RBC Immature Reticulocyte Fraction (IRF):
What does it mean and how does it help me understand my patient’s anemia?

Reticulocytes are immature RBCs that have extruded their nuclei but still have residual hemoglobin synthetic activity (i.e., ribosomal and messenger RNA see Laboratory newsletter April 2002). Once the nucleus has been extruded, reticulocytes take approximately 4 days to mature and complete synthesis of hemoglobin. During this time, there is a parallel decline in the reticulin or cytoplasmic RNA until all of the RNA is lost. Our automated method for quantifying peripheral blood reticulocytes is based on detecting RBCs containing RNA. In addition, the automated method has the capacity to quantify the relative amount of cytoplasmic RNA in each RBC and derive the proportion of all reticulocytes that are relatively RNA-rich or immature reticulocytes; this proportion is referred to as the IRF. Since most reticulocyte maturation occurs in the bone marrow, before being released into the peripheral blood near the end of their 4-day maturation, the fractional percentage of immature reticulocytes (IRF) in the peripheral blood is normally very low (~3–16%).

The absolute reticulocyte count and the IRF are etiologic, non-specific but important physiologic measures of erythropoietic bone marrow response to anemia or tissue hypoxia. These measures are useful indicators of suppressed, normal, or hyper-responsive marrow function. Most often, the IRF value and absolute reticulocyte count parallel each other, for example, when one is elevated, the other is also elevated and when one is depressed, the other is reduced. However, the IRF tends to respond more rapidly than the absolute reticulocyte count or percent reticulocytes to marrow stimulation or suppressive factors. Hence, the IRF is often an earlier indicator of acute blood loss, hemolysis, tissue hypoxia, marrow suppression, or marrow recovery following a myelosuppressive episode. In the setting of bone marrow transplantation, rising IRF values are the earliest indicator of engraftment preceding the rise in absolute neutrophil count by 1-5 days. Similar results showing an earlier rise in the IRF were reported in 2 non-transplant patients recovering from severe aplastic anemia treated with antithymocyte globulin, cyclosporin, and G-CSF. The IRF has also been reported to reflect increased erythropoietic activity that occurs in non-anemic patients with chronic pulmonary and cardiac conditions or successful renal engraftment that reflects restoration of normal renal erythropoietin production. The IRF may be a useful screening test for hypoxia in non-anemic patients and serial measurements might be useful for monitoring efficacy of treatment of the underlying cardiopulmonary condition. (Clin. Lab. Haem. 2001, 23: 27-37.)

In summary, the IRF may provide additional information to assess the erythropoietic response when results of the reticulocyte percentage or absolute reticulocyte counts alone are equivocal. A word of caution: The methodology is not standardized; therefore, clinical decision thresholds need to be institutionalized and practice-specific to reflect inter-laboratory variations. Serial testing is recommended for monitoring unstable patients or those with single equivocal results that do not give clear indication of the adequacy of the marrow response to hypoxia or anemia.
News From Chemistry
by Uttam Garg, PhD

Sweat Chloride Testing: Laboratory Diagnosis of Cystic Fibrosis

Sweat chloride analysis is used for the diagnosis of cystic fibrosis (CF) in children with a family history or clinical symptoms of CF, such as frequent and/or foul stools, diarrhea, malnutrition and failure to thrive, depletion of the fat-soluble vitamins, malabsorption, pancreatic insufficiency, history of meconium ileus, neonatal intestinal obstruction, rectal prolapse, infant celiac disease, chronic sinusitis and pulmonary disease, chronic cough, and Pseudomonas bronchitis. A number of diseases and conditions other than cystic fibrosis can cause an increased sweat chloride. These include anorexia nervosa, atopic dermatitis, autonomic dysfunction, ectodermal dysplasia, familial cholestasis, fucosidosis, glucose-6-phosphate dehydrogenase deficiency, glycogen storage disease type I, hypogammaglobulinemia, Klinefelter syndrome, long-term prostaglandin E1 infusion, Mauriac syndrome, mucopolysaccharidosis type I, nephrogenic diabetes insipidus, nephrosis, protein calorie malnutrition, pseudohypoaldosteronism, psychosocial failure to thrive, untreated adrenal insufficiency, and untreated hypothyroidism.

Sweating, generally on the forearms, is induced by pilocarpine iontophoresis, and the resulting sweat is collected on gauze. The sweat is then assayed for chloride. Some laboratories assay sweat for sodium, but chloride measurements have superior predictive values. Some laboratories/health facilities perform sweat conductivity. It should be kept in mind that sweat conductivity is a screening method only and positives should be confirmed by sweat chloride testing. The CMH Laboratory performs sweat chloride testing using Cystic Fibrosis Foundation and National Committee on Clinical Laboratory Standards guidelines.

Cystic Fibrosis Foundation guidelines for sweat chloride interpretation are:
- Negative: 0-40 mmol/L
- Borderline/indeterminate: 41-60 mmol/L
- Consistent with cystic fibrosis: >60 mmol/L

Also, it is recommended that all values be interpreted with family history and clinical presentation. Sweat chloride values <40 mmol/L have rarely been documented in patients with genetically proven CF; clinical correlation is necessary. The Cystic Fibrosis Foundation also recommends repeating all positives on a separate occasion.

Laboratory considerations: When ordering a sweat chloride test at CMH please keep in mind that it is a scheduled test and cannot be done on a walk-in basis. The sweat collection procedure is very involved and takes about 1 hour to complete. To keep sweat collection failure to minimum, we have a limited number of trained people; thus, scheduling is very important for the best patient care. Following are the phone numbers to schedule an appointment for sweat collection.
- Inpatients (CMH Main Campus): 816-234-3230
- Outpatients (CMH Main Campus): 816-234-1530
- Children’s Mercy South Laboratory: 913-696-8210

A small fraction of CF patients do not have diagnostic sweat chloride patterns or may not sweat enough to do a sweat chloride test. For these patients, DNA analysis of the cystic fibrosis gene may be helpful (contact the Laboratory for details).

Laboratory CME Series
Tuesday, November 19, 2002
12:00 p.m.-1:00 p.m.
Location: Laboratory Conference Room #2206.10
Speaker: Dr. David Zwick
Topic: “Flow Cytometry Minimal Residual Leukemia Testing”