New hematology lab instrumentation: what it means for your patients.

The main instruments in the hematology lab have been replaced by state of the art equipment that will improve accuracy, precision and turnaround times as well as provide a few new blood cell parameters that are helpful in monitoring erythropoietic activity.

1. **Extended Linearity for WBC and Platelet Counts**: This will improve turnaround times since there will be fewer occasions requiring diluting of samples for retesting or performing alternative time consuming manual testing for low blood counts.

2. **Fewer Manual WBC Differentials**: Any differential requested for WBC below 1000 cells/ul will now be done by the automated differential method. Consequently, turnaround times and test precision (reliability) are greatly improved. In addition, the improved reliability of automated differentials results in fewer flags and less manual confirmatory differential testing, thereby expediting turnaround times even when WBC is greater than 1000/ul.

3. **Less Interfering Substances in Hemoglobin Quantification (e.g., high WBC, lipemia and abnormal serum proteins)**: This will shorten turnaround times since samples will not need to be retested after altering samples to get rid of interference.

4. **Automated Reticulocyte Counts**: This will improve turnaround times and test precision. The test can be run on the same sample as the CBC. However, additional samples may be required for this test if it is requested as an "add on" and the CBC has already been done (additional sample is needed if there is no longer the 150/ul required for separate reticulocyte analysis). Results will be expressed as percentage value of all RBC as well as absolute number of reticulocytes per ul of whole blood. Normal ranges will no longer be provided for Retic % but will be provided for absolute retic counts (Normal absolute retic count = 0.020 – 0.080 x 10^6 reticulocyte/ul). In addition, the method allows determination of the proportion of immature reticulocytes (see article on “Immature Reticulocyte Fraction” in this issue).

5. **Automated NRBC Counts**: This results in improved accuracy and precision and reduced turnaround times. Results will be reported as # nbcs/100 wbcs as well as absolute number of nbcs/ul (e.g., 1.3 x 10^3/ul).

6. **Red Cell Distribution Width (RDW)**: a measure of RBC size variation, now reported as the standard deviation rather than as a coefficient of variation (CV). The conventional method of reporting variation of cell size by CV resulted in underestimating variation as mean cell size (MCV) increased (i.e., CV inversely proportional to MCV). Normal ranges RDW will change to 37 – 46.

There are a number of other benefits to the lab personnel in terms of data management and aids in recognizing cellular abnormalities. In addition, there are several potential new applications that need to be tested in order to determine utility in our patient. Normal ranges will remain the same.
News from Hematology

Immature Reticulocyte Fraction (IRF):

Reticulocytes are immature non-nucleated RBCs that have not completed production of hemoglobin and consequently contain residual hemoglobin synthetic machinery, mRNA and rRNA, commonly referred to as “reticulin.” Peripheral blood reticulocyte count is a measure of erythropoietic activity; rising counts in the face of anemia are regarded as an indicator of appropriate bone marrow response. Traditional manual methods rely on microscopic recognition and counting of retics and non-retic RBCs differentially stained by dyes specific for RNA. It is known that the maturation of reticulocytes, i.e., progressive accumulation of hemoglobin and loss of RNA, takes about 4 days. Conventional reticulocyte counts enumerate all RNA stained cells, including both heavy staining (immature) and weakly stained (mature) cells and simply lump immature and mature reticulocytes together. Measures of reticulocyte maturity provide complementary information to the absolute reticulocyte counts for assessing erythropoietic activity. Though manual methods are capable of quantifying a maturity fraction, they suffer from inordinately high imprecision that effectively negates test reliability. The new automated reticulocyte counting method overcomes these deficiencies and now provides a reliable measure of immature reticulocytes expressed as Immature Reticulocyte Fraction (IRF).

The IRF is a fractional percentage of all reticulocytes that have an intermediate and high content of RNA. IRF normal levels are reported to ranges from 5 to 22%; limited testing in our lab shows a somewhat narrower normal range of 3 to 16%. These are the reticulocytes that were most recently released from the bone marrow. The IRF measure has inherent methodologic variables that are well controlled within our lab but are not standardized between laboratories. In addition, RNA is inherently labile and there is currently no standardized reference material for establishing inter-laboratory comparison. Consequently, one cannot apply published data for determining normal ranges and clinically significant decision-making thresholds that were based on study of a different patient population without further validation measures.

There are a number of published studies that suggest that the IRF is a more sensitive and specific indicator than the reticulocyte count alone, of the following:

1. Adequacy of marrow response to anemia in patients with a variety of chronic diseases including chronic renal failure.
2. Adequacy of marrow response in neonates with anemia.
3. Response to anemia therapy including erythropoietin, iron, B12, and folate.
4. Signal of successful renal transplant engraftment and erythropoietin production.
5. Bone marrow recovery following myelosuppression and erythropoietin production.
7. Measure of chronic hypoxia and resultant increased erythropoietic activity in conditions such as chronic lung and cyanotic cardiac diseases.

Sequential testing or trending is recommended and provides more sensitive and reliable data for interpreting erythropoietic responses than a single measure. IRF will be reported routinely when a reticulocyte count is ordered. IRF is most useful as a measure of erythropoietic activity when correlated with the absolute reticulocyte count. These two parameters show a weak positive correlation and provide the similar clinical information as the calculated “corrected reticulocyte count” or the “reticulocyte production index (RPI).” RPI is a calculated estimate of marrow response and values > 2 are considered to reflect adequate marrow response to anemia whereas values equal to or < 2 reflect a nonresponsive or hyporesponsive marrow. An IRF is a direct measure of reticulocyte maturity, and values less than the upper limits of normal is an indicator, corresponding to RPI of 2 or less, of nonresponsive or hyporesponsive marrow to anemia.

Using a cut off value for IRF of 23%, one study of 132 patients demonstrated the following associations:

1. Increased Absolute Retic count and IRF > 23%: there were 26 patients, most with sickle cell disease with crisis and conditions in which one expects increased erythropoietic activity. Only 9 patients showed RPI > 2.
2. Increased Absolute Retic count and IRF < 23%: there were 19 patients with various disorders including hypothyroidism, sarcoidosis, eating disorders with malnutrition, and neurosyphilis. Seventeen of the 19 patients had RPI < 2.
3. Normal or Subnormal Absolute Retic count and IRF > 23%: there were 59 patients with this pattern of results and all showed a RPI < 2 indicating impaired erythropoietic activity. Patients suffered from anemia associated with underlying diseases known to lead to decreased erythropoietic activity such as chronic renal insufficiency.
4. Normal or Subnormal Absolute Retic count and IRF < 23%: 28 patients; and all had RPI < 2. These patients suffered from a variety of conditions including anemia associated with acute infection, iron deficiency anemia, HIV infection, SSD with crisis, pregnancy, and myelodysplastic syndrome.

Reference:
