Hemopoietic & Lymphatic Module
Clinical Biochemistry for 2nd Year Medical Students
Discussion : Gammopathy

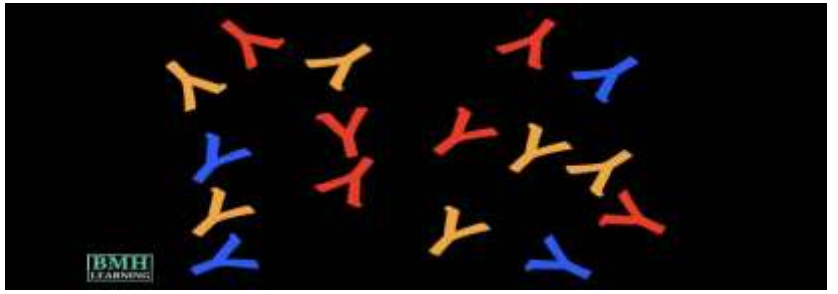
L . Dr. Raja Mohammed Ali

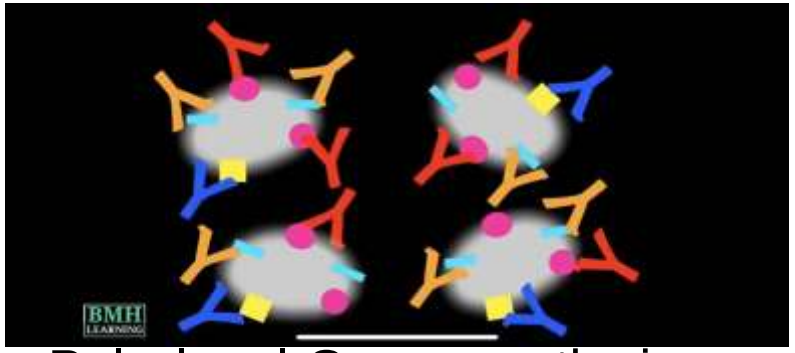
PhD. Medical chemistry

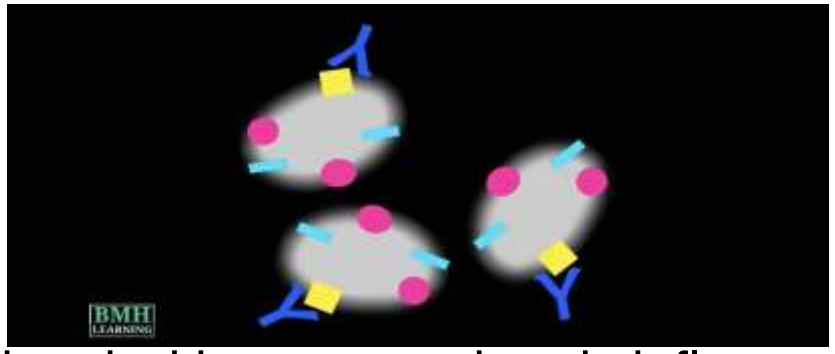
- **Objectives**
- **Gammopathy**
- **Define Gammopathy**
- **List the types and biochemical changes in Gammopathy**

What does gammopathy mean:

Gammopathy (condition or disorder) refers to over-production of one or more classes of immunoglobulin.
It may be polyclonal and monoclonal gammopathy.







- Polyclonal Gammopathy is associated with acute or chronic inflammation, such as infection, autoimmune disorders or some malignancies.
- Monoclonal gammopathy increase in a single immunoglobulin class may occur in association with normal or reduced levels of the other immunoglobulins.
- Monoclonal proteins (also called M-proteins, paraproteins or monoclonal gammopathies) occur as a feature of myeloma, lymphoma and amyloidosis, in connective tissue disease such as rheumatoid arthritis , in infection such as HIV, and in solid tumors. In addition, they may be present with no underlying disease.
- Gammopathies are detected by plasma immunoelectrophoresis.

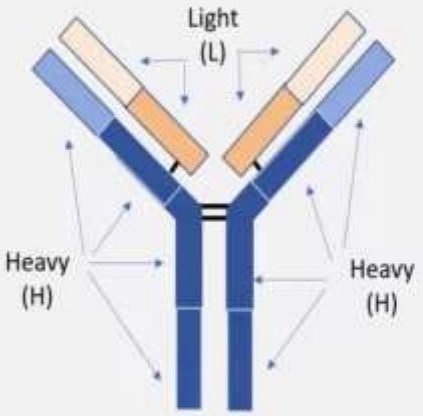
What is Hypergammaglobulinemia (polyclonal Gammopathy):

- **Liver disease, infection and autoimmune disease** gives **rise to** stimulation **of B lymphocytes** and an **increased production of γ-globulin** (**Immunoglobulins-Igs**), which on serum **protein electrophoresis** is revealed **as a broad (diffuse) band**.
- The **increase may affect all the Igs classes**, or it may **affect predominantly one class**.
- **Quantitation of the separate Ig classes** is only occasionally **helpful in diagnosis** since in **most cases the cause of the hypergammaglobulinemia is apparent**.

What is Hypergammaglobulinemia (monoclonal gammopathy):

- monoclonal – discrete – paraproteinemia

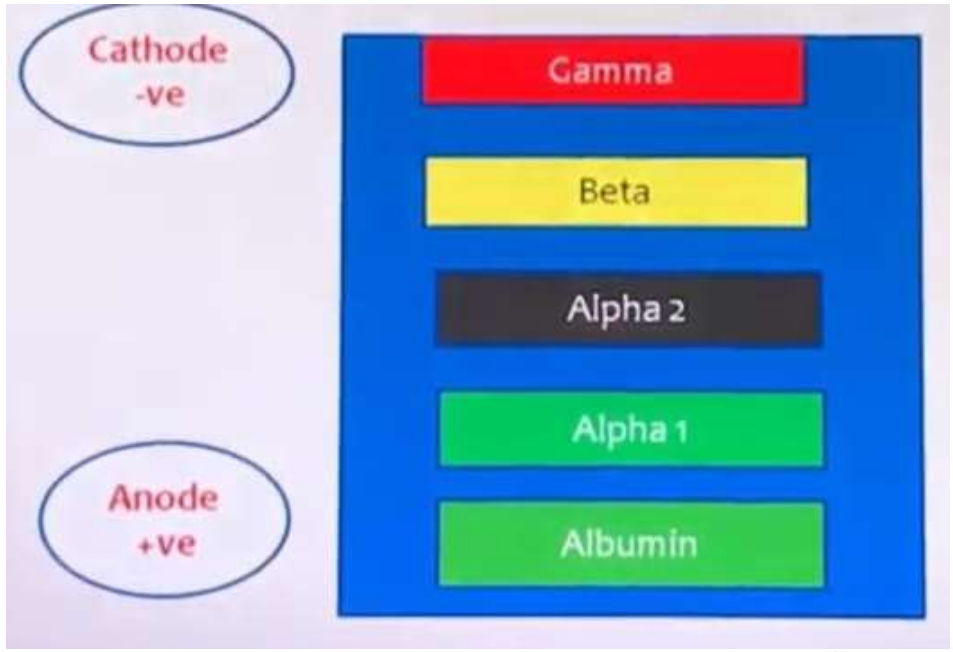
- A paraprotein is a monoclonal Ig or light chain produced by a clonal population of B cells.

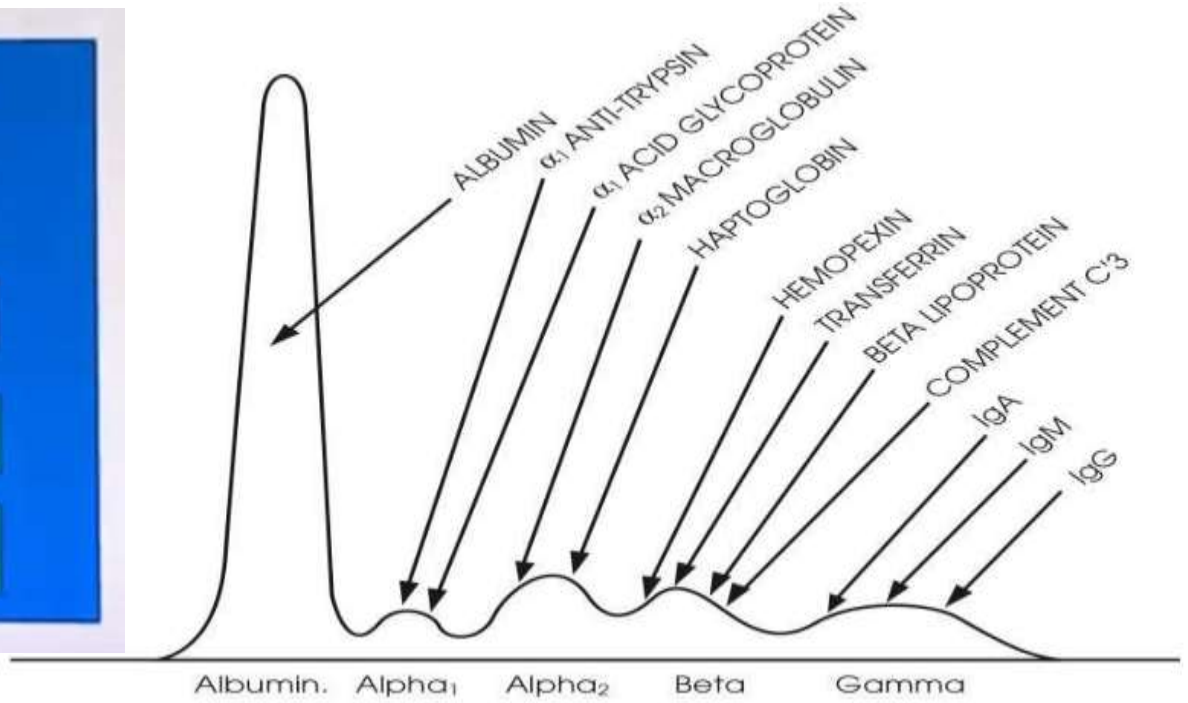


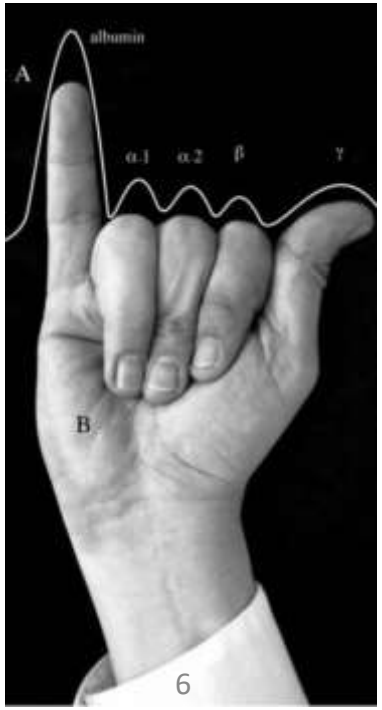
- They are often identified as a discrete Ig band on electrophoresis of serum .
- Plasma cell disorders are often associated with multiple myeloma and malignant lymphoid tumors, but benign causes are also described.
- The detection of a paraprotein in blood or urine requires further investigation to determine whether the paraproteinemia is malignant or benign .

What is Serum Protein Electrophoresis (SPEP)?

- Is a well established technique routinely used in clinical laboratories for screening of serum and some other fluids for protein abnormalities.
- It is based on separation of proteins by applying an electrical field across the medium. Proteins- negatively charged move toward the anode. The separated proteins are fixed and stained.
- Albumin carries the largest charge and therefore moves the fastest. The gamma globulins have the smallest net charge and move the least distance

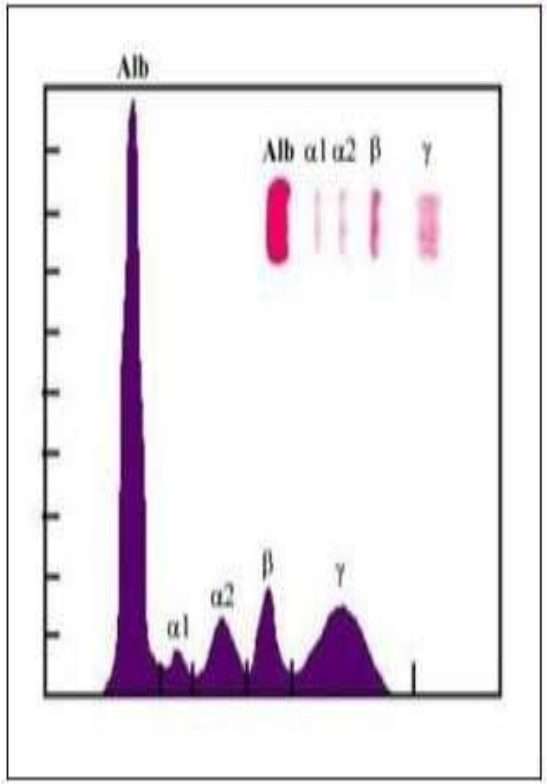






Serum proteins electrophoresis in diagnostics of diseases

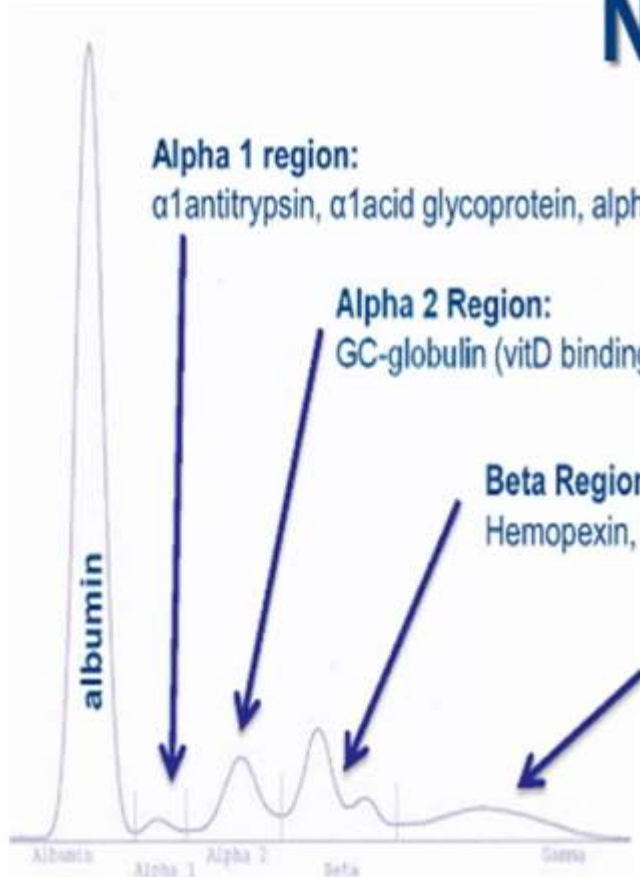
Normal pattern



Reference ranges:

Total protein	6.0 - 8.0 g/dL
Albumin	3.5 - 5.0 g/dL
α1-globulins	0.1 - 0.4 g/dL
α2-globulins	0.4 - 1.3 g/dL
β-globulins	0.6 - 1.3 g/dL
γ-globulins	0.6 - 1.5 g/dL

Normal SPE pattern

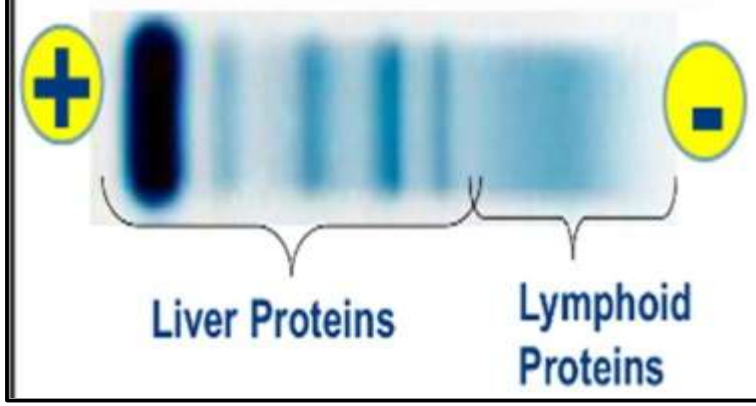


Alpha 1 region:
α1antitrypsin, α1acid glycoprotein, alpha- 1 anti- chymotrypsin, α1-Fetoprotein , SBG, TBG

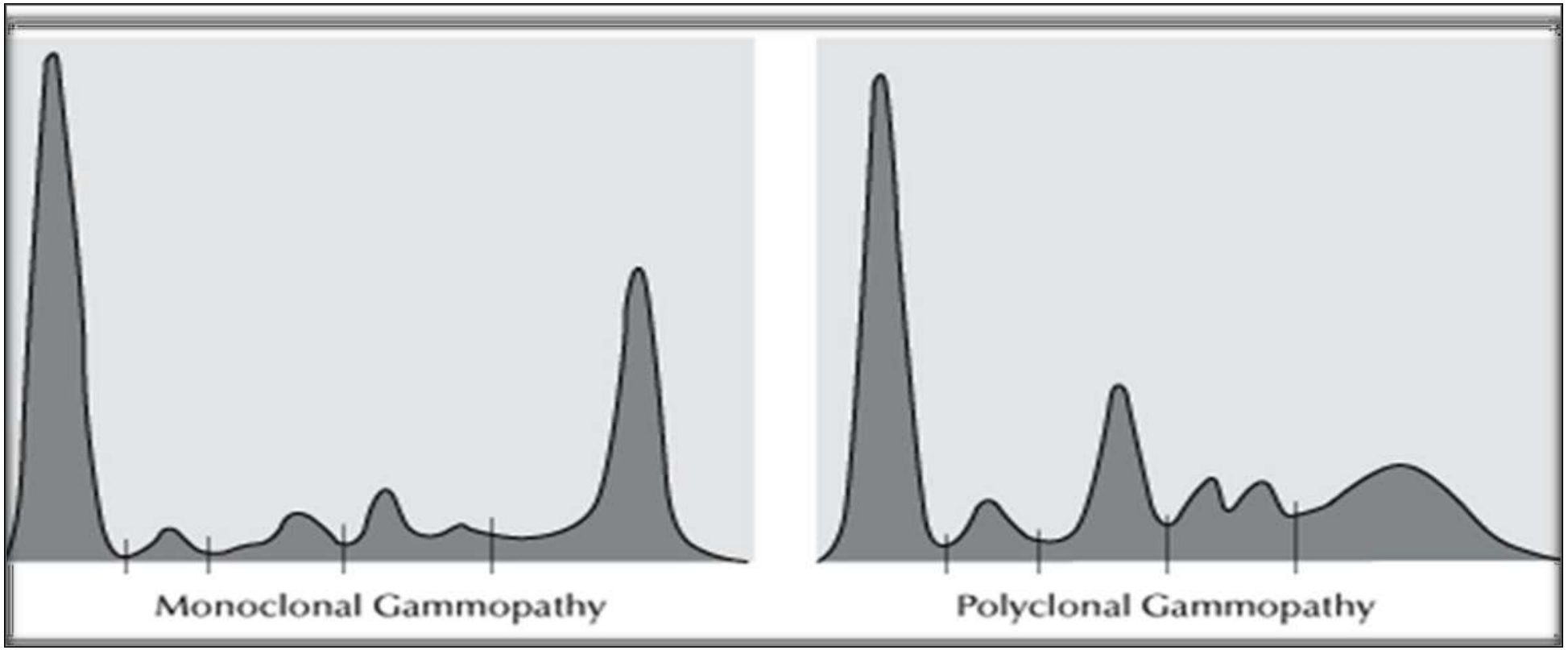
Alpha 2 Region:
GC-globulin (vitD binding), α_2 macroglobulin, haptoglobin, HDL, ceruloplasmin

Beta Region:
Hemopexin, transferrin, β-lipoprotein, LDL, C3, C4

Gamma Region:
Immunoglobulins , CRP, LDH, fibrinogen,



Location of some of the more commonly known proteins



Electrophoretic comparison between monoclonal and polyclonal gammopathy

Myeloma, lymphoid malignancies and paraproteinemia :

Malignant paraproteinemias are found in multiple myeloma and malignant lymphoid tumors .

The prevalence of paraproteinemia rises with age, and is about 3% in the geriatric population.

Multiple myeloma

Is the most common disorder associated with a paraprotein (M- protein) and is due to malignant proliferation of plasma cells. Which leads to bone destruction, impaired immune function, hyperviscosity and renal impairment.

The presenting features are given in Table1:

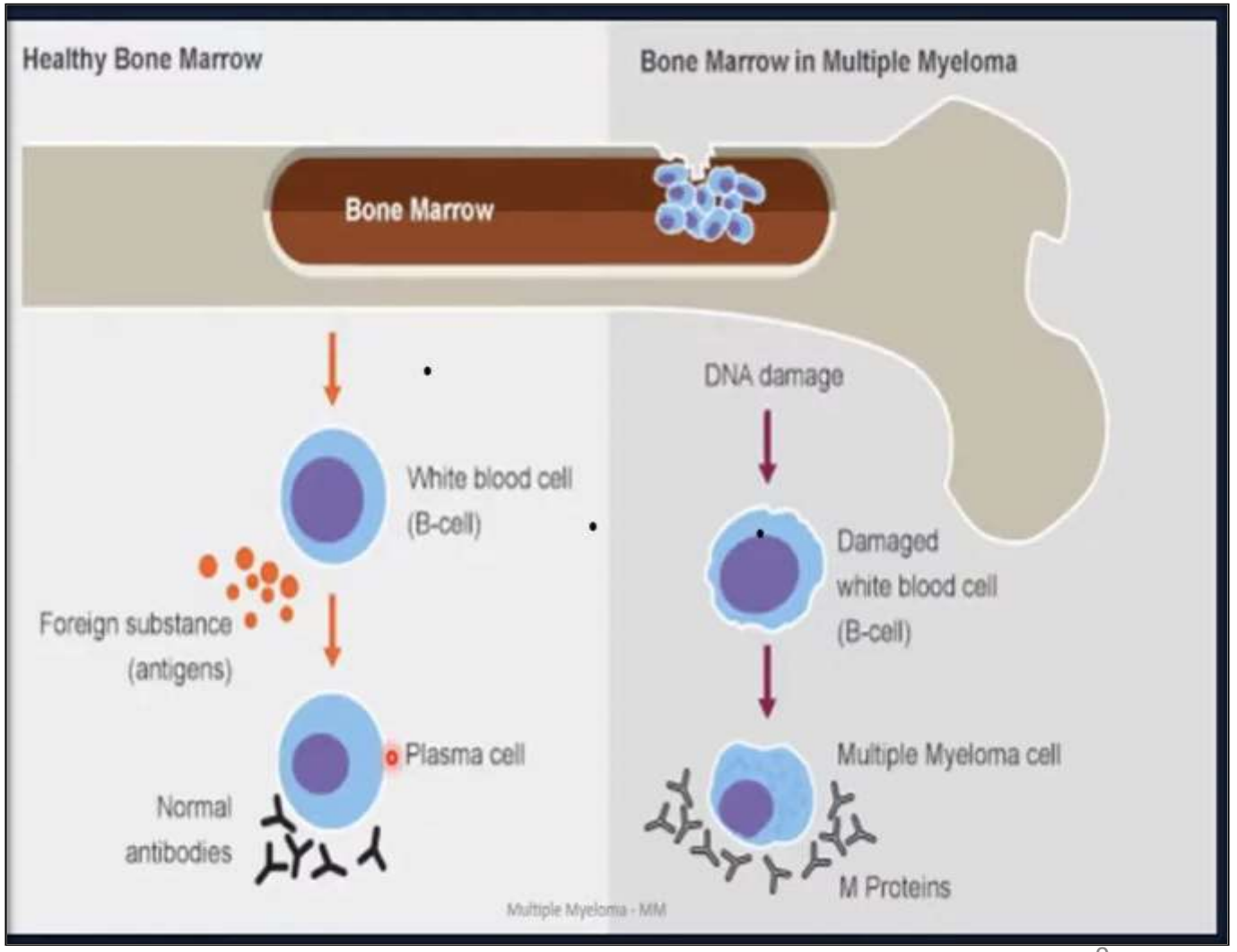


Table 1:Clinical presentation in myeloma

1. Bone pain – (with lytic areas on X-ray)
2. Impaired renal function
3. Anemia
4. Hypercalcemia
5. Recurrent Infection
6. Hyper viscosity

- Most myelomas produce complete Ig molecules, usually IgG, and the amount produced is often proportional to the tumor mass. Excessive amounts of light chains or parts of heavy chains are also produced in about 85% of cases.

- Dimers of light chains (M_r: 44 kDa) are usually found in urine, and are called '(Bence Jones proteins). In about 10–20% of cases of myeloma (usually the less
differentiated – termed 'light-chain disease' or 'Bence Jones myeloma'), excess light chains may be the only abnormality in serum.

Table 2. Recommended Testing in Patients with Suspected monoclonal gammopathy un sigineficant (MGUS).

1. History and physical examination
1. Hemoglobin concentration
1. Serum calcium and creatinine concentration
1. Protein studies • Total serum protein concentration and serum electrophoresis(serum monoclonal protein concentration) 24-hour urine protein excretion and urine electrophoresis (urine monoclonal protein concentration). Serum and urine immunofixation (Type of monoclonal protein) Determination of serum free light- chain ratio (kappa and lambda free light chains)* Examination of bone marrow aspirate ** Skeletal Survey **
* This determination is not yet standard procedure but is useful in assessing prognosis. **This is not recommended if the serum monoclonal protein concentration is below 1.5 g per deciliter

Table 2: Further investigations when myeloma has been diagnosed.

- Non-paraprotein Ig. – to assess the likelihood of intercurrent infection
- Serum [β2-microglobulin] – provides a prognostic index. Levels > 6 mg/L indicate a poor prognosis
- Serum creatinine and estimated GFR to assess renal function (RFT)
- Serum [calcium] – this may be raised due to increased release of calcium from bone
- Serum [albumin] – used for staging
- Hemoglobin and full blood count (CBC) – anemia is quite common
- Serum free light chains – for the diagnosis and monitoring of light chain disease

Investigation of suspected myeloma

- **Serum protein electrophoresis:**

shows a single discrete band, usually in the γ-globulin region but occasionally in the β-globulin or α2-globulin region. In patients in whom there is overproduction of complete Ig molecules; the concentrations of the other Igs may be reduced. Occasionally, a band due to the presence of light chains may be observed.

Plasma must not be used, since the fibrinogen band may obscure or mimic paraproteins.

Quantification of serum free light chain concentrations and the κ : λ ratio may also be helpful .

- **Urine protein electrophoresis :**

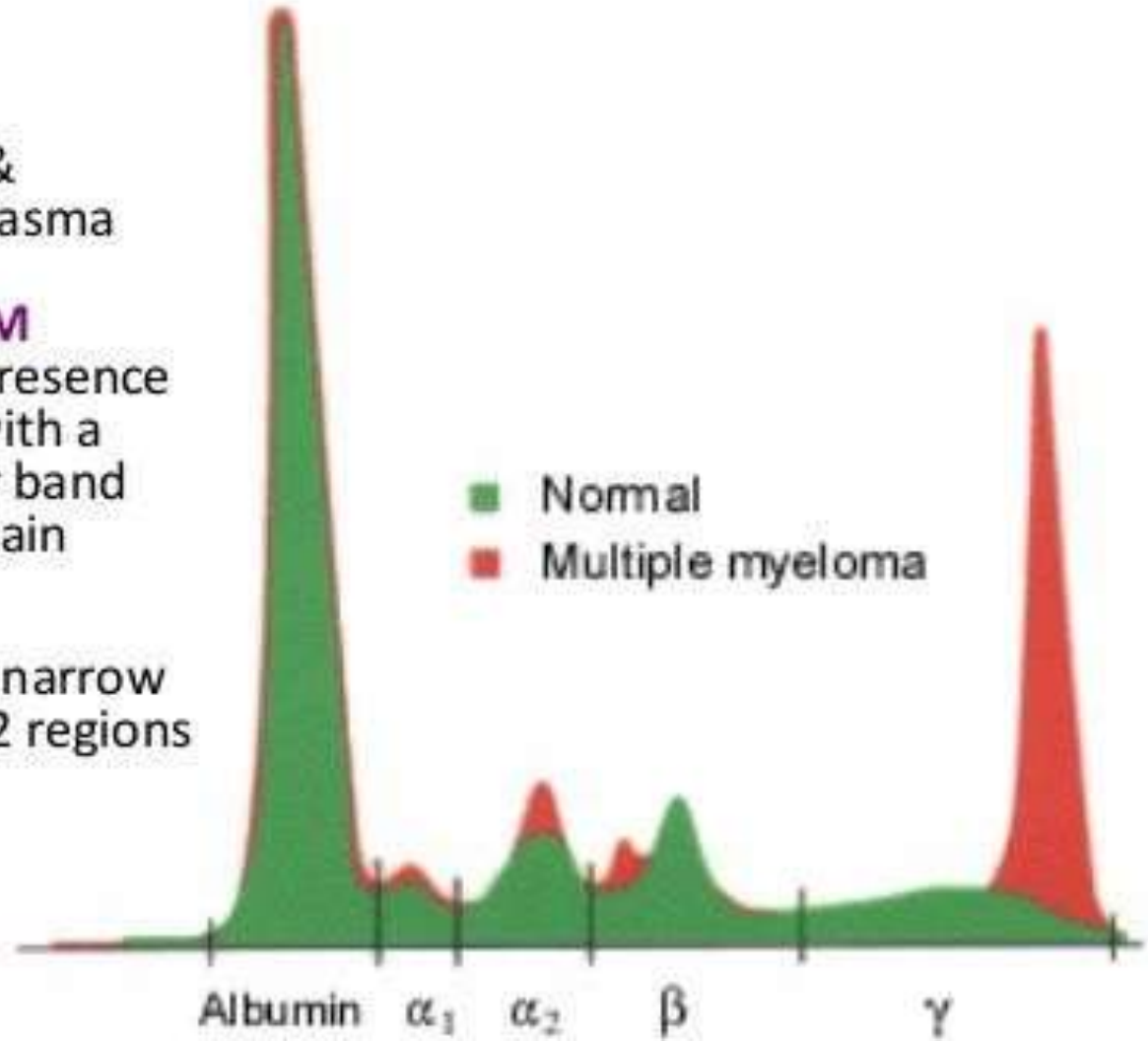
fresh early morning urine sample is needed to demonstrate Bence Jones protein; its small size (Mr 44 kDa) means that it is cleared rapidly by the kidney. If Bence Jones protein is detected, the monoclonal nature of the light chains can be confirmed by immunofixation.

In multiple myeloma, the light chains are nearly always dimers of type κ or type λ, but not a mixture of the two. Most cases of myeloma and many cases of macroglobulinaemia have Bence Jones proteinuria.

Serum Protein Electrophoresis

- In Monoclonal gammopathies & Myeloma the single clone of plasma cells produce a homogeneous monoclonal immunoglobulin **(M protein)** characterized by the presence of a sharp, well-defined band with a single heavy chain and a similar band with a kappa or lambda light chain

- The M protein is identified as a narrow peak or "spike" in the g, ß or a 2 regions



What is A Monoclonal gammopathy of unknown significance (MGUS)???

A paraprotein is found on electrophoresis in patients who have no symptoms and it may be unclear if this is due to early malignant disease or a benign disorder.

- MGUS is present in approximately 2% of individuals over 50 years of age and 3% of patients over 70.

- It is defined by a low concentration of paraprotein (<30 g/L), less than 10% of clonal bone marrow plasma cells and the absence of myeloma related organ or tissue damage .In MGUS the overall rate of progression to myeloma is in the order of 1% per year; long-term follow-up is thus required.

Diagnostic criteria for MGUS, asymptomatic myeloma and symptomatic myeloma

(International Myeloma Working Group, 2003)

MGUS	Asymptomatic myeloma	Symptomatic myeloma
M-protein in serum <30 g/l	M-protein in serum >30 g/l and/or	M-protein in serum and/or urine**
Bone marrow clonal plasma cells <10 % and low level of plasma cell infiltration in a trephine biopsy (if done)	Bone marrow clonal plasma cells >10 %	Bone marrow (clonal) plasma cells or biopsy proven plasmacytoma
No related organ or tissue impairment ((no end organ damage including bone lesions)	No related organ or tissue impairment (no end organ damage including bone lesions) or symptoms	Myeloma-related organ or tissue impairment (including bone lesions)

(Case Study 1)

A 70-year-old man complained to his doctor of back pain that he had for several months, and of feeling generally unwell. He appeared pale and he was tender over the lumbar spine. His urine contained protein (1 g/L) and his ESR was very high (90 mm in the first hour). The following abnormalities were reported: How would you interpret these results, and what further chemical investigations would you request in this patient?

Serum Result	**Reference range**
Albumin: 32	35–50 g/L
Calcium: 2.72	2.1–2.6 mmol/L
ALP :90	40–125 U/L
Creatinine: 180	60–120 μmol/L
Total protein: 84	60–80 g/L
IgA: <0.4	0.8–4.5 g/L
IgG: 37	6–15 g/L
IgM :<0.2	0.35–2.90 g/L

Comment:

- Serum and urine protein electrophoresis would both be indicated. The serum pattern showed a discrete band in the γ-globulin region, with marked reduction of the other Igs, and urine electrophoresis revealed the presence of Bence Jones protein, subsequently identified as of the λ type.
- The diagnosis of multiple myeloma was confirmed on X-ray examination (which demonstrated osteolytic lesions in the skull, vertebral column, ribs and pelvis) and by the finding of atypical plasma cells in the bone marrow. Hypercalcemia is present in about 30% of patients with multiple myeloma, and about 50% show some evidence of impaired renal function at the time of presentation; this is associated with a poor prognosis.
- Some serum paraproteins are not detected using the usual immunological methods even though they may be present in high concentration. Serum electrophoresis is thus a more reliable test than immunological methods for screening for paraproteinemia in symptomatic patients.

(Case Study 2)

72-year-old man presented to his general practitioner with back pain and weakness. The following are the results of some of his laboratory tests:

Plasma Sodium 136 mmol/L (135–145)

Potassium 4.9 mmol/L (3.5–5.0)

Urea 13.7 mmol/L (2.5–7.0)

Creatinine 160 µmol/L (70–110)

Estimated glomerular filtration rate (eGFR) 39 mL/min per 1.73 m2

Albumin-adjusted calcium 3.20 mmol/L (2.15–2.55)

Total protein 98 g/L (60–75) Albumin 34 g/L (35–45) Globulins 64 g/L (15–30)

DISCUSSION NOTE:

The impaired renal function, hypercalcemia, hypoalbuminemia and raised plasma globulins and raised total plasma protein concentration. The patient was also found to be anemic, with a hemoglobin concentration of 9.3 g/dL. Dipstick urine testing showed proteinuria. Serum and urinary protein electrophoresis showed an IgG k-paraprotein and Bence Jones proteinuria. Skeletal bone survey showed lytic bone lesions, and bone marrow biopsy suppor