Understanding Serum Protein Electrophoresis

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Serum protein electrophoresis (SPEP) is a critical diagnostic tool in clinical laboratories, widely employed for the separation and analysis of serum proteins. Initially SPEP was done using agarose or cellulose acetate gels, but nowadays automated analysers are used which works on principle of capillary zone electrophoresis. SPEP plays an essential role in diagnosing various conditions, including monoclonal gammopathies, liver diseases, and inflammatory states. This blog will deal into the advanced principles of SPEP, its clinical applications, interpretation of results, and emerging technologies enhancing its utility.

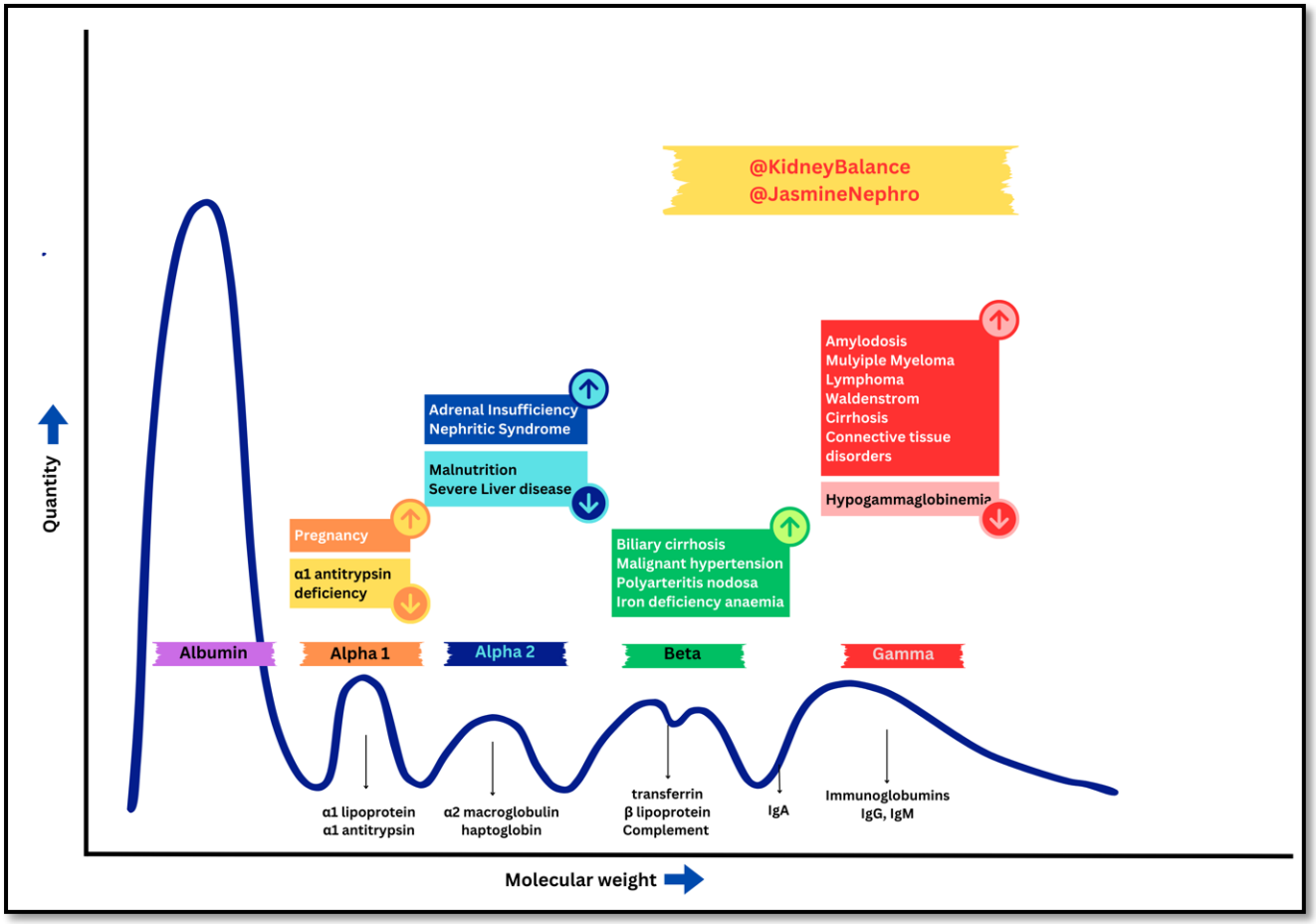
Principles of Serum Protein Electrophoresis

Serum proteins can be broadly categorized into albumin and globulins, with the latter further subdivided into alpha-1, alpha-2, beta, and gamma globulins. Further separation of different subsets of proteins can be done by immunofluorescence and immunofixation. SPEP employs an electric field to separate these proteins based on their size and charge. Proteins migrate through the gel at different rates due to their size and charge, forming distinct bands. Following electrophoresis, proteins are stained (commonly with Coomassie Brilliant Blue or Ponceau S) and visualized, allowing for band intensity and position analysis.

Components of serum protein electrophoresis

There is two major division of primary protein as interpreted by SPEP, Albumin and Globulins. Under physiological condition, Albumin is the major component. The interpretation of serum protein electrophoresis focuses on the subsets of these proteins and their relative quantities. Albumin, which appears as the largest peak, is located nearest to the positive electrode. The next five components, known as globulins, are identified as alpha1, alpha2, beta1, beta2, and gamma, with their peaks positioned closer to the negative electrode, where the gamma peak is closest. The below illustrates a typical normal distribution pattern of proteins as revealed by serum protein electrophoresis.

SPEP Interpretetion



Brief description of Components:

- ✓ **ALBUMIN-**The albumin band represents the largest protein component of human serum. In conditions like malnutrition, significant liver disease or renal loss (e.g., in nephrotic syndrome) albumin level decreases. Levels of albumin may be increased in patients with dehydration.

- ✓ **ALPHA-1 FRACTION-**The alpha1-protein fraction is comprised of alpha1-antitrypsin, alpha1 lipoprotein, thyroid-binding globulin and transcortin. Pregnancy or acute inflammation can increase the alpha1-protein band. A decreased alpha1-protein band may occur because of alpha1-antitrypsin deficiency or liver disease.

- ✓ **ALPHA-2 FRACTION-**Alpha2-macroglobulin and haptoglobin contribute to the alpha2-protein band. The alpha2 component is increased as an acute-phase reactant in Adrenal insufficiency and Nephritic syndrome. Its decreased in severe liver disease.

- ✓ **BETA FRACTION-**The beta fraction has two peaks labelled beta1 and beta2. Beta1 is composed mostly of transferrin, and beta2 contains beta-lipoprotein. IgA, IgM, and sometimes IgG, along with complement proteins, also can sometimes be identified in the beta fraction.

- ✓ **GAMMA FRACTION-**In the interpretation of SPEP, great attention focuses on the gamma region, which is predominantly composed of immunoglobulins of the IgG type. The various immunoglobulin classes (IgG, IgA, IgM, IgD and IgE) are usually make up most of the gamma band, but they can also be sometimes found in the beta-gamma and beta regions, and may occasionally even extend into the alpha2 -globulin area. Diseases that lead to an increase of gamma globulins include malignant lymphoma (multiple myeloma, Waldenström's macroglobulinemia, Hodgkin's disease, chronic lymphocytic leukemia), chronic infections, liver cirrhosis, amyloidosis, and rheumatological, granulomatous and connective tissue disorders (e.g. rheumatoid arthritis, systemiclupus erythematosus).

- ➢ **M for monoclonality-**While various conditions can lead to an increase in the gamma region, those that produce a homogeneous spike-like peak within the gamma globulin zone are particularly noteworthy. These monoclonal gammopathies represent a group of disorders characterized by the proliferation of a single, often malignant, clone of plasma cells that generate either a single class of intact immunoglobulins, heavy chains, light chains (Bence Jones protein), or a combination of these components. Collectively, these proteins are referred to as paraproteins or M (monoclonal) proteins. Multiple myeloma is the most common cause of IgA and IgG paraproteinemias, though chronic lymphocytic leukemia and lymphosarcoma can also produce M proteins, typically IgM. In Waldenström's macroglobulinemia, only IgM paraproteins are present .

- ❖ **M-band and M-Spike-**In serum protein electrophoresis, the terms "M-band" and "M-spike" are often used interchangeably but it refer to different aspects of monoclonal gammopathy.

- **M-Spike**: This term describes the distinct peak seen on the electrophoresis gel in the gamma region when there is an increase in monoclonal proteins. It appears as a narrow, sharp peak, indicating the presence of a high concentration of a single type of immunoglobulin produced by a clone of plasma cells. The M-spike is used to indicate the presence of disorders like multiple myeloma or other monoclonal gammopathies.
- **M-Band**: This term generally refers to the actual band seen on the gel that corresponds to the M-spike. It represents the area where the monoclonal proteins accumulate, visualized as a band or peak in the electrophoresis results. The M-band can be evaluated for its intensity and position, which helps in determining the specific type of paraprotein present.

Thus, M-spike indicates the presence and concentration of monoclonal proteins, while the M-band is the visual representation of these proteins on the electrophoresis gel.

Indications for ordering SPEP

- ✓ Suspected Multiple Myeloma, MGUS, MGRS, Waldenstrom's Macroglobinemia, AL Amylodosis and related disorders

- ✓ Unexplained peripheral neuropathy
- ✓ New onset anaemia with associated renal failure or bone pains
- ✓ Chronic Back pain
- ✓ Hypercalcemia attributed to possible malignancy
- ✓ Rouleaux formation noted on peripheral blood smear
- ✓ Renal insufficiency with serum protein elevation
- ✓ Unexplained pathological fractutes or lytic leisons on radiograph
- ✓ Bence Jones proteinuria
- ✓ Workup of elevated ESR

Emerging Technologies and Future Directions

While traditional SPEP remains invaluable, advancements in proteomics and mass spectrometry are paving the way for more comprehensive analyses. Techniques such as high-resolution capillary electrophoresis offer enhanced resolution and quantification capabilities. Furthermore, integrating SPEP with other biomarker analyses can provide a more holistic view of a patient's health status.

References

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