Data Interpretation of Hb and protein Electrophoresis

- Hemoglobin electrophoresis
  - Normal Hb
  - Pathological Hb
    - Sickle cell Anemia
    - α-thalassemia
    - β-thalassemia
- Protein electrophoresis
  - Normal
  - Pathological
    - multiple myeloma
    - other pathological profiles
Anemia

- Depletion anemia
- Production defect anemia
  - Aplastic anemia/ marrow replacement
  - Factor deficiency
    - Vitamin B12
    - Folic acid
  - Iron deficiency
  - Hemoglobinopathies

Table 3-4. Differential Diagnosis of Microcytic Hypochromic Anemia

<table>
<thead>
<tr>
<th>Condition</th>
<th>Serum Iron</th>
<th>TIBC</th>
<th>Serum Ferritin</th>
<th>FEP</th>
<th>HbA₂</th>
<th>HbF</th>
<th>RDW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>nl</td>
<td>nl-low</td>
<td>High</td>
</tr>
<tr>
<td>Alpha-thalassemia</td>
<td>High</td>
<td>nl</td>
<td>High</td>
<td>nl</td>
<td>nl</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>Beta-thalassemia</td>
<td>High</td>
<td>nl</td>
<td>High</td>
<td>nl</td>
<td>High</td>
<td>(varies)</td>
<td>high</td>
</tr>
<tr>
<td>Anemia of chronic disease</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>nl</td>
<td>nl</td>
<td>nl</td>
</tr>
<tr>
<td>Sideroblastic anemia</td>
<td>High</td>
<td>nl</td>
<td>High</td>
<td>Low</td>
<td>nl</td>
<td>High</td>
<td></td>
</tr>
</tbody>
</table>

TIBC = total iron-binding capacity; FEP = free erythrocyte protoporphyrin; nl = normal; HbA₂ = Hemoglobin A₂; HbF = Hemoglobin F; RDW = red cell distribution width.
Normal Hb in fetal stage

- Embryonic
  - Gower 1 = $\zeta_2\varepsilon_2$
  - Portland 1 = $\zeta_2\gamma_2$
  - Gower 2 = $\alpha_2\varepsilon_2$

- At birth
  - Hb A = $\alpha_2\beta_2$ (25%)
  - Hb F = $\alpha_2\gamma_2$ (75%)
Normal Hb in adult

- Hb A = $\alpha_2\beta_2$ (97%)
- Hb F = $\alpha_2\gamma_2$ (<1%)
- Hb A2 = $\alpha_2\delta_2$ (2.5%)
Pathological conditions

- Hemoglobinopathies
  - (a) Structural Hb variants
    - Substitution, addition, or deletion of one or more amino acids of the globin
  - (b) Thalassemias
    - Quantitative defect in globin chain production
  - (c) Combination of (a) and (b)
  - (d) Hereditary persistence of fetal Hb

Nomenclature of Hb variants

- HbA, HbF, and HbS were first discovered
- Additional variants start from HbC
- Too many variants were found (>500)
  - Hb with similar electrophoretic motility
  - distinguished by adding the place of discover
  - Some new Hb were named by pts' family
- New system
  - HbS B6 Glu → Val (E B6 V)
  - HbC B6 Glu → Lys (E B6 K)
Classification of Hb variants

- Amino acid substitution
  - e.g. Sickle cell anemia
- Deletion and insertion
  - e.g. thalassemia (α chain deletion)
- Unequal cross over (fusion genes)
- Chain elongation
- Frame shift variants

Table 36-2: Clinical manifestations associated with some abnormal hemoglobins

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Abnormal Hb</th>
<th>Structural change</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolytic anemia</td>
<td>H</td>
<td>αβ2 → ββ2</td>
<td>Unstable hemoglobin occurring in some forms of hemolytic anemia; precipitation of hemoglobin and hemoglobin is accelerated by certain drugs</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>β6 Glu → Val</td>
<td>Forms molecular aggregates when deoxygenated, producing sickle cell anemia in homozygotes</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>β6 Glu → lys</td>
<td>Low solubility lessens plasticity of red cells, causing hemolytic anemia in homozygotes</td>
</tr>
<tr>
<td>Carboxyhemoglobinemia</td>
<td>M</td>
<td>α58 His → Tyr</td>
<td>Methemoglobin causes cyanosis in heterozygotes; some also have evidence of hemolytic anemia</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>α87 His → Tyr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>β52 His → Tyr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kansas</td>
<td>β102 Asn → Thr</td>
<td>Decreased oxygen affinity of hemoglobin causes cyanosis in heterozygotes</td>
</tr>
<tr>
<td>Carboxyhemoglobinemia</td>
<td>I</td>
<td>α92 Arg → Gin</td>
<td>Increased oxygen affinity of hemoglobin hinders release of oxygen to tissues, causing compensatory polycythemia in heterozygotes</td>
</tr>
<tr>
<td></td>
<td>Chesapeake</td>
<td>α92 Arg → Kun</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rainier</td>
<td>β143 Thr → Cys</td>
<td></td>
</tr>
<tr>
<td>Carboxyhemoglobinemia</td>
<td>I</td>
<td>α92 Arg → Gin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bart's</td>
<td>α2,γ2,δ2 → δ4,γ4</td>
<td>Unstable hemoglobin with high oxygen affinity occurring in high concentration in all but fetuses with homozygous alpha-thalassemia</td>
</tr>
</tbody>
</table>

Note: The table is adapted from Schmidt RM, Brosious EH: Basic laboratory methods of hemoglobinopathy detection, Atlanta, 1978, Centers for Disease Control.
Clinical consequences of abnormal Hb

- Asymptomatic if they don’t interfere with Hb functions → unstable Hb
- Produce disease
  - affect the stability, shape, or function of Hb
- Homozygous for abnormal Hb
  - HbS
- Heterozygous are mostly mild
  - HbC, HbD, HbE but not HbA-HbS

Unstable Hb

- Hemolytic anemia
- Hemichrome formation
  (Heme iron form various side chain with globin)
- Inclusion body formation (Heinz bodies)
- Altered oxygen dissociation
- Altered hemoglobin stability → Increase methemoglobin (Fe^{++} → Fe^{+++}) and sufhemoglobin
- Altered solubility (Target cells)
Sickle cell anemia

- B chain 6th amino acid E → V substitution results in polymerization of deoxy form within the red cells
- The sickled-shape cells block microcirculation
- Stasis → hypoxia and ischemic infarction of liver, kidney, heart, bone, nervous system
- Hemolytic anemia or even DIC
Varying clinical severity of the different sickle syndrome

- Sickle cell trait
  - SA (30-40% HbS) (mild severity)
  - SF (70% HbS) (mild severity)
  - SC (50% HbS) (+++ severity)
- Sickle cell anemia
  - hemolytic anemia
  - aplastic crisis
  - vaso-occlusive
- Sickle cell-HbC disease
Thalassemia

- Decreased rate of globin chain production
  - thalassemia γ, ε, ζ → embryonic death
- Classification
  - Thalassemia major
  - thalassemia minor
  - thalassemia minima
- Alpha-thalassemia
- Beta-thalassemia
  - β+ or β0-thalassemia
Hemoglobin protein has two alpha subunits and two beta subunits.

- The two chromosomes #11 have one beta globin gene each (for a total of two genes).
- The two chromosomes #16 have two alpha globin genes each (for a total of four genes).
- Each alpha globin gene produces only about half the quantity of protein of a single beta globin gene.
Alpha-thalassemia

- **Defective α-chain synthesis**
  - No elevation of HbA₂ and HbF
    - (↑ in β-thalassemia)

- **Consequence of diminished α-chain synthesis**
  - Decrease production of HbA, HbF, HbA₂
  - Excess β-chain and γ-chain
  - Hb Bart’s (γ₄)
  - HbH (β₄)
The offspring that inherits the double deletion from one parent and the single from the other will have Hemoglobin H disease (Scenario 1).

The offspring who inherits no alpha genes from the parents dies in utero (Scenario 2; hydrops fetalis).

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Beta-thalassemia

- **Defective β-chain synthesis**
  - Diminished (β⁺ or β++) or absent (β⁰) of β-globin
  - elevation of HbA₂ & HbF in β-thalassemia

- **Consequence of diminished β-chain synthesis**
  - decrease production of HbA,
  - Excess γ-chain and δ-chain
  - HbF
  - HbA₂
  - No Hb Bart’s (γ₄)
  - No HbH (β₄)
Hb electrophoresis (alkaline)

- Separation on cellulose acetate (pH 8.4)
  (-) $A_2 \rightarrow S \rightarrow F \rightarrow A_1$ (+)
- Most frequently used
- Resolve most of the major Hbs (A1, A2, S, F)
- $A_2$ cannot be separated from $C$
- Cannot resolve HbD, G; and HbC, E
Hb electrophoresis (acidic)

- Separation on agarose gel (pH 6.0)
- Mostly used in confirmation
- E can be separated from C
- HbC, HbS migrate toward the Anode
- HbA, E migrate toward the cathode
Protein electrophoresis

- Multiple myeloma and Immunoglobulins
- Data of protein electrophoresis
  - Acute reaction pattern
  - Nephrotic syndrome
  - Chronic inflammation
  - Cirrhosis of liver
  - a1-antitrypsin deficiency (chronic diseases)
  - Polyclonal gammopathies
  - Hypogammaglobulinemia
  - Multiple myeloma (M spike)
Issues to be discussed

- Structure and function of Immunoglobulins
- Clonal deletion and clonal expansion
- Development of lymphocytic lineage
- Multiple myeloma
- Consequences of multiple myeloma
- Normal patterns of protein electrophoresis
- Pathological patterns
TABLE 2.1  Classes of Antibodies

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>IgE</th>
<th>IgD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy Chain</td>
<td>γ</td>
<td>µ</td>
<td>α</td>
<td>ε</td>
<td>δ</td>
</tr>
<tr>
<td>Light Chain</td>
<td>κ or λ</td>
<td>κ or λ</td>
<td>κ or λ</td>
<td>κ or λ</td>
<td>κ or λ</td>
</tr>
<tr>
<td>Molecular Formula</td>
<td>γδκε or γδκε</td>
<td>(μκλδκελκε) or (μκλδκελκε)</td>
<td>(ακδκεκε) or (ακδκεκε)</td>
<td>εκκε or εκκε</td>
<td>δκκε or δκκε</td>
</tr>
<tr>
<td>Y Structure</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Valency</td>
<td>2</td>
<td>10</td>
<td>2.4 or 6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Concentration in Serum Function</td>
<td>8–16 mg/ml Secondary response</td>
<td>0.5–2 mg/ml Primary response</td>
<td>1–4 mg/ml Protects mucous membranes</td>
<td>10–400 ng/ml Protects against parasites (?)</td>
<td>0–0.4 mg/ml</td>
</tr>
</tbody>
</table>

*n = 1, 2, or 3.
Multiple myeloma

- Maturation of plasma cells
- Proliferation of plasmacytoid lymphocytes
- Proliferation of plasma cells
- Principle of data interpretation
  - Blood
    - Hyperviscosity
    - Cryoglobulinemia
  - M spike
  - Bence Jones proteinuria
Bone marrow aspirate demonstrating plasma cells of multiple myeloma. Note the blue cytoplasm, eccentric nucleus, and perinuclear pale zone (or halo).

multiple punched-out lesions in a patient with multiple myeloma
Table 4-24. PRINCIPLES IN DIFFERENTIAL DIAGNOSIS OF MONOCLONAL GAMMOPATHIES

Establish presence of a monoclonal gammopathy by documentation of an M spike.
Serum protein electrophoresis
Urine protein electrophoresis
Serum immunoelectrophoresis
Establish nature of proliferating cell type.
Bone marrow aspirate/biopsy
Skeletal survey
Establish presence of entire or partial protein and activity of the protein.
Urine light chains
Quantitative analysis by radial immunodiffusion
Establish the degree of organ system involvement.

Serum Protein Electrophoresis

<table>
<thead>
<tr>
<th>Albumin</th>
<th>α₁</th>
<th>α₂</th>
<th>β</th>
<th>γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The multiple myeloma cell clone produces an excess of monoclonal (M proteins) and free light chain proteins. The M proteins may be recognized as IgA, IgD, IgG, IgE or IgM, depending on their heavy chain class. The light chain proteins may be designated as kappa or lambda. They may precipitate and deposit, producing organ damage. The organ most commonly affected is the kidney. When these monoclonal light chains appear in the urine, they are called Bence Jones proteins.
Routine laboratory

- Pancytopenia, abnormal coagulation, hypercalcemia, azotemia, elevated alkaline phosphatase and erythrocyte sedimentation rate, and hypoalbuminemia.
- Proteinuria, hypercalciuria, or both. Urine dipstick tests may not indicate the presence of Bence Jones proteinuria.
- All patients with suspected multiple myeloma require a 24-hour urinalysis by protein electrophoresis to determine the presence of Bence Jones proteinuria and kappa or lambda light chains.
Protein electrophoresis

- Supporting matrix
  - Molecular charge
    - Agarose, cellulose acetate
  - Molecular charge and size
    - Starch and polyacrylamide
- pH and ionic strength of buffer
  - pH 8.6 → most proteins are negative charge
  - barbital, Tris-barbital, boric acid, Tris and EDTA
- Visualization
  - Coomassie brilliant blue, Ponceus S (albumin>globulin)
  - Amido black (agarose gel), bromphenol blue,
Data interpretation of protein electrophoresis

- Protein electrophoresis
- Data interpretation of
  - Normal
  - Acute reaction pattern (2, 3)
  - Nephrotic syndrome (4)
  - Chronic inflammation (5)
  - Cirrhosis of liver (6, 7, 8)
  - a1-antitrypsin deficiency (chronic diseases) (9)
  - polyclonal gammopathies (10)
  - hypogammaglobulinemia (11)
  - Multiple myeloma (M spike) (12)
姓名 | 學號
---|---
1. Normal | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12