HEMATOLOGY LAB: HEMOGLOBIN ELECTROPHORESIS TESTING UPDATE SPECIAL
CONSIDERATION OF MICROCYTIC ANEMIA
By David Zwick, MD

Traditional methods of detecting structural hemoglobinopathies (i.e. those with globin chain amino acid substitutions) were based on electrophoretic separation of the hemoglobin variants on cellulose acetate and citrate agar and comparing the mobilities of unknown variants with known variants. Detection of Beta Thalassemias is based on reliable quantification of the levels of 2 normal minor hemoglobin variants, Hgb A2 and F. Because of the inherent high level of imprecision of densitometry scanning directly from the separation gels for quantification, particularly for hemoglobin accounting for less than 10%, precise and accurate quantification of hemoglobin A2 and hemoglobin F required labor intensive and time consuming affinity column chromatography and alkaline denaturation methods, respectively. All of these methods have been replaced in our laboratory by a single step high performance liquid chromatography (HPLC) method. The HPLC method is more rapid, less expensive, provides excellent separation of unknown variants and very reliable quantification of the hemoglobin A2 and hemoglobin F levels.

A practical approach and algorithms for differential diagnosis of anemias in children were discussed in previous newsletter (December 2003). Nearly half of all hemoglobin electrophoresis studies done in our laboratory are ordered for investigation of microcytic anemia. The two most common conditions leading to microcytic anemia in children are iron deficiency and Thalassemia. The detection of Beta Thalassemia is based on identification of elevated levels of Hemoglobin A2 and Hemoglobin F. Detection of Alpha Thalassemia in the newborn period is based on identification of elevated levels of Hgb Barts, a globin chain tetramer comprised of 4 gamma chains. Beyond the newborn period, hemoglobin Barts is lost since gamma chain production is markedly down regulated. Therefore, confirmation of Alpha Thalassemia after the newborn period requires either alpha globin gene molecular testing that can be done in our molecular genetics lab, or by globin chain synthesis studies on peripheral blood reticulocytes, which must be sent to a specialty laboratory.

NOTE OF CAUTION: Hemoglobin A2 and hemoglobin F levels are lower in iron deficiency than in an iron replete state. Hence the presence of coexistent iron deficiency may mask detection of underlying Beta Thalassemia. If suspected, it is recommended iron deficiency be excluded before doing quantitative hemoglobin A2 and hemoglobin F testing. Alternatively, testing should be repeated for Thalassemia if the patient remains microcytic after iron stores are replaced.

Lead toxicity, as a cause of microcytic anemia, is far less common than iron deficiency or Thalassemia. Lead toxicity has declined since the institution of public health measures to reduce lead exposure. Because anemia is an insensitive indicator of potentially serious and even life threatening exposure that may be too acute to manifest anemia, blood lead levels should be requested as part of normal screening or whenever the clinical circumstances warrant. There are very few structural hemoglobinopathies associated with microcytic anemias, e.g. Hgb C and Hgb E disease, which will be confidently detected by standard electrophoresis or HPLC testing.
Correction: HLA Association in Celiac Disease

The October Pathology and Laboratory Newsletter INCORRECTLY states celiac disease is associated with HLA B8 and A1. Celiac disease is associated with DQ2 or DQ8. The North American Society for Pediatric Gastroenterology, Hepatology and Nutrition states: “The development of celiac disease is clearly multigenic, with the presence of DQ2 or DQ8 being an essential component. Thus, probes for DQ2 and DQ8 have high sensitivity but poor specificity.”

Fresh Frozen Plasma Transfusion, Audit Criteria, and Surgery

By Marilyn Hamilton, MD, PhD & Kathy Perryman MD

It is common to order PT and PTT prior to surgery and, if elevated, to give FFP to correct the “problem”. In 1994, the American Society of Anesthesiologists Task Force on Blood Component Therapy developed evidenced-based indications for transfusing FFP. Their conclusion was that FFP is indicated for urgent reversal of warfarin therapy, correction of known factor deficiencies for which specific concentrates are not available and correction of microvascular bleeding when PT and PTT are > 1.5 normal. More recent “Guidelines for Blood Utilization Review,” published by the American Association of Blood Banks in 2001, confirms this recommendation: “PT or PTT > 1.5 times the mean of the reference range in a non-bleeding patient scheduled for or undergoing surgery or an invasive procedure.” With these recommendations, and after an extensive survey of other children’s hospitals, the CMH Transfusion Committee established Audit Criteria for FFP in patients with active bleeding or requiring an invasive procedure.

The PT and PTT measure different components of the coagulation cascade.

PT: Factor VII, Common Pathway Factors X, V, Prothrombin, Fibrinogen
PTT: Factors VIII, IX, XI, XII, Common Pathway X, V, Prothrombin, Fibrinogen

When using FFP to correct for one or more of these factors it VERY IMPORTANT to administer the FFP as close as possible to surgery to optimize its effectiveness. This is based on the short half lives of critical factors as shown below:

<table>
<thead>
<tr>
<th>Factor</th>
<th>Half Life</th>
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<tbody>
<tr>
<td>V</td>
<td>4.5-36 hours</td>
</tr>
<tr>
<td>VII</td>
<td>2-5 hours</td>
</tr>
<tr>
<td>VIII</td>
<td>8-12 hours</td>
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<tr>
<td>IX</td>
<td>18-24 hours</td>
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Normal Values

<table>
<thead>
<tr>
<th>Pts &gt; 4 Months</th>
<th>Calculated Values</th>
<th>Audit Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT 12-15</td>
<td>1.5 x 13.5 = 20</td>
<td>18</td>
</tr>
<tr>
<td>PTT 22.8-35.2</td>
<td>1.5 x 29 = 43</td>
<td>43</td>
</tr>
</tbody>
</table>

CME Series
Sponsored by Department of Pathology & Laboratory Medicine

Date: Tuesday, November 16, 2004
Time: Noon – 13:00
Location: Conference Room 2206.10 WT
Speaker: Greg Kearnes, FCP
Topic: Relevant Pharmacogenetics

Charles Barnes, PhD; Linda Cooley, MD; Uttam Garg, PhD; Marilyn Hamilton, MD, PhD; Joan Knoll, PhD; Rangaraj Selvarangan, BVSc, PhD; Carol Saunders, PhD; Lei Shao, MD; Vivekanand Singh, MD; Eugenio Taboada, MD; David Zwick, MD