Proteins

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Protein Trivia

- The most abundant organic molecule in cells (50% by weight)
- About 300 proteins have been identified in plasma
- Proteins can have a MW of greater than 1 million
- Albumin is the most abundant protein in humans and contains 550 amino acids
Structure of Amino Acids

ELECTROPHORESIS
Separation of a charged particle in an electric field

Rate of migration depends on:

- Charge of the molecule
- Size and shape of the molecule
- Voltage
- Support medium
- pH and ionic strength of the buffer
Optimizing electrophoresis

• Optimal electrophoretic separations must balance speed and resolution
  – Higher voltage increases speed, but heat causes evaporation of the buffer and may denature proteins
  – Higher ionic strength (buffer) increases conductivity.

Serum Protein Electrophoresis

• Apply samples 1 uL to the agarose gel
• Electrophoresis 21°C, 650v
• Dry 54°C
• Stain - Acid Blue
• Destain - Acetic Acid
• Dry 63°C
**Serum protein electrophoresis**

- **Albumin**
  - Most abundant protein in plasma (approximately half of total protein)
    - Synthesized in liver
    - $t_{1/2} = 15-19$ days
  - Principal functions
    - Maintaining fluid balance
    - Transport Protein
Clinical significance of albumin

- Hyperalbuminemia is rare and of no clinical significance
- Hypoalbuminemia
  - Increased loss (nephrotic syndrome)
  - Decreased synthesis (nutritional deficit, liver failure)
- Analbuminemia markedly decreased rare
- Bisalbuminemia, dimeric albumin with equal intensities
**Alpha 1 Proteins**

Alpha-1-Lipoprotein-HDL

Alpha-1-Antitrypsin-

protease inhibitor that binds to and inactivates trypsin
- Deficiency leads to destruction of the alveolar walls and is associated with pulmonary deficiency
- Deficiency also seen in cirrhosis
- Alpha-1-antitrypsin is an acute phase protein and is increased in acute episodes of tissue damage

**Other \( \alpha_1 \) proteins**

- \( \alpha_1 \)-Acid glycoprotein (orosomucoid) and alpha-1 anti-chromotrypsin are acute phase proteins

- \( \alpha_1 \)-Fetoprotein (AFP)
  - Principal fetal protein, used to screen for fetal abnormalities (neural tube defects)
Alpha-2-Proteins

Alpha-2-Macroglobulin - 720 Kda –
Large non-immunoglobulin in plasma
Synthesized in the liver
  Increased levels in nephrosis because its large size prevents passage into the urine. Also there is an increase in synthesis.
  – It is not an acute phase protein

(α2) Haptoglobin

• Synthesized in the liver
• Binds to, and preserves, hemoglobin
• Low Haptoglobin levels in intravascular hemolysis
• Increased haptoglobin levels because it is an acute phase
BETA PROTEINS

Transferrin - 77 Kda –
Iron transport protein, also binds copper
  Increased in iron deficiency anemia, pregnancy and estrogen therapy
  – Decreased in acute inflammation due to decrease synthesis of transferrin by the liver
  – Negative acute phase protein

Other (β) proteins

• Beta-1 Lipoprotein 2750Kda
• Increased in nephrosis and Type II hypercholesterolemia
• C3 and C4 migrate in the β region
• Compliment proteins are decreased in genetic deficiencies, and increased in inflammation. C3 is a late acute phase protein. C3 may not be detected if the sample is kept at room temperature
• IgA
**γ Region**

- Includes immunoglobulins (IgG, IgA, IgM, IgD and IgE)

- Single sharp peak indicates a paraprotein and is associated with a monoclonal gammopathy

- A small band is indicative of MGUS

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**Gamma Region**

IgG migrates in the gamma and beta regions and is increased in infections, autoimmune and liver disease

IgM migrates in the gamma region

IgA migrates in the alpha-2, beta and gamma regions

CRP is the most sensitive indicator of an acute phase reaction (inflammation, trauma, infection)
• Other ACPs include \( \alpha_1 \)-acid glycoprotein, haptoglobin, and ceruloplasmin.
**Nephrotic Syndrome**
- Decreased albumin
- Increased $\alpha_2$-macroglobulin
- Decreased gamma globulins

**Hepatic cirrhosis**
- Decreased albumin (synthesis)
- Increased gamma globulins (polyclonal gammopathy)
- "β-γ bridging"
**Immediate response pattern**

Decrease in albumin
Increase in haptoglobin and alpha 1-proteins

**Monoclonal gammopathy**

Albumin decreased
Sharp peak in gamma region
**Serum Proteins Sample 12**  12-06-2002 12:52:57.12

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<tr>
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<td>0.2-0.4</td>
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<tr>
<td>Alpha 2</td>
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<td>0.6-1.1</td>
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<tr>
<td>Beta</td>
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<td>Gamma</td>
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**Serum Proteins Sample 22**  03-07-2005 12:17:08.22

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<td>Beta</td>
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<td>Gamma</td>
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<td>3.4</td>
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<tr>
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<tr>
<td>Total</td>
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<td>6.7</td>
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</table>

Ratio

- ALB: 0.69
IMMUNOFIXATION ELECTROPHORESIS

- Dilute samples with saline
- Apply sample 1 uL to the agarose gel
- Electrophoresis 21°C, 650 v
- Apply antisera
- Blot and dry 50°C
- Stain - Acid Violet
- Destain - Acetic Acid
- Dry 60°C
MULTIPLE MYELOMA

Multiple Myeloma - proliferation of a single clone of plasma cells that produces a monoclonal protein

Annual Incidence - 4 in 100,000
Number of cases per year - 13,000
Represents 1% of all malignant diseases
Median age at diagnosis - 65 years
Median survival - 3 years
**DIAGNOSTIC CRITERIA FOR MULTIPLE MYELOMA**

- Bone Marrow Plasmacytosis >10% of Plasma Cells
- Serum Monoclonal Protein
- End Organ Damage
  - Lytic Bone Lesions
  - Renal Insufficiency
  - Anemia
  - Increased Calcium

**Clinical Laboratory in Multiple Myeloma**

- **Biochemical** -
  - Serum monoclonal proteins
  - Polyclonal Immunoglobulin Decreased
  - Proteinuria, Bence-Jones Protein present in urine
  - BUN, Creatinine ↑
  - Calcium ↑, N

- **Hematological** -
  - Hemoglobin Decreased
  - Anemia - Normochromatic, Normocyte
  - ESR Increased
  - Rouleaux Formation
Frequency of Monoclonal Proteins in Multiple Myeloma

- IgG - 58%
- IgA - 24%
- Light Chains - 15%
- Biclonal - 2%
- IgD - 1%
## Monoclonal Gammopathy of Undetermined Significance

Defined as the presence of a serum monoclonal protein at low levels

- **Number of cases per year**: 750,000-1,000,000
- **54% Men**  **46% Women**
- **Occurs in 2% of persons over 50 years, 3% over 70 years**
- **Median age at diagnosis**: 72 years
- **Median survival**: 12 years

### Criteria

- Serum monoclonal protein <3.0 g/dL
- Stability of monoclonal protein during long term follow-up
- <10% Plasma cells in bone marrow
- None or a small amount of Bence-Jones protein in urine
- Absence of lytic bone lesions
- Serum calcium, BUN, creatinine - Normal
- Hemoglobin - Normal
CLINICAL COURSE OF 241 PATIENTS WITH MGUS

- M Protein >3.0 g/dL, No Myeloma (23) 24%
- No Increase in M Protein (46) 19%
- Developed Myeloma & Unrelated Diseases (59) 47%
- Died of Unrelated Causes (173) 47%

Distribution Frequency of Monoclonal Proteins in MGUS

- IgG 73%
- IgM 14%
- IgA 11%
- Biclonal 2%
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<td>0.6</td>
<td>0.5 - 0.7</td>
</tr>
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<td>1.0</td>
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<tr>
<td>Gamma</td>
<td>1.1</td>
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<td>Total</td>
<td>7.1</td>
<td>6.7 - 8.6</td>
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Ratio:
- A/G: 1.92

Comments:
**BANDS MISTAKEN FOR MONOCLONAL IMMUNOGLOBULINS**

<table>
<thead>
<tr>
<th>BAND</th>
<th>CONDITION</th>
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<tbody>
<tr>
<td>Alpha-2-Macroglobulin</td>
<td>Nephrotic syndrome</td>
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<tr>
<td>Hemoglobin-haptoglobin</td>
<td>Hemolysis</td>
</tr>
<tr>
<td>Beta-1-Lipoprotein</td>
<td>Hyperlipidemia</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Inadequate clot</td>
</tr>
<tr>
<td>C-Reactive Protein</td>
<td>Acute inflammation</td>
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<tr>
<td>Immune complex pattern</td>
<td>Inflammation</td>
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![Image showing a gel electrophoresis pattern with bands labeled](image-url)
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Ratio
A/G 0.84