Biochemistry

Plasma proteins

Hypergammaglobulinaemia: -

- Determination of individual Ig classes for diagnosis of immunodeficiency states, for hypergammaglobulineaemia, and diagnosis of allergies.
- Increase Ig levels may result from stimulation of many clones of B cells (polyclonal hypergammaglobulinaemia), few clones (oligoclonal) or monoclonal proliferation (paraproteinaemia).
- Acute and chronic infections produce wide range of antibodies (polyclonal hypergammaglobulinaemia). Also liver disease.

Multiple myeloma: a disseminated malignancy of plasma cells produces a wide range of mature Ig, usually IgG or IgA and their plasma concentration is related to the tumor mass. Also fragments of Ig including dimmers of free light chains ***(Bence – Jones protein)*** may be produced.

- Plasmacytoma, macroglobulinaemia, non – Hodgkin's lymphoma. All these malignant disease causes of paraproteinaemia where a single B – cells clone produce a single Ig called paraprotein.

Other plasma globulin which are diagnostically important plasma proteins:

Note: **Dysproteinemia:** qualitative or quantitative changes in the protein composition of serum.

1:	C – reactive protein {CRP}
	Serum amyloid A {SAA}
	α_1 – acid glycoprotein
	Haptoglobin

These proteins belong to the group of acute phase proteins. The concentration of these proteins increase in tissue injuries, the extant of elevation depends on the severity of tissue damage as well as the synthesis rate and half – life of each of these proteins.

2:	α_1 – antitrypsin (α_1 - AT)
	α_2 – macroglobulin (α_2 - M)

These proteins are proteinase inhibitors.

- α_1 – AT: belong to the group of Serine protease inhibitors (serpins) → are inactivated by forming irreversible complex with Ser proteases such as elastase, chymotrypsin, trypsin, and thrombin.
- α_1 – AT: is an acute phase protein, homozygous deficiency may be associated with emphysema ***(pulmonary disease) or liver cirrhosis or hepatitis.***
- α_2 – M: this protein together with C_1 – esterase inhibitor, regulate the formation of kinins. Decrease α_2 – M concentration are found in condition that are associated with a release of ***e.g. acute pancreatitis***.

- α_1 – AT concentration determined in serum electrophoresis in radial immunodiffusion, immunoturbidmetry, immuonephelometry.

3:	Transthyretin {TTR}
	Transferrin {Tf}
	α_1 – microglobulin { α_1 – M}

These proteins belong to the group of transport proteins.

TTR: this protein was formerly referred to as ***prealbumin***. It binds thyroids hormones and retinol – binding protein (RBP). TTR is an anti – acute phase protein whose concentration ***decrease in the presence of inflammation and protein deficient diet*.**

Tf: this iron – transporting protein is also a negative acute phase protein. Its concentration is determined in order to calculate transferrin saturation (Tfs); ***the level of Tfs is a measure of the iron availability for erythropoiesis***.

α_1 – M: the function of this protein has not been determined, its produce by the liver and to a minimal extent by lymphocytes. The determination in urine serves to assist in the ***diagnosis of tubular proteinurea***.

Carbohydrate deficiency transferrin {CDT}:

CDT represents the two isoforms of the iron transporting protein, transferrin with defective glycosylation. CDT has been shown to be more useful than any other biochemical test for alcohol abuse [this condition is usually identified on the basis of clinical judgment, alcoholism – related questionnaires and laboratory test

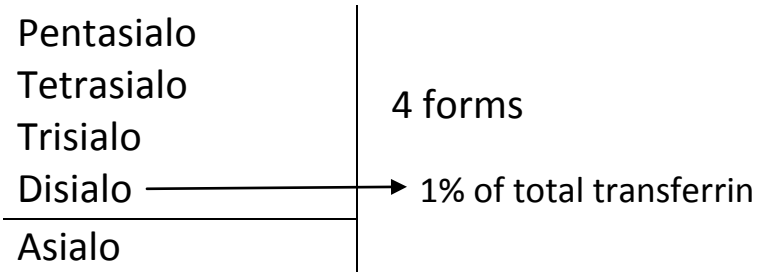
like: 6 – glutamyl transferase (6GT), Aspartate aminotransferase (AST) or mean cellular volume (MCV)].

- But more specific test is (CDT).

Isomers of transferrin: -

- There are isoforms of transferrin in a high resolution separation system such as isoelectric lowering and HPLC.
- There are 3 causes of transferrin heterogeneity: -
  - The iron content.
  - The differences in the content of sialic acid.
  - Variation of the amino acid structure of the polypeptide chain due to genetic polymorphism.
- There are 4 different forms of transferrin with respect to the iron content: -
  - Apotransferrin lacking iron.
  - Monoferric forms with iron in N – terminal.
  - Monoferric forms with iron in C – terminal.
  - Diferric transferrin.

Transferrin separated into 4 isoforms according to their approximate (pI): -



- Disialo and asialo fractions together are named carbohydrate – deficiency transferrin (CDT).

4:

C_3, C_4, C_1 – esterase inhibitors (C_1 – 1NH)

These proteins are factors of the complement system.

C_3: is represents the central component of the complement system. Cleavage of C_3 results in the formation of onaphylatoxins, opsonins, and the membrane attack complex; all of which serve an important function in the inflammatory process that is part of infection.

C_4: important component for activation of the classical pathway of the complement cascade. C_4 deficiency is the most common hereditary deficiency within the complement system.

C_1 – 1NH: this protein belongs to the group of serpins and is an inhibitor of C_1r and C_1s fragments. C_1 – 1NH deficiency is the second most common genetic defects in the complement system and result in an uncontrolled activation of the classical pathway, clinically; the picture of hereditary angioneurotic edema may occur.

5: Lipoproteins (Lp)

Water – insoluble lipids are bound to proteins or so – called apolipoproteins (apoLp); this results in the formation of water – soluble Lp particle. Changes in serum apolipoproteins concentration may be indicators of certain defects in fat metabolism.

6: Ceruloplasmin (Cp)

Cp also refer to as plasma ferroxidase, is important for transport and availability of iron in tissue. In the presence of Cp, Fe^{2+} is

oxidized to Fe^{3+}. Besides the determination of copper in serum and urine, Cp is also a parameter employed in diagnosis of Wilson's disease.

7:	Cystatin C

Cystatin C is a cationic polypeptide, because of its constant synthesis rate and free glomerular filtration, Cystatin C is an endogenous marker of the glomerular filtration rate {GFR}.

8:	Haptoglobin (Hp)
	Hemopexin (Hx)

Hp is an acute phase protein and a transporter protein. Its function is to transport intravascular, free hemoglobin to its degradation site in the reticulo – endothelial system. Hp is subject to genetic polymorphism, Hp diagnosis monitoring of hemolytic diseases.

Hx is not acute phase protein. It has high affinity towards heme derivatives and transports these substances, Hx estimation of extent of intravascular hemolysis.

9:	Cryoglobulins

Are immunoglobulins (Ig) that become insoluble at temperatures below (37℃) and dissolve on rewarming.

Indication: purpura, neurological disorders, renal disorders, arthritis, and chronic hepatitis C.

10:	Lysozyme

Lysozyme is a bacteriologic enzyme and is cell – bound in lysosomes of body cells.

Indication: -

- Early detection of renal transplant rejection.
- Differentiation of leukemias and their monitoring.
- Disease monitoring and therapy, assessment in children with urinary tract infections.
- Differentiation between bacterial and abacterial meningitis in children.

Serum protein electrophoresis (SPE)

- For medical diagnosis purposes, proteins are separated either on a cellulose acetate support into 6 classical microbands or on agarase gel where 8 – 11 bands can be separated.
- The electrophoresis patterns of these bands can be altered in various disease.

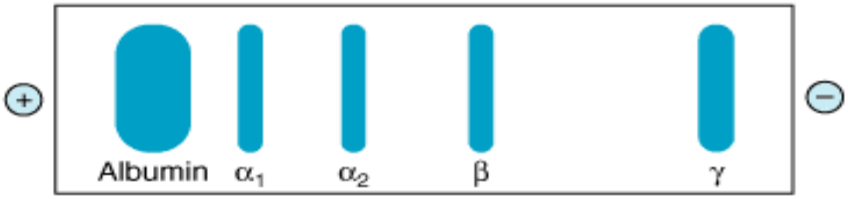
Indication: SPE used for diagnosis and disease monitoring in patients with: -

- Acute and chronic inflammatory disease.
- Protein losing syndromes (kidneys, gastrointestinal tract, skin, exudates and transudates).
- Monoclonal gammopathies.

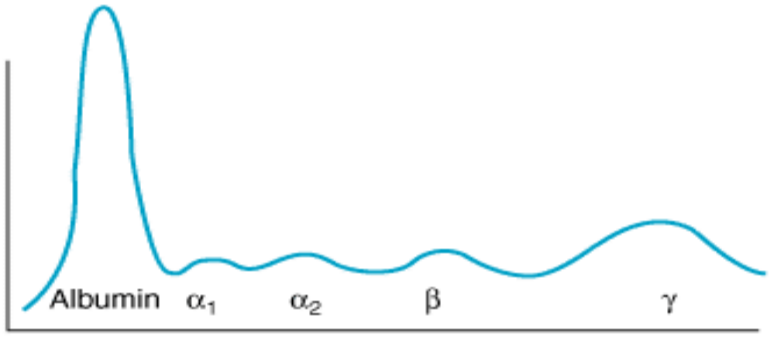
Note: electrophoresis is usually carried out using serum rather than plasma since fibrinogen (removal during clotting) which

appears as a discrete band between the β and γ regions and may thus resemble a paraprotein band.

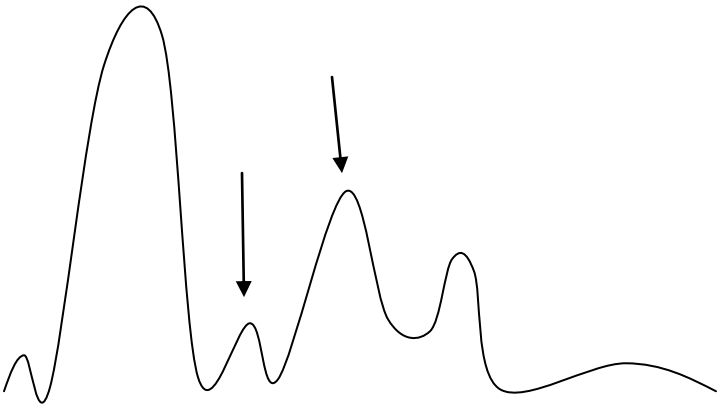
- Abnormal result in basic investigations –
  - Increase erythrocytes sedimentation rate.
  - Proteinuria.
  - Increase or decrease total protein level in serum



- α_1
  - α_1 – lipoprotein HDL
  - α_1 – glycoprotein
  - α_1 –antitrypsin
- α_2
  - α_2 – macroglobulin
  - Haptoglobin
  - Pre – β – lipoprotein
- β
  - Transferrin
  - β – Lipoprotein
  - Complement IgA
- γ
  - IgM
  - IgG



Normal SPE → normal serum protein electrophoresis in paper E

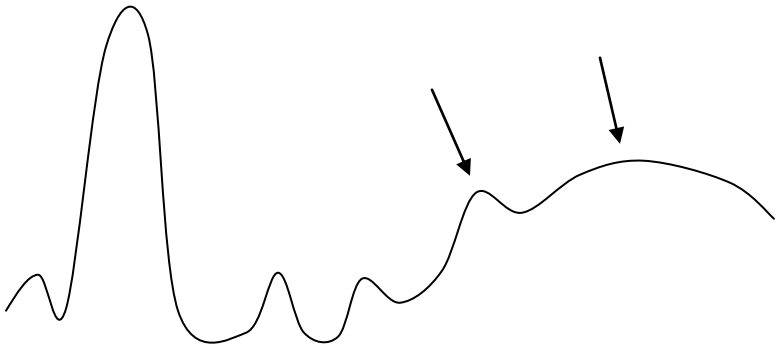


↓ Albumin
↓ α_1 – globulin
↑ α_2 – globulin
n γ – globulin

Acute inflammation

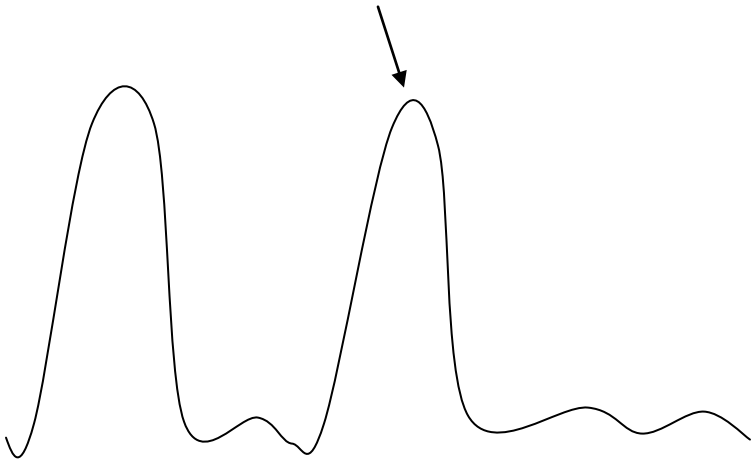
↓ Albumin
n α_1 - globulin
↑ α_2 – globulin
n β – globulin
↑ γ – globulin

Chronic inflammation



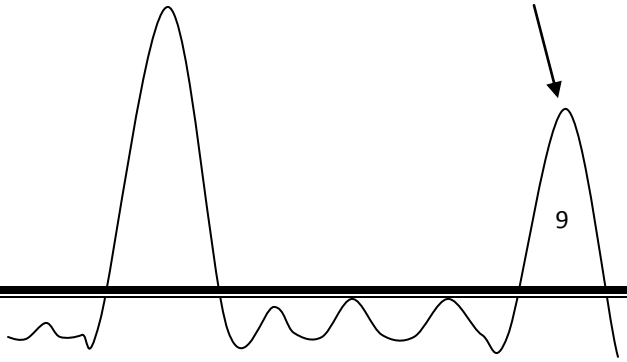
↓↓ Albumin
n α_1 - globulin
n α_2 – globulin
n β – globulin
↑↑ γ – globulin

Liver cirrhosis

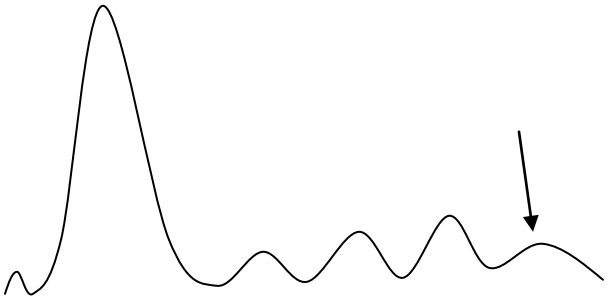


↓↓ Albumin
n α_1 - globulin
↑↑ α_2 – globulin
↑↑ β – globulin
↓ γ – globulin

Nephrotic syndrome



Monoclonal gammopathy



Antibody deficiency syndrome

Q: SPE pattern of paraproteinaemia?

Or of hypergammaglobulineaemia?