News from the Coagulation Laboratory
By Marilyn Hamilton, MD, PhD

Platelet Function Screening (PFA)-A NEW TEST
PFA testing is a screening test and diagnosis of a specific platelet defect will require additional testing. PFA testing provides better quality results than Bleeding Time and will eventually replace the Bleeding Time test at CMH.

O/E ordering mnemonic: LAB PFA
Specimen Requirements: two 2.7 mL light blue* and 1-3 mL in a lavender top
*coagulation testing fill requirements apply to the light blue tubes
Availability: 24/7 Routine status with a 4 hour turn around time
Special Instructions: Specimens must have the time of collection written on the tube and be delivered to the lab immediately at room temperature. Testing must be completed with-in 4 hours of collection.
Ranges:
- COL/EPI 83-170 seconds
- COL/ADP 55-115 seconds
Important Notes:
- The PFA can monitor patients with type 1 vWD after therapeutic treatment
- It does not detect fibrinogen or coagulation factor defects

Questions- contact the laboratory at X 3831.

Pre-analytical Considerations
Platelet count: If the platelet count is <100, PFA testing will not be performed. Testing will be performed when the platelet count is in the range of 100-150 and will be reported if normal. Abnormal PFA results will not be reported when the platelet count is in this range; abnormal results could reflect the platelet count, not function.
Hematocrit: Specimens with a hematocrit <30 will not be tested. Testing will be performed when the hematocrit is in the range of 30-35 and will be reported if normal. Abnormal PFA results will not be reported when the Hct is in this range; abnormal results could reflect the Hct, not platelet function.
Platelet activation: Testing is sensitive to a traumatic stick or the sheer force associated with using a small needle. Venipuncture should be performed using a 23 g needle or larger. Hemolyzed specimens will not be tested as hemolysis indicates a traumatic stick or severe sheer forces have been applied to the specimen.
Coumadin/Heparin: Coumadin and low levels of heparin do not affect the results. However, specimens should not be collected through a heparinized line. Following cardiac surgery, specimens should only be sent after protamine has been administered.

Methodology and Discussion
The PFA-100 is an instrument in which platelet adhesion and aggregation and is used to detect platelet dysfunction. The PFA simulates in-vivo vascular injury and blood flows through an aperture in a manner similar to that of a cut vessel. The aperture membrane is coated with collagen and epinephrine (EPI) or collagen and ADP (ADP) which activate platelets. Platelet function is measured by the time it takes the platelet/RBC thrombus to occlude the aperture and stop the blood flow, measured as the Closure Time (CT). The EPI membrane is the primary test used to detect platelet dysfuctions such as von Willebrand’s disease, Glazmann’s disease, uremia, or exposure to platelet inhibiting agents such as acetyl salicylic acid (aspirin). A platelet dysfunction is associated with a prolonged CT. The ADP cartridge is used to discriminate between a platelet defect and a drug effect. The CT using the ADP cartridge will be prolonged with a platelet defect (including uremia) but will be normal in the presence of aspirin.

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal</th>
<th>Aspirin</th>
<th>vWD</th>
<th>Uremia</th>
<th>Glanzmann’s</th>
<th>Rare Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI</td>
<td>Norm</td>
<td>Elev</td>
<td>Elev</td>
<td>Elev</td>
<td>Elev</td>
<td>Normal</td>
</tr>
<tr>
<td>ADP</td>
<td>Norm</td>
<td>Norm</td>
<td>Elev</td>
<td>Elev</td>
<td>Elev</td>
<td>Elev</td>
</tr>
</tbody>
</table>
Homocysteine: Risk Factor for Vascular Disease
And a Marker of Folate and Cobalamin Deficiency
By Uttam Garg, Ph.D.

Homocysteine is a sulfur containing amino acid not included in 20 amino acids that serve as protein precursors. It exists in three forms: free homocysteine, disulfide homocysteine and mixed disulfide homocysteine-cysteine. Almost all plasma homocysteine is bound to proteins. It is an intermediate in the formation of cysteine from methionine. Increased homocysteine is a risk factor for occlusive arterial and venous disease, adverse pregnancy outcome and impaired cognitive function. Homocysteine is making its way into pediatrics (1,2)

Hyperhomocysteinemia may be inherited due to enzyme defects involved in homocysteine or cofactors metabolism or it may be acquired due to deficiency in enzyme cofactors. Table 1 lists some of the inherited or acquired factors leading to increased homocysteine.

Inherited factors:
- Cystathionine β-synthase deficiency
- Methylene tetrahydrofolate reductase deficiency
- Methionine synthase deficiency
- Vitamin B12

Cofactors deficiency:
- Vitamin B12
- Folate deficiency
- Pyridoxine (B6) deficiency

Other factors:
- Smoking
- Renal Failure
- Malignant disease
- Hypothyroidism
- Methotrexate
- Nitrous oxide
- Anticonvulsants
- Cyclosporine A
- HIV infection

Homocysteine metabolism requires several enzymes and cofactors (Figure). Deficiency of enzymes (generally due to inherited defects) or cofactors (inherited or acquired) leads to hyperhomocysteinemia. There is a strong relationship between serum folate and homocysteine indicating that homocysteine can be used as a marker of folate deficiency.

Recent reports demonstrate that homocysteine measurement in conjunction with the cobalamin deficiency-marker (methylmalonic acid) or with vitamin concentrations are an effective way of diagnosis of deficiencies of cobalamin and folate. These metabolites determinations are particularly useful for the diagnosis of sub clinical deficiencies, which lack the typical clinical signs of anemia and megaloblastosis. The recent data indicate that folate and cobalamin deficiencies are more common in children than recognized.

Figure: Simplified homocysteine metabolism. MTHFR (methylene tetrahydrofolate reductase, folate dependent); MS (methionine synthase, Vitamin B12 dependent); CBS (cystathionine β-synthase, Vitamin B6 dependent).

References: