Lab 1A and B: Deficiency and Hemolytic Anemias

Hematology Unit
2017
Objectives

Laboratory Instructor will:
• Provide overview of normal morphology of erythroid lineage
• Review morphologic and laboratory features of deficiency anemia and hemolytic anemia
• Assist students during self study

Students will:
• Study the case histories provided for MD Lab 1
• Examine the pathological material related to each case using virtual microscopy
• Answer the questions related to each case
Erythropoiesis

- Characterized by:
  - Increasing hemoglobin synthesis
  - Decreasing cell size
  - Decreasing cytoplasmic basophilia
  - Progressive chromatin condensation
  - Extrusion of nucleus (orthochromatic stage)
  - Extruded nuclei are subsequently phagocytized
  - Loss of mitotic capability after the early stage of polychromatophilic normoblast

- Average of 4 cell divisions during maturation
  [One pronormoblast gives rise to 16 red cells]

- Pronormoblast $\rightarrow$ reticulocyte = 7 days
  Reticulocytes $\rightarrow$ mature RBC = 1-2 days
Erythropoiesis (Cont.)

- Erythroid progenitors (normoblasts) cluster around macrophages (arrows) in the bone marrow and spleen.
- Macrophages store iron.
- Iron is transferred from macrophages to erythroid cells.
- Iron is used by normoblasts for hemoglobin synthesis.
Mature Red Blood Cell

7-8 microns; round / ovoid biconcave disc with orange-red cytoplasm, no RNA, no nucleus; survives ~120 days in circulation
Classification of Anemia by Morphology

1. Microcytic, hypochromic anemia
   MCV < 80
   MCH < 27
   MCHC < 32

2. Normocytic, normochromic anemia
   MCV and MCH within normal range

3. Macrocytic
   MCV > 100
Normal Red Blood Cell Morphology

Size of normal RBC is comparable to the nucleus of a small lymphocyte
Normocytic = Normal size

Normochromic (vs Hypochromic) RBCs:
Defined by area of central pallor:
Up to 1/3 the size of RBC = Normochromic
>1/3 of RBC size = Hypochromic
Megaloblastic Anemia: Bone Marrow

Megaloblastic Erythropoiesis characterized by:
- Hypercellular BM; decreased M:E ratio
- Delayed nuclear maturation compared with cytoplasmic maturation
- Larger cell size with open chromatin network of the nucleus
- Howell-Jolly bodies (DNA fragments in the cytoplasm)
- Giant metamyelocytes
The peripheral smear shows:

- Aniso- and poikilocytosis
- The red blood cells are microcytic since many are smaller than the nucleus of the lymphocyte
- The erythrocytes are hypochromic with an increased central pallor
- Elliptocytic and pencil-shaped forms are present.
Iron Stained Bone Marrow

Prussian Blue Stain

Normal bone marrow biopsy:
Iron (Blue stain) is present in the reticuloendothelial cells

Iron deficiency:
There is no iron staining
Classification of Hemolytic Anemias

• **Intracellular Causes**
  – Red cell membrane defects
  – Enzyme defects
  – Hemoglobin defects
    • Thalassemia
    • Sickle cell disease
    • Hemoglobin C

• **Extracellular causes**
  – Autoimmune
  – Microangiopathic
Laboratory Markers of Hemolysis

- Anemia: CBC shows low Hb and red blood count
- Evidence of marrow activity: high reticulocyte count (and normoblasts in extreme cases)
- Increased breakdown products of Hb (High bilirubin, lactate dehydrogenase), hemoglobinuria
- Decreased binding protein (haptoglobin-consumed)
# Red Blood Cell Shapes

<table>
<thead>
<tr>
<th>Red cell abnormality</th>
<th>Causes</th>
<th>Red cell abnormality</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>Microspherocyte</td>
<td>Hereditary spherocytosis, autoimmune haemolytic anaemia, septicaemia</td>
</tr>
<tr>
<td>Macrocyte</td>
<td>Liver disease, alcoholism. Oval in megaloblastic anaemia</td>
<td>Fragments</td>
<td>DIC, microangiopathy, HUS, TTP, burns, cardiac valves</td>
</tr>
<tr>
<td>Target cell</td>
<td>Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy</td>
<td>Elliptocyte</td>
<td>Hereditary elliptocytosis</td>
</tr>
<tr>
<td>Stomatocyte</td>
<td>Liver disease, alcoholism</td>
<td>Tear drop poikilocyte</td>
<td>Myelofibrosis, extramedullary haemopoiesis</td>
</tr>
<tr>
<td>Pencil cell</td>
<td>Iron deficiency</td>
<td>Basket cell</td>
<td>Oxidant damage—e.g. G6PD deficiency, unstable haemoglobin</td>
</tr>
<tr>
<td>Echinocyte</td>
<td>Liver disease, post-splenectomy. storage artefact</td>
<td>Sickle cell</td>
<td>Sickle cell anaemia</td>
</tr>
<tr>
<td>Acanthocyte</td>
<td>Liver disease, abetalipoproteinaemia, renal failure</td>
<td>Microcyte</td>
<td>Iron deficiency, haemoglobinopathy</td>
</tr>
</tbody>
</table>
RBC Inclusions

- The reticulocytes and Heinz bodies are only demonstrated by supravital staining (like new methylene blue, bottom 2 pictures).
- Heinz bodies are oxidized denatured hemoglobin
- Reticulocytes contain remnant RNA
- Pappenheimer bodies are siderotic granules (contains iron)
- The Howell-Jolly body is DNA remnant
- Basophilic stippling is denatured RNA

Red Cell Membrane Defects

(a) Hereditary Spherocytosis

Note the small deeply staining red cells without area of central pallor (thick arrow) (spherocytes). At the top, note the larger polychromatic reticulocyte (confirmed by a supravital stain).

(b) Hereditary Elliptocytosis

Note the oval, elongated red cells with rounded ends distinguishing them from pointed ends in sickle cells.
Anemia from Enzyme Defect: G6PD Deficiency

Note loss of cytoplasm in some cells due to oxidant stress (thin arrow). Also, separation of hemoglobin from cell membrane (hanging basket cells) (thick arrow).

Heinz bodies representing denatured hemoglobin shown by supravital stain. Heinz bodies are also seen in Thalassemia due to excess globin chains.
Hemoglobin Defects: Sickle Cell Disease

- Sickled cell
- Nucleated Red Blood Cell (Normoblast)
- Target Cell
- Howell-Jolly bodies
Hemoglobin Defects: β-Thalassemia Major

Blood film in β-thalassaemia major post-splenectomy. There are hypochromic cells, target cells and many nucleated red cells (normoblasts). Howell-Jolly bodies are seen in some red cells.

Hemoglobin Defects: Homozygous C Disease

- Hemoglobin C produces an envelope shaped or rhomboidal shaped cells (arrows) as opposed to the sickled shaped cell
- Target cells and microspherocytes are common.
Extracellular causes of Hemolysis: Autoimmune

(a) Note the microspherocytes (small deeply staining cells without central pallor) and the larger polychromatic reticulocytes. This picture is usually associated with warm antibody hemolysis.

(b) Note the clumping (agglutination) of red cells usually associated with cold agglutinin disease.
Extracellular Causes of Hemolysis: Microangiopathic

Blood film in microangiopathic hemolytic anemia. Note the numerous contracted and deeply staining cells (spherocytes) and broken RBCs (schistocytes)
Laboratory Testing in Hemolytic Anemias: Coombs Test

- Determines the presence of immunoglobulins (Ig)/or complement on the red blood cell surface (direct) or the presence of anti red blood cell Ig in the serum (indirect)
Direct Coombs

The patient erythrocytes are incubated with Coombs reagent which contain:

- Broad spectrum, or
- Type-specific **antibodies** (Anti-IgG, -IgM, or -Complement)

If the corresponding **antigens** (broad spectrum, complement or IgG, IgM) are present on the red cell surface, there will be red cell agglutination
Indirect Coombs

- Patient serum is incubated with 0 Rh negative reagent RBCs
- The RBCs are then washed and incubated with anti-human IgG antibodies
- If the patient’s serum has antibodies, which react with the reagent RBCs, the anti-IgG antibodies will cause the reagent red cells to agglutinate
Laboratory Testing in Hemolytic Anemias: Hemoglobin Solubility Test

- Demonstrates the presence of a sickling hemoglobin
- The clear tube contains a nonsickling hemoglobin. The hemoglobin is soluble in the buffer as demonstrated by the visible lines, which can be seen through the tube.
- In the turbid tube the lines on the grid cannot be seen. This indicates the presence of hemoglobin S which is insoluble in this reagent.
- The sickle solubility test is positive.
The electrophoresis runs left to right; samples loaded at the origin (arrow)
- Hemoglobins are separated by their net electric charge
- Hb C (crawls), A², E and O co-migrate near the origin
- Hb S (slow), D and G are next
- Hb F (fast) runs between S and A
- Hb A (accelerated)
- Note that in ‘trait’, the A is more concentrated than the abnormal Hb
Radiologic feature in β Thalassemia major
The expanded marrow shows a “Hair on End” appearance