Hemolytic Anemias

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Disclosure Information

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No speaker bureaus or ongoing consultative relationships

No discussion of unlabeled uses
Synthesis of Hemoglobin
Hematopoiesis

It all starts here
Red Blood Cells
RBC

- Biconcave disc; diameter: 7.5 um
- Shape allows deformability as the erythrocytes move through capillaries
- Normal life span 100 – 120 days
RBC Structure

**Cell membrane**
- Lipid bilayer
- Transmembrane proteins anchored to cytoskeleton
- Band 3, glycophorins

**Cytoskeleton**
- Spectrin, ankyrin, actin

**Hemoglobin**

**Others**
- Enzymes, electrolytes
RBC Metabolism

- No nucleus, mitochondria, ribosome
  - So, depends on anaerobic metabolism

- Requires continuous supply of $O_2$ for energy

- Energy needs & enzyme activities ↓ with age of RBC

- Energy supplied by glycolysis & used to:
  - Maintain membrane shape & cationic balance
  - Prevent oxidative damage (by providing reduced glutathione)
  - Provide 2,3-DPG
  - Maintain Hb in functional form
Anaerobic Glycolysis in RBC

Hexokinase
Glucosephosphate isomerase

Hexose to pentose Monophosphate Shunt produces NADPH & reduced Glutathione

Met Hb Reduction

Pyruvate Kinase
Lactate Dehydrogenase

Pyruvate

Net result 2 mols of ATP/Glucose

Glucose

G6P

F6P

F1,6DP

G3P

1,3DPG

3DPG

2DPG

PEP

Pyruvate

Lactate
Hemoglobin Molecule

- α chain
- β chain
- iron
- heme group

red blood cell
helical shape of the polypeptide molecule
Genetics of Hemoglobin
Globin Polypeptide Chains

- α – alpha
- β – beta
- γ – gamma
- δ – delta

Heme
Note: There are 2 beta globin genes and 4 alpha globin genes.
Synthesis of Hemoglobin

Beta Globin Genes

Hemoglobin Protein

Alpha Globin Genes

Chromosome 11

Chromosome 16
<table>
<thead>
<tr>
<th>Types of Hb</th>
<th>Globin Chains Synthesized</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb F</td>
<td>α2, γ2</td>
<td>(Fetal) 1%</td>
</tr>
<tr>
<td>Hb A</td>
<td>α2, β2</td>
<td>(Adult) &gt;95%</td>
</tr>
<tr>
<td>Hb A2</td>
<td>α2, δ2</td>
<td>(Adult) ≤3.5%</td>
</tr>
</tbody>
</table>
Hemoglobin

- The red substance in the Red Blood Cells
- Carries Oxygen from lungs to rest of the body
- Brings back CO$_2$ in exchange
Hemoglobin-Oxygen Dissociation Curve

\[ P_{50} = \]

Partial Pressure of Oxygen Required to Saturate 50% of Hemoglobin

HbF:

Binds less to 23DPG so ↑ affinity to O\textsubscript{2}.
Left shifted curve.
Favors O\textsubscript{2} shift
Mother → Fetus
Definition of Anemia

Hemoglobin level too low to meet cellular Oxygen demand

Hemoglobin level at least 2 SD below mean value for age, gender and race (& altitude & tanner stage)
From: Dallman, J.Pediatr. 94: 26, 1979
## Physiologic Anemia of Infancy

<table>
<thead>
<tr>
<th></th>
<th>Age (wk)</th>
<th>Hb Nadir Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term</td>
<td>12</td>
<td>9.5 gm/dl</td>
</tr>
<tr>
<td>Premature</td>
<td>6 – 8</td>
<td>7 gm/dl</td>
</tr>
</tbody>
</table>

From: Dallman, J.Pediatr. 94: 26, 1979
Physiologic Anemia

- Short RBC lifespan

- ↓ erythropoiesis
  - ↑ oxygen availability
  - ↑ 2,3 DPG; ↓ HbF levels → ↓ $O_2$ affinity
  - ↑ cardiac output, ↑ $O_2$ extraction

- Nutritional:
  - ↓ iron, folate, Vit E

Classification of Anemias

**Pathology**
- ↓ Production
- Bleeding
- Hemolysis

**RBC size**
- Microcytic
- Normocytic
- Macrocytic
Classification of Anemias
Pathologic

↓ Production
- Marrow infiltration / injury
- Nutritional deficiency
- Erythropoietin def
- Ineffective erythropoiesis

Acute Bleeding

Hemolysis
- Acquired (Extrinsic to RBC)
- Inherited (Intrinsic)
Classification of Anemias
↓ ed Production

**Marrow infiltration / injury**
- Malignancies
- Infections
- Drugs / chemicals
- BM failure syndromes (*Aplastic, DBA..*)

**Erythropoietin def**
- Renal failure

**Ineffective erythropoiesis**
- Hemoglobinopathies (*sickle cell, thalassemia..*)
Hemolytic Anemia

↑ Destruction of erythrocytes

Bone Marrow Compensatory Response

↑ Production of erythrocytes
# Classification of Hemolysis

<table>
<thead>
<tr>
<th></th>
<th><strong>Intravascular</strong></th>
<th><strong>Extravascular</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location of</strong></td>
<td>Bld vessels</td>
<td>Spleen &amp;/or Liver (RES)</td>
</tr>
<tr>
<td><strong>RBC Clearance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Antibody Type</strong></td>
<td>IgM (Occ IgG)</td>
<td>IgG, Non-autoimmune</td>
</tr>
<tr>
<td><strong>Mechanism of</strong></td>
<td>Complement or shear mediated</td>
<td>Macrophage</td>
</tr>
<tr>
<td><strong>Hemolysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lab Findings</strong></td>
<td>Hemoglobinuria</td>
<td>↑ Bilirubin</td>
</tr>
<tr>
<td></td>
<td>↓ Haptoglobin</td>
<td>↑ LDH</td>
</tr>
<tr>
<td><strong>Examples</strong></td>
<td>PCH*, PNH</td>
<td>AIHA*, HDN*, HS</td>
</tr>
</tbody>
</table>
Etiology

**Intrinsic factors**
- Membrane disorders
- Enzyme deficiencies
- Hemoglobin Disorders

**Extrinsic factors:**
- Antibodies
- Toxins
- Mechanical destruction
- Hypersplenism, DIC, other MAHAs
Clinical Presentation

- Pallor (suspect aplastic crisis)
- Icterus, jaundice
- Fatigue
- Splenomegaly
- Gallstones
- Dark urine
Intrinsic Defects

Red Cell Membrane Defect
Hereditary Spherocytosis

- Most common cause of non-immune hemolytic anemia
- Autosomal dominant 75%
  Sporadic mutations 25%
- Abnormalities of spectrin (and/or ankyrin, protein4.2, Band 3)
- Loss of membrane surface area relative to intracellular volume → Spherocytes, ↓ deformability
Clinical Manifestations of HS

- Hemolytic anemia
- Pallor, fatigue
- Jaundice (neonatal jaundice on Day 1)
- Splenomegaly
- Gallstones
- Positive family history (75%)
- Risk for Parvovirus associated aplasia
Lab Findings

- Spherocytes on peripheral smear
- ↑ Reticulocyte, Bilirubin, LDH
- ↑ Osmotic fragility
- Negative DAT
- ↑ MCHC due to relative cellular dehydration
Peripheral smear: HS
Osmotic Fragility
Graphical illustration of Ektacytometer

Yao et al, Journal of Biomechanics 2001; 34, 1501
Hereditary Elliptocytosis

- Cigar shaped RBCs (>25%)
- Autosomal dominant
- Southeast Asian Ovalocytosis (SAO) is a variant, hyperstable cells, confers malaria protection
HE: Peripheral Blood Smear
Hereditary Pyropoikilocytosis

- Rare cause of severe hemolytic anemia
- Bizarre shaped cells in smear
- Family history of HE
Ehh, What's Up, Doc?
RBC Enzyme Disorders
G6PD Deficiency

- Most common enzyme deficiency
- X linked inheritance
- Anemia: low grade, chronic or acute
- Africans have a milder variant (A)
- More severe in Mediterranean population
G6PD Deficiency

- Oxidative stress → Hemolysis
  - Infection
  - Drugs
  - Fava beans (favism)
  - Naphthelene (moth balls)

- Denatured Hb = Heinz bodies
  (Need special staining)

- Reticulocytes have higher G6PD levels, so assay after resolution of hemolytic crisis
G6PD Deficiency: Blood Smear
Pyruvate Kinase Deficiency

↓ ATP production
→ Loss of membrane stability
→ Water loss
→ Cell shrinkage
→ Hemolysis
Other Rare Enzymopathies

- Triose Phosphate Isomerase Deficiency
- Phosphoglycerate Kinase Deficiency
- Pyrimidine 5′-Nucleotidase Deficiency
  - basopholic stippling, associated with lead
- Aldolase A deficiency
Hemoglobin Disorders
Methemoglobinemia

- Normal heme group is in Fe$^{2+}$ (ferrous) state which combines with oxygen to form oxyhemoglobin

- When Hgb is oxidized it becomes Fe$^{3+}$ (Ferric) heme or Methemoglobin

- Methemoglobin **DOES NOT** bind to oxygen → poor tissue oxygen delivery

- Oxygen dissociation curve: Left shifted
Methemoglobinemia

- Congenital

- Acquired
  - Drugs: Lidocaine, Pyridium
  - Aniline dyes
  - Nitrates, nitrites (well water)
  - G6PD deficiency in oxidative stress
Methemoglobinemia

- Chocolate color blood
- Normal PO$_2$, but ↓ O$_2$ sats on Oxymetry
- MetHb levels >40-50%
  → cardiopulmonary, neurologic symptoms
- Rx: Remove cause
  Methylened blue (Contraindicated in G6PD)
Extrinsic Causes of Hemolytic Anemia
Neonatal Alloimmune Hemolytic Anemia (Erythroblastosis Fetalis)

Rh or ABO incompatibility (or other blood groups)
+ Feto-maternal hemorrhage (spontaneous, amniocentesis, abortion, trauma, external cephalic version)

→ Maternal immune response (IgG)
→ Transplacental passage of maternal alloantibody directed against fetal antigens
→ Hemolysis of fetal RBCs
→ Anemia, hyperbilirubinemia
→ Risk of hydrops fetalis, kernicterus
Extrinsic Causes of Hemolytic Anemia

- **Immune mediated**
- **Mechanical destruction**
  - Microangiopathic Hemolytic Anemia (MAHA)
  - DIC, TTP, HUS
  - Drugs: Methyldopa
  - Thermal burns
  - Toxins
  - Hypersplenism
- **Complement mediated**
  - Paroxysmal Nocturnal Hemoglobinuria
Rh Hemolytic Disease

- Rh: The most immunogenic blood groups
- Hemolysis does not occur with 1st pregnancy (Alloimmunization does occur)
- Infant’s Direct Antiglobulin Test (DAT): +
- RhIgG is given to Rh-ve mothers to prevent alloimmunization @ 28wks GA and after any invasive procedure
Direct (Coomb’s) Antiglobulin Test
Indirect Antiglobulin test
Warm Reactive Autoimmune Hemolytic Anemia (AIHA)

- IgG mediated
- Extravascular clearance via reticuloendothelial system (Spleen)
- Primary / Idiopathic
- Secondary
  - SLE, lymphoid malignancies, immunodeficiency, Evans Syndrome*
- DAT positive
- Treatment:
  - Transfusion with least incompatible blood
  - Steroids, splenectomy, IVIG, immunosuppressives
Blood Smear: AIHA
Cold Agglutinin Disease

- IgM mediated
  - IgM-RBC immune complex forms @ 4°C
  - Activates complement when warmed
- Associated with Mycoplasma, EBV
- DAT + for C3
- Intravascular hemolysis
- Treatment:
  Keep patient warm, supportive
Microangiopathic Hemolytic Anemia (MAHA)

- Vasculitic disorders
- Burns
- DIC
- TTP
- Pregnancy
- Drug: Cyclosporine, tacrolimus, cocaine
- Congenital heart disease
Blood Smear: MAHA
Mechanical fragmentation of RBCs
(Schistocytes, Spherocytes)
Thrombotic Thrombocytopenia Purpura (TTP)

Classical Pentad of:
- Fever
- MAHA
- Thrombocytopenia
- Renal dysfunction
- Neurological changes

May be congenital or autoimmune process

Treatment:
Plasmapheresis or FFP infusion with steroids, other immunosuppressants
DONOT give platelets (its like adding fuel to fire)
Paroxysmal Nocturnal Hemoglobinuria

- Hemoglobinuria due to intravascular hemolysis
- Thrombosis (intraabdominal, cerebral veins)
- Pancytopenia
- ↑ risk of Leukemia
- Lab Testing:

  Flowcytometry: Absence of PI-linked proteins CD55 & CD59 on leukocytes → complement mediated hemolysis
Wilson's Disease

- Wilson's Disease must be considered in a patient with unexplained liver disease and new hemolysis.
- Copper and ceruloplasmin levels should be obtained immediately before irreversible hepatic disease.

Clostridium Sepsis
That's all Folks!
Thank you

Questions..

See you next time...until then.
Keep reading
(thalassemia, sickle cell..)