HEMATOLOGY/
HEMATOPOIESIS

Introduction
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 & CO₂ 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
Fig. 1.4 Haemopoiesis occurs in a suitable microenvironment provided by a stromal matrix on which stem cells grow and divide. There are probably specific recognition and adhesion sites (see p. 10); extracellular glycoproteins and other compounds are involved in the binding.
HEMATOPOIESIS

Pluripotent stem cell

CFU<sub>GEMM</sub>
Mixed myeloid progenitor cell

CFU<sub>GMEo</sub>

CFU<sub>M</sub>

CFU<sub>G</sub>

BFU<sub>E</sub>
Erythroid progenitors

CFU<sub>E</sub>

CFU<sub>Meo</sub>
Megakaryocyte progenitor

CFU<sub>GM</sub>
Granulocyte-monocyte progenitor

CFU<sub>Lo</sub>
Eosinophil progenitor

CFU<sub>baso</sub>

Lymphoid stem cell

Thymus

Red cells
Platelets
Monocytes
Neutrophils
Eosinophils
Basophils
Lymphocytes
NK cell
HEMATOPOIESIS – GROWTH FACTORS

Fig. 1.6 A diagram of the role of growth factors in normal haemopoiesis. Multiple growth factors act on the earlier marrow stem and progenitor cells. EPO, erythropoietin; PSC, pluripotent 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

PPSC Accelerates Maturation
Reticuloerythroblast 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

Fig. 1.10 Control of haemopoiesis by growth factors. The factors act on cells expressing the corresponding receptors. Binding of a growth factor to its receptor activates Janus-associated kinases (JAKs) which then phosphorylate signal transducer and activators of transcription (STATs) which translocate to the nucleus and activate transcription of specific genes (see text). JAKs may also activate other pathways, e.g. RAS/RAF/MAP kinase, concerned with cell proliferation by activating nuclear transcription factors which stimulate the cell to enter the cell cycle. E2F is a transcription factor needed for cell transition from G1 to S phase. E2F is inhibited by the tumour suppressor gene Rb (retinoblastoma) which can be indirectly activated by p53. The synthesis and degradation of different cyclins (not shown) stimulates the cell to pass through the different phases of the cell cycle. The growth factors may also suppress apoptosis by activating protein kinase B.
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

Fig. 2.4 The production of erythropoietin by the kidney in response to its oxygen (O₂) supply. Erythropoietin stimulates erythropoiesis and so increases O₂ delivery. (From A.J. Erslev and F. Gebuzda 1985.)
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
RETILOCYTOCYTE

- 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

- = Retic % x RBC Count
  - eg 0.01 x 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>
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<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
HYPOPROLIFERATIVE ANEMIAS

Maturation Disorders

Hemolytic Anemias
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

- Serum Haptoglobin
- Hemoglobinuria
- Urine Hemosiderin

Acute Hemolytic Event
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