Hemoglobinopathies
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NORMAL HEMOGLOBINS
- Consist of 2 alpha chains and 2 non alpha chains
- Hb A = α2β2
- Hb F = α 2γ2
- Hb A2 = α2δ2

Hemoglobin Variants
Altered the conformational dynamic of globin: globin interaction
Altered oxygen affinity
Altered the rheology and physiology of the red cell

Principle of Hemoglobin Electrophoresis
- Hemolysate is prepared
- Globin is run on a gel at acid and alkaline pH
- Mobility based on charge

Abnormal Hb variant
- Hemolysate run on HPLC column (cation)
- Molecule is split into heme and globin
- Globins are dissociated but not denatured
- Globins eluted based on mobility (charge difference)
- As Hb elutes, it passes through a photometer generating a chromatogram
New Born Screening

- New York State one of 43 states that test for hemoglobin variant as part of newborn screening
- NYS Newborn screening for sickle hemoglobin began in 1976

Genetics of Sickle Cell Disease

- Autosomal recessive inheritance
- 8-10% African American (AA) carry the Hb S gene
- Gene arose as evolutionary selection for resistance to falciparum malaria
- Approximately 1 in 600 AA are affected with SCD
- The offspring of two carriers has 1:4 chance being affected

Molecular Genetics

- SSD is due to the substitution of thymine for adenine in the glutamic acid DNA codon (GAG \rightarrow GTG) resulting in valine substitution at the \( \beta \)6 position
- \( \text{HbS} = \alpha_2\beta_2^{(\text{glu} \rightarrow \text{val})} \)

Hb S

- Hb S tends to aggregates (valine)
- Deoxygenated Hb S in solution forms gel causes elongation of the cells and resulting in a distorted red cell
- Sickling is also influenced by amount and type of Hb in the cell
- 2,3 DPG
Clinical Laboratory SCD

- Chronic hemolytic anemia
- Steady state Hb 5-11gm/dl
- Normochromic anemia
- Reticulocytosis
- Normal MCHC
- Decrease amount of erythropoeitin relative to degree of anemia

Table 1. Sickle Hemoglobinopathies: Neonatal Screening and Diagnostic Test Results.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Hb F (%)</th>
<th>Hb A2 (%)</th>
<th>MCV (fL)</th>
<th>Mean corpuscular volume (fL)</th>
<th>Hemoglobinopathies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancellular</td>
<td>&lt;25</td>
<td>&lt;2.5</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>FS</td>
<td>N or D</td>
<td></td>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-HPFH</td>
<td>Heterocellular</td>
<td>&lt;25</td>
<td>&gt;3.6</td>
<td>D</td>
<td>FS</td>
</tr>
<tr>
<td>Heterocellular</td>
<td>Heterocellular</td>
<td>&lt;25</td>
<td>&gt;3.6</td>
<td>D</td>
<td>FS</td>
</tr>
<tr>
<td>NA</td>
<td>&lt;15</td>
<td>&lt;25</td>
<td>N or D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSA or FS 7</td>
<td>S beta-thal</td>
<td>&lt;25</td>
<td>&gt;3.6</td>
<td>N or D</td>
<td>FSC</td>
</tr>
<tr>
<td>SC</td>
<td>SS</td>
<td>&lt;25</td>
<td>N or I</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

New Model of Vaso-occlusion

- Unlike normal red cells, sickle cells adhere to the endothelium cells in vitro
- Membrane damage induces oxygen radical generation
- Increased expression of adhesion molecules

Role of Leukocytes

- Suggestion by clinical observation
- Severity of disease
- Increased leukocytes associated with increased mortality
- Increased silent infarcts
- Neutrophils binds sickle cell in vitro

Natural History of SCD

- FIRST DECADE
  - Infection/Sepsis
  - Cerebral infarct
  - ACS
  - Arthropathy
  - Cholelithiasis
  - Priapism
  - Poor growth/Development

- SECOND DECADE
  - Chronic organ damage
  - Renal disease
  - Leg ulcers
  - Neurologic disease
  - Sudden death

Functional asplenia

- The red pulp of the spleen is hypoxic and and acidic and favors sickling.
- Sickle cells lack the deformability necessary for passage through the splenic sinus gaps.
- Sluggish flow, increased viscosity and low O2 and pH lead to chronic congestion of the red pulp and results in multiple microinfarcts and hemorrhages.
Splenic Sequestration

- Occurs when there is rapid enlargement of the spleen accompanied by an acute fall in hemoglobin.
- The mechanism for the triggering of these episodes are not clear but be accompanied by viral illnesses.
- 10-20% of children with sickle cell disease between the ages of 6 mons to 3 years will have at least one episode.

Transcranial Doppler (TCD) in SCD

- A measure of cerebral blood flow velocity.
- Abnormal TCD predicts increased risk of stroke in SCD.
- Velocity in excess of 200cm/sec is associated with increased risk of stroke.

Stroke in Sickle Cell Disease:

- Affects as many as 15-25% of patients with SS disease (rare in Hgb SC).
- Hemorrhage or thrombosis.
- Peak age for cerebral thrombosis and infarction is 7-10 years.
- Subarachnoid hemorrhage more common in the second decade.

Patho-physiology of Stroke in Sickle Cell Disease:

- Intimal and medial hyperplasia and proliferation secondary to damaged endothelial from sickle cells.
- Narrowing or complete occlusion of the cerebral vessels; anterior and and/or middle cerebral arteries.
- Multi-vessel involvement is common even in patients will unilateral findings.

Prevention of Stroke in SCD

STOP I (Stroke Prevention) Study:A randomized clinical trial of patients with abnormal TCD, compared chronic transfusion to reduce Hb S level ≤ 30% to a non transfusion arm.

- There were 92% fewer strokes in the transfusion arm compared to the non transfused arm.

STOP II

- Patients with abnormal TCD who received transfusion therapy for 36 months randomized to continue or discontinue Tx after 36 months.
- Study was halted early because of increase stroke in discontinued Tx arm.
Acute Chest Syndrome

- Pulmonary disease (ACS) is the second most common reason for admission to the hospital in patients with SCD.
- It accounts for 25-40% of premature deaths in SCD.

Indications for Episodic Transfusion

- ACS
- Surgery
- Erythroid aplasia
- Splenic/ Hepatic sequestration
- Intractable Pain
- Priapism

Patho-physiology of ACS:

Pathogenesis

Increased Permeability (Pulmonary Edema)
Vasospasm
Pulmonary Hypertension
Platelet/WBC activation
Thrombosis/Inflammation
Endothelial Injury (Cytokines)

Transfusion for SCD

- Goals
  - Improve anemia and oxygen carrying capacity
  - Reduce or prevent the occurrence of the complications
  - Reduce Hb S levels
- Methods
  - Episodic
  - Chronic
  - Exchange

Indications: Chronic Transfusion

- Primary Stroke Prevention
- Prevention of recurrent stroke
- Intractable pain
- Symptomatic anemia
- Pulmonary Hypertension/Sickle Cell Chronic Lung
- Recurrent Splenic Sequestration

Exchange Transfusion

- Advantages
  - Fast: allows emergent intervention
  - Eliminates risks of increase in viscosity and volume
  - Decreased iron loading compared to simple transfusion
- Major role
  - Treatment/prevention of life threatening events
Complications of Transfusion

• Transfusion reactions
• Iron Overload
• Alloimmunization
• Hypersplenism
• Hyperviscosity
• Transfusion transmitted infections

Pharmacological Agents that Stimulate Fetal Hemoglobin Production

• 5-Azacytidine
• Hydroxyurea
• Butyrate
• Erythropoietin
• Combinations

Why is Fetal Hemoglobin Beneficial in Sickle Cell Disease

• Hb F interferes with polymerization of Hb S in vitro.
• Infants with SCD do not have complications during the first 6 months of life.
• SCD patients from Saudi Arabia and India with high Hb F levels have milder clinical disease.
• CSSCD showed an inverse correlation between Hb F levels and clinical severity

Hydroxyurea

• Hydroxyurea (HU) is widely used in the treatment of sickle cell disease (SCD) in the US and abroad.
• The Multi-center Hydroxyurea Study (MSH), a randomized clinical trials demonstrated a reduction in frequency and severity of ACS and vaso-occlusive crisis by 30% and 50% respectively.
• Clinical benefit in the HU treated patients correlated with cyto-reductive effects

MSH follow-up Study

299 of the 500 patients enrolled in the original MSH study 9 year follow-up period

• -40% decrease in mortality in patients treated with HU for at least 3 yrs
• -28% of all deaths were pulmonary

PHT : Risk Factor of death in SCD

Gladwin et al NEJM 350:9 2004
-Prevalent in 32 % adult pts with SCD

-Development of PHT in SCD associated with anemia, chronic hemolysis and either existing cardiac or renal disease
- Hb F levels or HU therapy did not appear to have protective effect.
Summary of HU + BU Combination Therapy

Patho-physiology Thalassemia

- Disease results from and imbalance in the synthesis of alpha and beta globin chains

Molecular Genetics of Thalassemia

- There are over 200+ different thalassemia mutations.
- Beta thalassemia from a single base substitution

Beta Globin Gene Expression

- Genes arranged in the order they are expressed during development
- Developmental switching of human globin

Clinical Manifestation

- When Hb F levels fall which are normal offset by increased Hb A levels

Findings:
- Extra-medullary hematopoeisis
- Hemolysis
- Hyperbilirubinemia
- Congestive heart failure.

Types of Beta Thalassemias

<table>
<thead>
<tr>
<th>Type</th>
<th>Hb levels</th>
<th>Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta^0\beta^0$ Thal Major</td>
<td>Hb F 100%</td>
<td>Tx dependent</td>
</tr>
<tr>
<td>$\beta^+\beta^0$ Thal intermedia</td>
<td>Hb F 40-80%</td>
<td>+/- Tx</td>
</tr>
<tr>
<td>$\beta^+\beta^+$ Thal intermedia</td>
<td>Hb F 30-95%</td>
<td>+/- Tx</td>
</tr>
</tbody>
</table>
Complications of Disease

• Bone expansion
• Short stature
• Osteoporosis/Arthopathy
• Delayed puberty
• Endocrinopathy/Hypopituitary
  -IDDM, Hypothyrodism, Hypo
  -Testicular and ovarian failure
• Cardiomyopathy
• Pulmonary hypertension

Transfusion therapy

• 10-20 cc/kg every 4-6 weeks to maintain Hb in 9.5-11 gm/dl range.
• Suppression of the erythropoietic drive
• Decrease GI iron absorption, less bone demineralization and
• Splenectomy can reduce transfusion requirements.

Therapy

Transfusion:
Mainstay of the therapy to correct the anemia.
Suppress the ineffective erythropoiesis
Prevent excessive marrow expansion

Iron Balance in Thalassemia

Other Therapies for Thalassemia

• Erythropoetin
• Fetal Hb Augmentation
• Antioxidant
  Curative: BMT/HPSC

When to transfuse?

• Thal intermedia Hb levels 6-9 gm/dl
• Thal Major Hb levels 3-4 gm/dl

Hb Level that are insufficient and that lead to cardiovascular compromise, impaired linear growth and excessive marrow expansion.
Support Therapies

- Chelation Therapy
  - Subcutaneous Desferoxamine
  - Deferipone, L1
  - Deferasirox (oral chelator)
- Osteoclast replacement therapy
  - Bisphosphonates and Vitamin D
- Hormonal replacement