Hemolytic Anemias

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Anemia

- Reduced red cell mass below the normal limit for age and sex of patient
  - In practice, a hemoglobin level below the normal limit for age and sex of patient
Classifications of Anemias

- **Morphological classification** - Based on size of RBCs and their hemoglobin content
  - Normocytic vs Microcytic vs Macrocytic
  - Normochromic vs Hypochromic

  NOTE: The morphological classification suggests an etiologic differential which is confirmed by additional tests

- **Etiological Classification**
  - Decreased Hgb and/or RBC production
  - Defects of red cell survival *(Hemolytic Anemias)*
# Automated Blood Count

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEMOGLOBIN</td>
<td>15.5</td>
<td>g/dL</td>
<td>[13.7-17.3]</td>
</tr>
<tr>
<td>HEMATOCRIT</td>
<td>0.46</td>
<td>L/L</td>
<td>[0.39-0.49]</td>
</tr>
<tr>
<td>RED BLOOD CELL COUNT</td>
<td>4.95</td>
<td>X10^12</td>
<td>[4.37-5.74]</td>
</tr>
<tr>
<td>MCH</td>
<td>31.3</td>
<td>pg</td>
<td>[26.5-34.0]</td>
</tr>
<tr>
<td>MCHC</td>
<td>33.4</td>
<td>%</td>
<td>[31.5-36.3]</td>
</tr>
<tr>
<td>RDW-CV</td>
<td>13.4</td>
<td>%</td>
<td>[11.5-14.5]</td>
</tr>
<tr>
<td>MCV</td>
<td>94</td>
<td>fL</td>
<td>[80-98]</td>
</tr>
<tr>
<td>NUCLEATED RBC %</td>
<td>0.0</td>
<td>/100WC</td>
<td></td>
</tr>
<tr>
<td>NUCLEATED RBC COUNT</td>
<td>0.00</td>
<td>X10^9</td>
<td>[0.00-0.00]</td>
</tr>
<tr>
<td>PLATELET COUNT /L</td>
<td>171</td>
<td>X10^9</td>
<td>[150-450]</td>
</tr>
<tr>
<td>WHITE BLOOD CELL COUNT</td>
<td>4.5</td>
<td>X10^9</td>
<td>[3.2-9.8]</td>
</tr>
<tr>
<td>NEUTROPHIL %</td>
<td>60.2</td>
<td>%</td>
<td>[37.0-80.0]</td>
</tr>
<tr>
<td>LYMPHOCYTE %</td>
<td>26.8</td>
<td>%</td>
<td>[10.0-50.0]</td>
</tr>
<tr>
<td>MONOCYTE %</td>
<td>10.1</td>
<td>%</td>
<td>[0.0-12.0]</td>
</tr>
<tr>
<td>EOSINOPHIL %</td>
<td>2.7</td>
<td>%</td>
<td>[0.0-7.0]</td>
</tr>
<tr>
<td>BASOPHIL%</td>
<td>0.2</td>
<td>%</td>
<td>[0.0-2.0]</td>
</tr>
<tr>
<td>NEUTROPHIL COUNT</td>
<td>2.7</td>
<td>X10^9</td>
<td>[2.0-8.6]</td>
</tr>
<tr>
<td>LYMPHOCYTE COUNT</td>
<td>1.2</td>
<td>X10^9</td>
<td>[0.6-4.2]</td>
</tr>
<tr>
<td>MONOCYTE COUNT</td>
<td>0.5</td>
<td>X10^9</td>
<td>[0.0-0.9]</td>
</tr>
<tr>
<td>EOSINOPHIL COUNT</td>
<td>0.12</td>
<td>X10^9</td>
<td>[0.00-0.70]</td>
</tr>
<tr>
<td>BASOPHIL COUNT</td>
<td>0.01</td>
<td>X10^9</td>
<td>[0.00-0.20]</td>
</tr>
</tbody>
</table>

Mean cell Hb
Mean cell Hb concentration
Red cell distribution width
Mean cell volume
### Table: Hematological Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Range</th>
<th>Decreased below lower limit =</th>
<th>Increased above upper limit =</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb g/dL</td>
<td>M: 14 - 18, F: 12 - 16</td>
<td>Anemia</td>
<td>Polycythemia</td>
</tr>
<tr>
<td>MCV in fL</td>
<td>80 - 98</td>
<td>Microcytic</td>
<td>Macrocytic</td>
</tr>
<tr>
<td>MCH in pg</td>
<td>27 - 34</td>
<td>Hypochromic</td>
<td>Hyperchromic</td>
</tr>
<tr>
<td>Reticulocyte:</td>
<td>% 0.5 – 1.5, Abs /c mm 20k – 100k</td>
<td>(usually seen in aplastic anemia or myeoldysplasia)</td>
<td>(usually seen in hemolytic anemia)</td>
</tr>
</tbody>
</table>
Use of reticulocyte count in the evaluation of anemias

- **HIGH Retic Index >2% or Absolute count >100,000/ul**
  - HIGH Production Anemias
  - RESPONSE TO BLOOD LOSS
  - HYPERSPLENISM
  - HEMOLYTIC ANEMIAS

- **Low Retic Index <2% or Absolute count <100,000/ul**
  - LOW Production Anemias
  - HYPOPROLIFERATIVE
  - MATURATION DEFECTS
Classification of Hemolytic Anemias

- Red cell abnormality
  - Hereditary
    - Hemoglobin Abnormalities
      (thalassemias, sickle cell anemia)
    - Membrane defect (spherocytosis, elliptocytosis etc)
    - Enzyme defect
      (Glucoze-6-Phosphate-Dehydrogenaze (G6PD) deficiency, Pyruvate kinase (PK) deficiency)
  - Acquired
    - Membrane abnormality-paroxysmal nocturnal hemoglobinuria (PNH)
Classification of Hemolytic anemias

- Extracorporeal factors
  - Immune hemolytic anemias
    - Autoimmune hemolytic anemia
    - Transfusion of incompatible blood
  - Nonimmune hemolytic anemias
    - Chemicals
    - Bacterial infections, parasitic infections (malaria), venoms
    - Hemolysis due to physical trauma
      - hemolytic - uremic syndrome (HUS)
      - thrombotic thrombocytopenic purpura (TTP)
      - prosthetic heart valves
  - Hypersplenism
Hereditary Anemias

- Affect over 400 million people worldwide

- Basic mechanisms
  - Reduced hemoglobin synthesis
  - Reduced life span of red cells
  - Reduced / abnormal stem cells
Basic Mechanisms in Hereditary Anemias -1

- Reduced hemoglobin synthesis
  - Quantitative defect of globin chain synthesis = **Thalassemias**
  - Note: Iron deficiency is an acquired cause of reduced Hgb synthesis
Basic Mechanisms in Hereditary Anemias -2

- Reduced life span of red cells
  - **Qualitative** detects
    - Gobin chains = **Hemoglobinopathies**
    - RBC membrane or cytoskeleton = Abnormal shape
    - RBC enzymes

- Hemolysis may occur predominantly in the spleen (=extravascular) or in the vessels (=intravascular)
  - With intravascular hemolysis,
    - Why are serum **haptoglobin** levels are reduced?
    - How can iron deficiency develop in chronic cases?
Basic Mechanisms in Hereditary Anemias -3

- Reduced / abnormal stem cells
  - Rare disorders like Diamond Blackfan syndrome and congenital dyserythropoietic anemia, etc.
Quantitative defects of Hgb production: **Thalassemia**

- Thalassemias produce hypochromic and microcytic anemia
- Etiological differential diagnosis of hypochromic/microcytic anemias
  - Iron deficiency anemia
  - Thalassemia
  - Anemia of chronic inflammation
  - Sideroblastic anemia
  - Lead poisoning
Hemoglobin

- >90% of an RBC mass is hemoglobin
- Heme – four pyroll rings and a central ferrous iron (Fe$^{2+}$)
- Globin genes – Alpha and similar chains on chromosome 11 and beta and similar chains on chromosome 16
- Hemoglobin: 4 globin chains + 4 heme molecules. Subtypes
  - Adult Hb = $\alpha_2\beta_2$
  - Fetal Hb = $\alpha_2\gamma_2$
  - Hb A2 = $\alpha_2\delta_2$
Human Globin Genes

α-Like Genes

β-Like Genes

Hemoglobins

Hb Gower 1
(ζ2ε2)

Hb Gower 2
(α2ε2)

Hb Portland
(ζ2γ2)

Hb F
(α2γ2)

Hb A2
(α2δ2)

Hb A
(α2β2)

Developmental Period

Embryonic

Fetal

Adult

Chromosome 11

Chromosome 16

Kilobases

0 10 20 30 40 50 60
Thalassemia - pathogenesis

- Genetic alterations in promoter region of globin genes cause decreased amount of globin chain synthesis
- Decreased globin >> decreased hemoglobin synthesis >> small RBC (microcytic) with less Hgb (hypochromic)
  - The thalassemias are named after the affected globin chain – Alpha thalassemia and Beta thalassemia
  - The unaffected chain is produced in relative excess
Thalassemias – Mechanisms of anemia

- Beta Thalassemias
  - Decreased synthesis of β chains – compensatory increase in γ and δ chains – increased levels of fetal Hb (α2γ2) and Hb A2 (α2δ2)
  - The excess α chain are toxic to RBC -- markedly reduced life span of RBC (= hemolytic anemia)
  - Tetramers of α chains have very high O₂ affinity – poor delivery of oxygen to tissues

Adult Hb = α2β2
Fetal Hb = α2γ2
Hb A2 = α2δ2
Thalassemias – Mechanisms of anemia

- Alpha Thalassemias
  - Alpha chains required for all types of hemoglobin.
    - Complete absence of alpha chains is incompatible with normal fetal development.
  - Excess β chains are mildly toxic to RBC (= hemolytic anemia).
  - Tetramers of γ chains (Hb Bart) and β chains (Hb H) have very high O₂ affinity – poor delivery of oxygen to tissues.

Adult Hb = α2β2
Fetal Hb = α2γ2
Hb A2 = α2δ2
Molecular Basis of Thalassemias

<table>
<thead>
<tr>
<th></th>
<th>β-Thalassemia</th>
<th>α-Thalassemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Globin Genes</strong></td>
<td>2 (1 per chromosome 11)</td>
<td>4 (2 per chromosome 16)</td>
</tr>
<tr>
<td><strong>Genetic abnormality</strong></td>
<td>Point mutations in promoter region</td>
<td>Gene deletions</td>
</tr>
<tr>
<td><strong>Molecular consequence</strong></td>
<td>Either complete absence ($\beta^0$) or reduced transcription ($\beta^+$).</td>
<td>No transcription from affected gene(s).</td>
</tr>
<tr>
<td><strong>Clinical Severity</strong></td>
<td>Depends on number of β genes affected and type of abnormality</td>
<td>Proportional to number of affected α genes (1 to 4).</td>
</tr>
</tbody>
</table>
Thalassemia: Clinical consequences

- **Pathophysiology:**
  - Anemia of varying severity (reduced Hb synthesis + Hemolysis)
  - Tissue hypoxia – increased erythropoietin
  - Hyperplasia of bone marrow
  - Consequences of treatment (↑ iron from repeated transfusions)

- **Severity of anemia varies greatly, depending on precise genetic defect**
  - Normal >> asymptomatic microcytic anemia
    >> severe anemia >> intra-uterine death
Clinical Syndromes in Thalassemias:

- **β-Thalassemia**
  (type of genetic abnormality in parenthesis. Remember, \( \beta_0 \) is complete absence of \( \beta \) chain synthesis from that allele, \( \beta^+ \) is reduced synthesis of \( \beta \) chain and \( \beta \) is normal level synthesis)

  - **Minor** (\( \beta^0/\beta \) or \( \beta^+/\beta \))
  - **Intermedia** (\( \beta^0/\beta \) or \( \beta^+/\beta^+ \))
  - **Major** (\( \beta^+/\beta^+ \) or \( \beta^0/\beta^0 \)) – Also called Cooley’s anemia

- Key to color coding: Normal -- asymptomatic microcytic anemia -- severe anemia -- fetal death
Clinical Syndromes in Thalassemias:

- **α-Thalassemia**
  (number of α genes with mutations shown in parenthesis)
  - Silent carrier state (1)
  - α-Thalassemia trait (2)
  - HbH (=β4) disease (3)
  - Hydrops fetalis (4)

- Key to color coding: Normal -- asymptomatic microcytic anemia -- severe anemia -- fetal death
1. Severe hypochromic anemia – tissue hypoxia – stunted growth etc
2. Damage to RBC by excess α chains
   a. Destruction in spleen – enlarged spleen
   b. Compensatory increase in red cell production - marrow hyperplasia
   c. Dependence on blood transfusions – iron overload - cirrhosis

A characteristic “hair on end” abnormality produced by bone marrow hyperplasia and diploetic expansion
Qualitative Hgb Change: Hemoglobinopathies

- Sickle Cell Disease:
  - Chronic hemolytic anemia characterized by sickle-shaped red cells caused by homozygous inheritance of Hemoglobin S
  - Commonest type of hereditary anemia in US

  - The sickle-cell gene occurs widely throughout Africa and in countries with African immigrant populations, some Mediterranean countries, the Middle East, and parts of India
# Prevalence of Thalassemia and Hemoglobinopathies

<table>
<thead>
<tr>
<th></th>
<th>US</th>
<th>World</th>
<th>Maximum incidence area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalassemia</td>
<td>Carriers</td>
<td>Very low</td>
<td>40 million Beta thal carriers</td>
</tr>
<tr>
<td></td>
<td>Disease</td>
<td>1,000</td>
<td>??</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mediterranean countries, Indian subcontinent, far East</td>
</tr>
<tr>
<td>HbS</td>
<td>Trait</td>
<td>2.5 million</td>
<td>&lt;1% to &gt;15%</td>
</tr>
<tr>
<td></td>
<td>Disease</td>
<td>90,000</td>
<td>“millions”</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Equatorial Africa</td>
</tr>
<tr>
<td>HbC</td>
<td>Trait</td>
<td>&gt;500,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Disease</td>
<td>~6,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Equatorial Africa</td>
</tr>
</tbody>
</table>
Sickle Cell trait = shown in orange stripes
High prevalence of malaria = shown in green
Prevalence of Alpha Thalassemia
Qualitative Hgb Changes: Hemoglobinopathies

- Usually single nucleotide difference in the coding region of the globin gene leads to single amino acid change -
  - In sickle Hgb, **valine** replaces **glutamic acid** in the 6th position of β chain
  - In Hgb C, **lysine** replaces the same glutamic acid
  - In Hgb E, lysine replaces glutamic acid at 26th position

- Over 1100 genetic variants of Hgb described
  - [http://globin.cse.psu.edu/](http://globin.cse.psu.edu/) for a comprehensive database
Hemoglobinopathies: Basic facts

- Hgb S, C, E and D are the most prevalent
  - Heterozygous state = Trait
    - Denoted – AS, AE, AD etc. Or double heterozygous state such as SC, Sickle-Thal, etc
    - Offers some protection against falciparum malaria (Sickle cell trait)
      - 8% of African Americans are heterozygous for Hgb S
    - Can increase the severity of thalassemia (E, D)
  - Homozygous state = Disease
    - Hemolytic anemia (severe in Hb SS, mild in Hb CC)
    - Microcytosis due to reduced Hgb synthesis (E)
Hemoglobinopathies: More facts

- Other effects of abnormal hemoglobins
  - Altered Oxygen affinity
  - Hemoglobin with oxidized iron (methemoglobin)
  - Unstable hemoglobins.
  - Abnormal chain termination due to “new” stop codon
    >> abnormal length of globin chain and reduced amount of globin chain (= like thalassemia).
Sickle Cell Anemia – Laboratory Findings

- Anemia-normocytic or slightly macrocytic
- Leukocytosis (chronic neutrophilia)
- Thrombocytosis-usually mild < 1000k/cmm
- Reticulocytosis
- Peripheral smear: few sickle shaped red cells, polychromatophilia, Nucleated RBC, Howell-Jolly bodies
- Hb - electrophoresis
Hemoglobin Electrophoresis

At Alkaline pH

At Acid pH

C = A2, E
S = D, G
Sickle Cell Anemia - Clinical Features

- Due to severe hemolytic anaemia
  - slow growth and development in children
  - bilirubin stones
  - congestive heart failure from chronic anemias and cardiac overload compensation

- Consequences of vaso-occlusion of the microcirculations (tissue ischemia and infarction)
  - infarction of spleen (“auto-spelnectomy”), brain, marrow, kidney, lung, aseptic necrosis of bone, and ophtalmic vascular lesions
Occlusion of Vessels

Ischemia

Pain, infarcts, ulcers, papillary necrosis

RBC Sequestration

Splenic sequestration, Acute lung syndrome, Priapism

Reduced elasticity of red cells

Hemolysis

RBC Sequestration

Jaundice

Anemia

Increased Erythropoiesis

Stunted Growth

Parvovirus

Aplasia

Anand Lagoo/Hereditary Anemias RS/5-12
Sickle Cell Anemia - Therapy

- Preventive measures:
  - Infections (penicillin prophylaxis and pneumococcal vaccination)
  - Fever
  - Dehydration
  - Acidosis
  - Hypoxemia
  - Cold exposure

- Blood transfusions for severe anemia

- New approaches to therapy
  - Activation of Hb F synthesis - 5-azacytidine
  - Antisickling agents acting on hemoglobin or membrane
  - Stem cell transplantation, including umbilical blood stem cells
Hereditary Hemolytic Anemias: Other Qualitative Defects

- Red cell membrane/ cytoskeleton
- Enzymes
Hemolytic Anemias due to RBC Membrane/ Cytoskeleton Defects

- Spherocytosis
- Elliptocytosis/Ovalocytosis
- Stomatocytosis

Increased Reticulocytes = Increased RBC production in response to hemolysis
## RBC Membrane Defects

<table>
<thead>
<tr>
<th>Condition</th>
<th>Genes involved</th>
<th>Population Affected</th>
<th>Mutation Frequency</th>
<th>Severity of Anemia</th>
<th>Splenectomy effective?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherocytosis</td>
<td>Ankyrin, Spectrin, Band 3</td>
<td>N European</td>
<td>1 in 3000</td>
<td>Mild 20%, Mod 60%, Severe 20%</td>
<td>Yes</td>
</tr>
<tr>
<td>Elliptocytosis</td>
<td>Spectrin</td>
<td>W Africa</td>
<td>1 in 50</td>
<td>No/mild 90%, Severe 10%</td>
<td>Yes</td>
</tr>
<tr>
<td>Ovalocytosis</td>
<td>Band 3</td>
<td>S Asia</td>
<td>1 in 20</td>
<td>No/minimal</td>
<td>Some</td>
</tr>
<tr>
<td>Stomatocytosis</td>
<td>Ion transporters</td>
<td>Worldwide</td>
<td>Rare</td>
<td>Mild</td>
<td>Contraindicated</td>
</tr>
</tbody>
</table>
RBC Enzyme Defects

- **G-6-PD Deficiency**
  - Affects about 10% of US black population. Also many in Africa, middle east, and south Asia – offers protection against falciparum malaria
  - Enzyme required to regenerate NADPH and glutathione, which are critical to avoid oxidative injury
  - Mutation leads to shorter half life of enzyme in RBC
  - No protein synthesis in mature RBCs. Older RBC become deficient in G-6-PD
  - Exposure to oxidative drugs or certain beans precipitates attack of hemolysis
    - Hemoglobin is oxidized and precipitates as Heinz bodies.
    - Self-limited because only older RBCs are eliminated but young ones are unaffected
Acquired Hemolytic Anemias

- **Immune:**
  - Autoimmune
  - Allo-immune
    - Transfusion
    - Feto-maternal

- **Non-immune**
  - Infections
  - Mechanical
  - Others
Autoimmune Hemolytic Anemia

- Warm AIHA:
  - Abs bind at 37 °C
  - Usually IgG and not C’ binding,
  - Extravascular hemolysis.

- Cold AIHA:
  - Abs bind 4-30 °C.
  - Usually IgM and fix C’.
  - Usually Intravascular hemolysis.
# Autoimmune Hemolytic Anemia

## Warm Antibody Type

<table>
<thead>
<tr>
<th>Type</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>Idiopathic</td>
</tr>
<tr>
<td>Secondary</td>
<td>Lymphoproliferative disease (e.g. CLL, lymphoma) Autoimmune disorders (e.g. SLE) Drugs (e.g. penicillin, quinidine or α-methyldopa)</td>
</tr>
</tbody>
</table>

## Cold Antibody Type *(less dangerous if cold exposure can be avoided)*

<table>
<thead>
<tr>
<th>Type</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Mycoplasma infection, infectious mononucleosis</td>
</tr>
<tr>
<td>Chronic</td>
<td>Lymphoproliferative diseases.</td>
</tr>
</tbody>
</table>
TEST EARLY FOR SICKLE CELL
Brief History of HbS

- 1910: Chicago physician, James B. Herrick, described patient of anemia with "sickle shaped." red cells.
- 1927, Hahn and Gillespie showed that sickling was related to low oxygen.
- 1940, Sherman noted alteration of Hgb due to low O2
- 1948, Janet Watson noted that fetal Hgb does not cause sickling
- 1948, Linus Pauling and Harvey Itano showed HbS to be different by protein electrophoresis
- 1956, Vernon Ingram and J.A. Hunt sequenced sickle hemoglobin. This made sickle cell disease the first genetic disorder whose molecular basis was known.
Percentage of persons considered anemic according to age and sex