>85.8</td>
<td>100</td>
<td>93</td>
<td>0</td>
<td>97.6</td>
<td>100</td>
</tr>
</tbody>
</table>

* Adapted from Favaloro, 2008
Platelet Defects
Glanzmann thrombasthenia (GT) is an autosomal recessive disease in which glycoprotein IIb/IIIa is either deficient or dysfunctional, leading to defective platelet aggregation and bleeding, typically presenting in infancy. However, for those that present at an older age, the symptoms may include excessive bleeding after dental extractions, menorrhagia, epistaxis, and ecchymoses.

Bernard-Soulier syndrome (BSS) is also autosomal recessive but has absent or decreased expression of glycoprotein Ib/IX/V, resulting in defective platelet adhesion to vWF. Patients generally have thrombocytopenia, giant platelets, and easy bruising, epistaxis, menorrhagia, and mucoasal/gastrointestinal bleeding. The PFA test has excellent sensitivity for both platelet defects. (Table 2) Definitive diagnosis requires flow cytometry of the platelet membrane glycoproteins.

Antiplatelet Agents
The other category with this CT pattern is patients on GPIIb/IIIa inhibitors (abciximab, tirofiban, and eptifibatide), which block platelet aggregation, prolonging the CT.

CEPI normal; CADP > 110 s – Rare result
This rarely occurs, as the CEPI is more sensitive and is not characteristic of a clinical scenario but can be seen with hemolyzed samples (released ADP may prolong the CADP). Repeat testing on a new sample is recommended.

The PFA result is dependent on platelet function, von Willebrand factor level, platelet number, and hematocrit. While the manufacturer recommends that the platelet count should be above 150,000/uL and the hematocrit should be above 35%, numerous studies have found that valid CT results with platelets >100,000/uL and hematocrit >25%.

Table 4. What extrinsic effects or conditions can affect the CT results?

<table>
<thead>
<tr>
<th>Falsely elevated CT results</th>
<th>Low hematocrit (&lt;25%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low platelet levels (&lt;100,000/uL)</td>
</tr>
<tr>
<td>Invalid CT results</td>
<td>Hemolyzed samples</td>
</tr>
<tr>
<td></td>
<td>Clotted samples</td>
</tr>
<tr>
<td></td>
<td>Samples drawn through IV lines</td>
</tr>
<tr>
<td>Normal CT results but still with bleeding tendency</td>
<td>Fibrinogen deficiency or defect</td>
</tr>
<tr>
<td></td>
<td>Factor deficiencies</td>
</tr>
<tr>
<td></td>
<td>Heparin (unless at very high levels)</td>
</tr>
<tr>
<td></td>
<td>Warfarin</td>
</tr>
</tbody>
</table>

MONITORING PATIENTS WITH THE PFA
von Willebrand disease
When pretreatment CT is abnormal, DDAVP is generally able to normalize the CT for most patients with type I VWD and some with type 2, and this parallels normalization of plasma VWF. However, the PFA-100 is not reliable for monitoring factor concentrate therapies.

Antiplatelet therapy
Daily intake of 100 mg of aspirin prolongs the CEPI-CT by days 3 and 4 of use. However, most CT results are prolonged beyond the maximum detection limit (>300 sec), so the degree of antiplatelet activity cannot be quantified. There is no established role as yet for the PFA-100 in monitoring antiplatelet therapy other than aspirin.

How to order a PFA-100 analysis
The PFA-100 analysis can be ordered through SCM or Centricity. Blood should be collected through venipuncture into a light blue-top tube (same as for PT/aPTT). Expedited delivery to the laboratory is recommended because the test should be run within 4 hours of sample collection. The test is available between 7:30 am and 11 pm, and results are generated within 30 minutes.
Katy Van Patten, MD
Laboratory Medicine and Pathology Resident, PGY4

Henry Rinder, MD
Laboratory Medicine Attending, Hematology Laboratory Director

References:
Coagulation Factor Assays: Approaches to Testing

Coagulation disorders challenge both the clinician and laboratory physician not only in managing the clinical aspects of bleeding, but also in pursuing an efficient diagnostic testing scheme (Figure 1). A patient's clinical, medication and transfusion history is often the best screening test in assessing a coagulopathy. If the patient has a personal or family history of bleeding or bruising, a more detailed history of the nature of bleeding episodes (e.g. onset, severity, site, and duration) should be taken. The clinical history must exclude common acquired conditions such as disseminated intravascular coagulation or anticoagulant therapy.

While the severity of bleeding varies depending upon the specific factor deficiency, a history of repeated bruising or prolonged bleeding warrants further testing, in the absence of underlying conditions such as liver disease or vitamin K deficiency. Factor deficiencies are traditionally classified as type 1 (quantitative) or type 2 (qualitative) deficiencies, with most acquired and inherited disorders classified as type 1. Acquired factor deficiencies are subclassified as immune mediated or non-immune mediated with the latter resulting from increased destruction or abnormal production of factors.

In conjunction with the clinical history, laboratory screening should include a prothrombin time (PT) with international normalized ratio (INR), activated partial thromboplastin time (aPTT) and fibrinogen (Table 1). Investigation of a prolonged PT and/or PTT begins with a mixing study. Patient plasma is mixed with pooled normal plasma and the PT or PTT is repeated on the mixture, immediately and after incubation for 1 hour at 37°C. If the clotting time normalizes, factor deficiency is indicated; if the clotting time of the mixture remains prolonged, a circulating inhibitor may be present. (Note: The presence of heparin should be ruled out as a cause of the prolonged PTT, as heparin will prolong the mixing study.)

Factor activity is assessed by one-stage clotting assays; Factors II, V, VII and X activity are measured by PT assays, and factors VIII, IX, XI and XII by aPTT assays. In the one-stage factor assays used at YNHH the clotting times of patient plasma mixed with specific factor-deficient plasma are measured against a standard curve prepared from normal plasma with a known quantity of the factor in question. The clotting times are converted to percent from the standard curve, and an average percent activity is reported. Reference range for most factors is 50-150%; although factor VIII (an acute phase reactant) may be 50-200%

Though not performed at YNHH, chromagenic factor assays are also available, most commonly outside of the US. More costly than the clotting method, the chromagenic assay employs a factor-specific substrate that emits color when cleaved; the rate of change and intensity of color is proportional to the factor activity. This type of assay is also calibrated using pooled normal plasma with a known concentration of factor activity. In the presence of an extremely potent lupus anticoagulant, a chromagenic factor assay may be less affected than a clotting assay.

In the case of a circulating inhibitor, a specific low factor activity (0-5%) should be identified through factor assays as described above. Once the low factor is known, an inhibitor titer to that factor can be determined through factor assays performed on a series of dilutions of patient plasma mixed with pooled normal plasma.

Specimen quality is extremely important, since factor assays are subject to the same interferences as the PT and aPTT testing. Appropriate specimen collection is crucial. A 4.5-mL blue-top (sodium citrate) tube is preferred although a 2.7-mL pediatric blue-top tube is also acceptable; either tube must be filled completely. A discard tube should be collected before the blue top tube and, when possible, the draw should be by venipuncture rather than through a line to avoid heparin contamination. The sample should then be inverted several times without shaking. Samples should be received within four hours of collection for appropriate processing. Factor assays are performed Monday through Friday, 8:00 am to 3:00 pm; however, samples may be collected at any time and sent to the Hematology laboratory, where they will be spun and the plasma frozen for later testing.

Cross-reacting factor inhibitors, lupus anticoagulant or large amounts of heparin can lead to falsely decreased factor activity levels due to artificial prolongation of either the PT or aPTT. However, because the one-stage assay involves multiple dilutions, circulating non-specific inhibitors are diluted out and rarely cause significant interference. In reporting results in patients with possible interferences, only those dilutions which accurately reflect factor activity are reported.
<table>
<thead>
<tr>
<th>Findings</th>
<th>Differential Diagnosis</th>
<th>Additional Factor Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ PT (normal aPTT)</td>
<td>Vit K deficiency/warfarin</td>
<td>Mixing study</td>
</tr>
<tr>
<td></td>
<td>Factor VII deficiency/ inhibitor</td>
<td>Factor V &amp; VII activity</td>
</tr>
<tr>
<td></td>
<td>Liver disease: mild to moderate</td>
<td>Lupus anticoagulant panel</td>
</tr>
<tr>
<td></td>
<td>Lupus anticoagulant (LA) - rare</td>
<td></td>
</tr>
<tr>
<td>↑ aPTT (normal PT)</td>
<td>Von Willebrand disease</td>
<td>Mixing study</td>
</tr>
<tr>
<td></td>
<td>Factor VIII/IX/XI /XII deficiency/inhibitor</td>
<td>If appropriate clinical history,</td>
</tr>
<tr>
<td></td>
<td>Lupus anticoagulant- common</td>
<td>Factor VIII/IX/XI activity with inhibitor study;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>if negative clinical history, vWD/LA panel</td>
</tr>
<tr>
<td>↑ PT &amp; aPTT</td>
<td>Vit K deficiency - severe</td>
<td>Mixing studies</td>
</tr>
<tr>
<td></td>
<td>Liver disease: Severe</td>
<td>Factor V/X activity</td>
</tr>
<tr>
<td></td>
<td>DIC</td>
<td>LA panel</td>
</tr>
<tr>
<td></td>
<td>Lupus anticoagulant (LA) - rare</td>
<td>D-dimer</td>
</tr>
<tr>
<td></td>
<td>Factor V/X deficiency/inhibitor</td>
<td></td>
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</tbody>
</table>

While clinical presentation and history are paramount in management, appropriate laboratory testing is equally important. Factor testing should target the involved factor(s) based on the clinical presentation and laboratory screening tests as summarized in Figure 1. Samples must be drawn appropriately and sent to the laboratory in a timely manner to ensure sample integrity for accurate results. Those assay results, interpreted in the context of a patient’s clinical history, will then help direct further testing and appropriate therapy.

James Ziai, MD, Nancy Kriz, BS and Henry M. Rinder, MD

References:


Abnormal Bleeding

PT/aPTT/INR

↑ PT

History: Prior bleeds/Familial/Medication/Transfusion
Lab Testing: vitK, liver function tests, fibrinogen, d-dimer, etc.

↑ aPTT

↑ PT & aPTT

Factor V & VII activity

↓ Factor V & VII activity

Liver disease

Isolated ↓ FVII activity

Congenital deficiency/Inhibitor

Vitamin K/Warfarin

Factor VIII and IX activity

↓ Factor VIII

Hemophilia A/Acquired inhibitor

Hemophilia B/Acquired Inhibitor

Normal Factor VIII/IX activity

Factor XI activity

↓ Factor XI

Congenital deficiency/Inhibitor

Factor II, V, VII, X activity

↓ Factor II

Recommend lupus anticoagulant (lupus cofactor effect)

Isolated ↓ Factor V

Factor V inhibitor

Isolated ↓ Factor X

Inhibitor (amyloid, malignancy, resp infection)

↓ Factor X, VII and II

Vitamin K deficiency/Warfarin

↓ Factor V activity

Congenital deficiency/Inhibitor

Mixing Study

Immediate correction: deficiency

Prolonged after 1 hr: inhibitor

Immediate correction: deficiency

Prolonged after 1 hr: inhibitor

Immediate correction: deficiency

Prolonged after 1 hr: inhibitor

Figure 1: Suggested Coagulation Factor Testing Algorithm
Adapted from Wagenman et al, 2009