Paraproteinaemia (PP)

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Outline

• Definition
• Incidence of paraproteinemia
• Disorders that associated with paraproteinemia
• Indication of screening for paraproteinemia
• Investigation of paraproteinemia
• MGUS, multiple myeloma
• A paraprotein is a monoclonal immunoglobulin or immunoglobulin light chain (Bence Jones protein) present in the blood or urine and arising from clonal proliferation of mature B-cells, most commonly plasma cells or B-lymphocytes.

• Alternative terms include monoclonal protein or M-band.
**Immunoglobulins**

- **Heavy Chains:**
  - IgG, IgA
  - IgM, IgD

- **Light Chains:**
  - Kappa (κ)
  - Lambda (λ)
Immunoglobulins

IgG  IgE  IgD

IgM  IgA

<table>
<thead>
<tr>
<th>Class</th>
<th>Light Chains</th>
<th>Heavy Chains</th>
<th>Tetramer</th>
<th>Molecular Weight</th>
<th>Sedimentation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>k or λ</td>
<td>γ</td>
<td>k₂γ₂ or λ₂γ₂</td>
<td>150,000</td>
<td>7 S</td>
</tr>
<tr>
<td></td>
<td>k or λ</td>
<td>α</td>
<td>(k₂α₂₃)ₙ or (λ₂α₂₃)ₙ</td>
<td>180,000 to 500,000</td>
<td>7 S, 10 S, 13 S</td>
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<tr>
<td>IgA</td>
<td>k or λ</td>
<td>µ</td>
<td>(k₂µ₂₅)ₙ or (λ₂µ₂₅)ₙ</td>
<td>950,000</td>
<td>18 S to 20 S</td>
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<tr>
<td>IgM</td>
<td>k or λ</td>
<td>δ</td>
<td>k₂δ₂ or λ₂δ₂</td>
<td>175,000</td>
<td>7 S</td>
</tr>
<tr>
<td>IgD</td>
<td>k or λ</td>
<td>ε</td>
<td>k₂ε₂ or λ₂ε₂</td>
<td>200,000</td>
<td>8 S</td>
</tr>
<tr>
<td>IgE</td>
<td>k or λ</td>
<td></td>
<td></td>
<td></td>
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</table>
## Immunoglobulins

<table>
<thead>
<tr>
<th>Name</th>
<th>Properties</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>Found in mucous, saliva, tears, and breast milk. Protects against pathogens.</td>
<td><img src="image" alt="IgA Structure" /></td>
</tr>
<tr>
<td>IgD</td>
<td>Part of the B cell receptor. Activates basophils and mast cells.</td>
<td><img src="image" alt="IgD Structure" /></td>
</tr>
<tr>
<td>IgE</td>
<td>Protects against parasitic worms. Responsible for allergic reactions.</td>
<td><img src="image" alt="IgE Structure" /></td>
</tr>
<tr>
<td>IgG</td>
<td>Secreted by plasma cells in the blood. Able to cross the placenta into the fetus.</td>
<td><img src="image" alt="IgG Structure" /></td>
</tr>
<tr>
<td>IgM</td>
<td>May be attached to the surface of a B cell or secreted into the blood. Responsible for early stages of immunity.</td>
<td><img src="image" alt="IgM Structure" /></td>
</tr>
</tbody>
</table>
Incidence of Paraproteinaemia

- Elderly > Young

- In individuals aged >50 yrs the overall incidence is (3.2%)

- Those with age>70 yrs is (5.3%)

- Men > women (4.0%:2.7%)

- African Americans > Caucasians
Consequences of paraproteinemia

- Increased serum viscosity
- High serum proteins (>TP) concentration
- Pseudo-hyponatraemia
- Bence Jones protein:
  - found in the urine of patient with B-cell malignancy
  - Consists of free monoclonal L-chains or their fragments
  - Low MW (20-40 kDa)
- Amyloidosis can also occur
Disorders associated with paraproteinemia

Mayo Clinic Reports:

- Experience
- 1684 pt with PP
- Kyle and Kumar, British
- Journal of Haematology 2007; 139, 730–743

Fig 1. Causes of 1684 cases of monoclonal gammopathy diagnosed at Mayo Clinic, 2006. Macro, Waldenström macroglobulinaemia; MGUS, monoclonal gammopathy of undetermined significance; SMM,
Disorder associated with a paraprotein

1. **Malignant B-cell disorder:**
   - Multiple myeloma (IgG, IgA, IgD, IgM or, λ,K free light chains)

2. **MGUS:**
   - Paraprotein detected with no evidence of other B-cell disorder
Indication of testing of PP

- Multiple myeloma suspected
- Both urine and blood should be screened
- Patient should be screened for presence of M-protein when presenting with any of the clinical findings listed below:
Clinical Indications for screening for M-protein

- Malaise and fatigue
- Bone disease (persistent back pain, osteopenia or lytic lesions)
- Impaired renal function
- Normochromic normocytic anaemia ± pancytopenia
- Hypercalcaemia
- Recurrent bacterial infections
- Hyperviscosity
- Nephrotic syndrome, cardiac failure, malabsorption
- Peripheral neuropathies, carpal tunnel syndrome
- Incidental persistent elevated erythrocyte sedimentation rate
Investigation of paraproteinemia

- Serum protein electrophoresis (SPE)
- Serum immunofixation electrophoresis (IFE)
- Serum free light chain (SFLCA)
- Quantitative Immunoglobulins
Serum & urine protein electrophoresis

**SPE:**
- Semi-Quantitative
- M-band quantification

**UPE:**
- Qualitative
- Requires 24 hrs urine collection

**Immunoglobulin levels:**
- IgA, IgG, IgM, IgD
Serum & urine protein electrophoresis

**Advantages:**
- Simple, cheap
- Commercially produced gels
- Multiple samples in parallel on the same gel
- Low amount of sample

**Disadvantages:**
- Low resolution
- Not fully automated
- Time consuming
- Gel must be stained to visualize
- Semi-quantitative
- Maxi. Volts used 100 v
Immunofixation (IFE)

- Permits the detection and typing of monoclonal antibodies or Immunoglobulins in serum, CSF and urine.

**Principle:**
- The method detects by precipitation: when soluble Ag is brought in contact with corresponding Ab, precipitation occurs, which may be visible only after staining.
Immunofixation (IFE)

IFE:

- Qualitative

- Identifies heavy chain (IgG, IgA, IgM, IgD, IgE)

- Identifies light chain (λ, κ protein) type
Immunofixation tends to replace protein electrophoresis

- It is faster
- It is somewhat more sensitive and reveal an immunoglobulines at lower concentration (less than 1gm /L)
- It can be partially automated
- It is more easily read and interpreted
Serum Free light chain (SFLC)

- Daily production 0.5-1 gm
- Freely filtered by glomeruli
- Reabsorbed by proximal tubules
- Half life 3-6 hrs
Figure 1  Immunoglobulin-free light chain assay. (a) Shows the location of the hidden light chain determinants in the intact immunoglobulin model. (b) Shows the location of the hidden light chain determinants in the free light chain model.
Serum Free Light Chain (SFLC)

- Assay detects only free LC
- Quantitative
- Can detect mildly increased levels of FLC even when these levels are undetectable by SPEP and IFE
- Excellent for following disease progression in MGUS, and disease response in myeloma
The Kappa/Lambda Ratio

• When the level of either kappa or lambda is very high and the other chain is normal or low, then the ratio is abnormal → myeloma is active

• If levels of both kappa and lambda are increased, the ratio maybe within the normal range → indicates a disease other than myeloma, such as poor kidney function
The Kappa/Lambda Ratio

- If the kappa and lambda levels are both within the normal range, sometimes the ratio may be abnormal → persistent low level of active myeloma with excess production of the abnormal light chains.

- Normal kappa/lambda ratio after treatment → good remission
Once a PP is found what further workup should be performed?

- Depending on clinical sings and symptoms and on types of M-protein (IgG, IgA, IgM, IgD) indentified by IFE:

**Differential Dx IgM:**

- Waldenstroms macroglobulinaemia (WM)
- Chronic lymphoid leukemia (CLL)
- Non- hodgkins lymphoma (NHL)
- Monoclonal gammopathy of undetermined significance (MGUS)
- Amyloidosis
Once a PP is found what further workup should be performed?

**Dx of high IgA:**
- Gamma A myloma
- Chronic infection
- Chronic liver disease
- Sarcoidosis
- RA
- SLE

**Dx of high IgG:**
- IgG myloma
- Chronic infection
- Chronic liver diseases
- Sarcoidosis
- Autoimmune disease
- Parasitic diseases
Disorders associated with M-protein

Patients with plasma cell disorder and a circulating paraprotein is subdivide into three groups:

- MGUS
- Asymptomatic myeloma (previously termed indolent or equivocal myeloma)
- Symptomatic myeloma
Disorders associated with M-protein

Three variables define these groups:

(1) M-protein level
(2) bone marrow plasma cell percentage
(3) the presence or absence of myeloma related organ or tissue impairment (ROTI)
Table 1  Diagnostic criteria for monoclonal gammopathy of undetermined significance (MGUS), asymptomatic myeloma and symptomatic myeloma (based upon BCSH guidelines 2005)

<table>
<thead>
<tr>
<th></th>
<th>MGUS</th>
<th>Asymptomatic myeloma</th>
<th>Symptomatic myeloma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraprotein</td>
<td>&lt;30 g/l</td>
<td>&gt;30 g/l</td>
<td>Variable*</td>
</tr>
<tr>
<td>Bone marrow clonal</td>
<td>&lt;10%</td>
<td>&gt;10%</td>
<td>&gt;10%</td>
</tr>
<tr>
<td>plasma cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bence Jones protein</td>
<td>Possible</td>
<td>Possible</td>
<td>50% of cases</td>
</tr>
<tr>
<td>Immune paresis</td>
<td>Possible</td>
<td>Probable</td>
<td>Probable</td>
</tr>
<tr>
<td>ROTI:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lytic Lesions</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Anaemia</td>
<td>Absent</td>
<td>Absent</td>
<td>66% of cases</td>
</tr>
<tr>
<td>Renal dysfunction</td>
<td>Absent</td>
<td>Absent</td>
<td>30% of cases</td>
</tr>
<tr>
<td>Hypercalcaemia</td>
<td>Absent</td>
<td>Absent</td>
<td>30% of cases</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Absent</td>
<td>Absent</td>
<td>Frequent</td>
</tr>
</tbody>
</table>

*Diagnosis made in conjunction with other features.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased calcium levels</td>
<td>Corrected serum calcium $&gt;0.25$ mmol/l above the upper limit of normal or $&gt;2.75$ mmol/l</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>Attributable to myeloma</td>
</tr>
<tr>
<td>Anaemia</td>
<td>Haemoglobin $2$ g/dl below the lower limit of normal or haemoglobin $&lt;10$ g/dl</td>
</tr>
<tr>
<td>Bone lesions</td>
<td>Lytic lesions or osteoporosis with compression fractures (MRI or CT may clarify)</td>
</tr>
<tr>
<td>Other</td>
<td>Symptomatic hyperviscosity, amyloidosis, recurrent bacterial infections ($&gt;2$ episodes in $12$ months)</td>
</tr>
</tbody>
</table>

CT, computed tomography; MRI, magnetic resonance imaging.
Monoclonal gammopathy of undetermined significant (MGUS)

- Usual presentation is asymptomatic elevation of total protein

Diagnosis of exclusion:

- R/O Myeloma
- R/O other plasma cell abnormalities
- the risk of progression of MGUS to myeloma or related disorder is 1% per year
Monoclonal gammopathy of undetermined significance (MGUS)

- Not found in children
- Common over the age of 50 yrs
- Risk increased 3.7 folds higher for individuals with 1st degree relative with disease
Multiple Myeloma (MM)
Multiple Myeloma

- 1% of all malignancies
- 10% of hematological malignancies (2nd most common)
- 3-4 per 100,000 population
- 16,000 new cases/yr; 11,000 deaths/yr
- M>F
- Risk: radiation exposure
Symptoms

- Bone pain (70%)
- Hypercalcaemia (30%)
- Fever (15%)
- Renal failure (20%)
- Infection (10%)
- normochromic normocytic anemia (66%)
- Peripheral neuropathy
- Hyperviscosity
- Impaired coagulation

It can be summaries as **CRAB**
Paraprotein picture in MM

- Serum/urine paraprotein seen in 97% of MM at diagnosis
- 75% of MM patient have BJP in their urine
- 80% have a serum M-protein & can be identified on SPE
- >90% have an M-protein identified via serum IFE (some are hidden in other normal bands)
- 15-20% of MM produce light chains only, which may not be present in serum and presented only in urine
Types of monoclonal proteins in MM

- Based on IG isotypes with frequency parallel to normal serum percentages
  - IgG kappa (30%) or lambda (18%)
  - IgA kappa (10%) or lambda (6%)
  - Free kappa or lambda (15% to 20%)

- Bence-Jones proteins
  - IgM (< 1%)
  - IgD (<1%)
  - IgE (<1%)
Rx of Multiple Myeloma

• No cure for MM

• Median survival time:
  – Stage I
    • 60 months
  – Stage II
    • 45 months
  – Stage III
    • 30 months
Treatment option for Multiple Myeloma

- **Chemotherapy:**
- **Immunotherapy:**
  - Thalidomide (Thalomid)
  - Lenalidomide (Revlumid)
  - Bortezomib (Velcade)
- **Corticosteroids:**
  - Prednizone
- **Stem cell transplantation:**
  - Autologous
  - Allogenic
- **Radiation therapy:**
Cases

- **Case1:**
  - 71 yrs male has
  - target organ damage (anemia, renal failure and bony lytic lesion)
  - MRI showed infiltration of T5, T12, L5
  - BM: plasma cell of 30%
Serum protein electrophoresis

On agarose gel (Hydragel)

Total Protein = 102.0 g/l
A/G = 0.58

<table>
<thead>
<tr>
<th>Fraction</th>
<th>%</th>
<th>g/l</th>
<th>Normal %</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>36.7</td>
<td>37.4</td>
<td>60-71</td>
<td>43-51</td>
</tr>
<tr>
<td>Alpha 1</td>
<td>3.1</td>
<td>3.2</td>
<td>1.4-2.9</td>
<td>1-2</td>
</tr>
<tr>
<td>Alpha 2</td>
<td>7.3</td>
<td>7.4</td>
<td>7-11</td>
<td>5-8</td>
</tr>
<tr>
<td>Beta</td>
<td>8.7</td>
<td>8.9</td>
<td>8-13</td>
<td>6-9</td>
</tr>
<tr>
<td>Gamma</td>
<td>43.6</td>
<td>44.5</td>
<td>9-16</td>
<td>6-11</td>
</tr>
</tbody>
</table>
Case 2:

- 37 yrs old female diagnosed on May 2013 to have plasma cell myeloma. Presented to hospital having lower back pain. MRI done showed multiple sacral lesion.
- BM done showed 20% plasma cell with CD138 expression
THANK YOU