HEMATOLOGY

Introduction

- Study of blood & its components
- Window of rest of body
BLOOD
*Raison d’etre*

- Delivery of nutrients
  - Oxygen
  - Food
  - Vitamins
- Removal of wastes
  - Carbon dioxide
  - Nitrogenous wastes
  - Cellular toxins
- Repair of its conduit
- Protection *versus* invading microorganisms
- Multiple cellular & acellular elements

HEMATOLOGY
*Divisions*

- Red Blood Cells/Oxygen & CO$_2$ transport
- Coagulation/platelets/Maintenance of vascular integrity
- White Blood Cells/Protection *versus* pathogens/microorganisms
HEMATOLOGY

Hematopoiesis

- In humans, occurs in bone marrow exclusively
- All cellular elements derived from pluripotent stem cell (PPSC)
- PPSC retains ability to both replicate itself and differentiate
- Types of differentiation determined by the influence of various cytokines

PLURIPOTENT STEM CELLS

- Self-renewal
- Differentiation and development
- Stroma cells
- Progenitor cells (recognized by culture techniques)
- Recognizable precursors (marrow precursors)
- Endothelial cell
- Macrophage
- Fat cell
- Extracellular matrix

Fig 1.4. Hematopoiesis occurs in a suitable microenvironment provided by a stromal matrix on which specific cell types depend. There are probably specific recognition and adhesion sites in this microenvironment. Adhesion molecules and other compounds are highlighted in the diagram.
HEMATOPOIESIS

HEMATOPOIESIS – GROWTH FACTORS

Fig. 1.6: A diagram of the role of growth factors in normal haemopoiesis. Multiple growth factors act on the early marrow stem and progenitor cells. EPO: erythropoietin; PSC: pluripotential stem cell; SCF: stem cell factor; TPO: thrombopoietin. For other abbreviations see fig. 1.2.
RED BLOOD CELLS

Introduction

• Normal - Anucleate, highly flexible biconcave discs, 80-100 femtoliters in volume
• Flexibility essential for passage through capillaries
• Major roles - Carriers of oxygen to & carbon dioxide away from cells
ERYTHROPOIETIN

• Cytokine - Produced in the kidney
• Necessary for erythroid proliferation and differentiation
• Absence results in apoptosis of erythroid committed cells
• Anemia of renal failure 2° to lack of EPO

ERYTHROPOIETIN

Mechanism of Action

PPSC → BFU-E → CFU-E

EPO Stimulates Proliferation

Accelerates Maturation

Reticulocye

Mature RBC
ERYTHROPOIETIN

Mechanism of Action

- Binds specifically to Erythropoietin Receptor
- Transmembrane protein; cytokine receptor superfamily
- Binding leads to dimerization of receptor
- Dimerization activates tyrosine kinase activity

GROWTH FACTORS - Mechanisms of Action
ERYTHROPOIETIN

Mechanism of Action

• Multiple cytoplasmic & nuclear proteins phosphorylated via JAK-STAT pathways
• Nuclear signal sent to activate production of proteins leading to proliferation and differentiation
• Signal also sent to block apoptosis

ERYTHROPOIETIN - Regulation of Production/Mechanism of Action
Erythropoietin Response to Administration

- rhuEPO 150 u/kg 3x/wk
RBC Precursors

- Pronormoblast
- Basophilic normoblast
- Polychromatophilic Normoblast
- Orthochromatophilic Normoblast
- Reticulocyte
- Mature Red Blood Cell
- 5-7 days from Pronormoblast to Reticulocyte
RETICULOCYTE

• Important marker of RBC production
• Young red blood cell; still have small amounts of RNA present in them
• Tend to stain somewhat bluer than mature RBC’s on Wright stain (polychromatophilic)
• Slightly larger than mature RBC
• Undergo removal of RNA on passing through spleen, in 1st day of life
• Can be detected using supravital stain

RETICULOCYTE COUNT

**Absolute Value**

• \( \text{Absolute Value} = \text{Retic \%} \times \text{RBC Count} \)
  - eg 0.01 \times 5,000,000 = 50,000
• Normal up to 100,000/µl
• More accurate way to assess body’s response to anemia
RBC Assessment

- Number - Generally done by automated counters, using impedance measures
- Size - Large, normal size, or small; all same size versus variable sizes (anisocytosis). Mean volume by automated counter
- Shape - Normal biconcave disc, versus spherocytes, versus oddly shaped cells (poikilocytosis)
- Color - Generally an artifact of size of cell
### Red Blood Cells

**Normal Values**

<table>
<thead>
<tr>
<th>RBC Parameters</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>35-47%</td>
</tr>
<tr>
<td>Males</td>
<td>40-52%</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>12.0-16.0 gm/dl</td>
</tr>
<tr>
<td>Males</td>
<td>13.5-17.5 gm/dl</td>
</tr>
<tr>
<td>MCV</td>
<td>80-100 fl</td>
</tr>
<tr>
<td>Reticulocyte Count</td>
<td>0.2-2.0%</td>
</tr>
</tbody>
</table>
ANEMIA

Causes

- Blood loss
- Decreased production of red blood cells (Marrow failure)
- Increased destruction of red blood cells
  - Hemolysis
- Distinguished by reticulocyte count
  - Decreased in states of decreased production
  - Increased in destruction of red blood cells
RBC DESTRUCTION - EXTRAVASCULAR

Markers

- Heme metabolized to bilirubin in macrophage; globin metabolized intracellularly
- Unconjugated bilirubin excreted into plasma & carried to liver
- Bilirubin conjugated in liver & excreted into bile & then into upper GI tract
- Conjugated bilirubin passes to lower GI tract & metabolized to urobilinogen, which is excreted into stool & urine

RBC DESTRUCTION - INTRAVASCULAR

- Free Hemoglobin in circulation leads to
  - Binding of hemoglobin to haptoglobin, yielding low plasma haptoglobin
  - Hemoglobin filtered by kidney & reabsorbed by tubules, leading to hemosiderinuria
  - Capacity of tubules to reabsorb protein exceeded, yielding hemoglobinuria
INTRAVASCULAR HEMOLYSIS

Acute Hemolytic Event

Serum Haptoglobin

Hemoglobinuria

Urine Hemosiderin

HEMOLYTIC ANEMIA

Commonly used Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulocyte Count</td>
<td>Increased</td>
</tr>
<tr>
<td>Unconjugated Bilirubin</td>
<td>Increased</td>
</tr>
<tr>
<td>Lactate Dehydrogenase</td>
<td>Increased</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>Decreased</td>
</tr>
<tr>
<td>Urine Hemoglobin</td>
<td>Present</td>
</tr>
<tr>
<td>Urine Hemosiderin</td>
<td>Present</td>
</tr>
</tbody>
</table>

Problems with sensitivity & specificity