EDUCATIONAL COMMENTARY – LABORATORY EVALUATION OF IMMUNOPROLIFERATIVE DISORDERS

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**Florida licensees, please note: This exercise is NOT intended to fulfill your state education requirement for Molecular Pathology. It will fulfill requirements for Immunology.

Learning Objectives

On completion of this exercise, the participant should be able to

- describe characteristics typical of immunoproliferative disorders;
- list three categories of immunoproliferative disorders;
- describe laboratory findings in each of the four plasma cell dyscrasias discussed in this commentary;
- compare the similarities and differences between multiple myeloma and Waldenstrom macroglobulinemia;
- describe laboratory methods used to aid in diagnosis of the plasma cell dyscrasias and describe the purpose of each;
- recall examples of leukemias and lymphomas classified as immunoproliferative disorders; and
- identify laboratory methods that are used to classify the different leukemias and lymphomas.

Introduction

Immunoproliferative disorders, also known as immunoproliferative neoplasms, are disorders of the immune system that are characterized by the abnormal proliferation of the primary cells of the immune system: B lymphocytes, T lymphocytes, and natural killer cells. Immunoproliferative diseases may also be characterized by excessive production of immunoglobulins. These diseases are usually classified as leukemias, lymphomas, and plasma cell dyscrasias. They arise from a malignant genetic transformation in one cell that produces progeny that do not respond normally to growth signals and controls.

Lymphomas and leukemias are classified by morphologic features, cytochemical staining, and immunophenotype as determined with flow cytometry, cytogenetic testing, and sometimes molecular testing. Examples of leukemias and lymphomas classified as lymphoproliferative disorders are Hodgkin lymphoma, non-Hodgkin lymphoma, acute lymphocytic leukemia, chronic lymphocytic leukemia, and hairy cell leukemia.
Plasma cell dyscrasias result in an overproduction of a single immunoglobulin component by a clone of malignant plasma cells. The result is the presence of a myeloma protein, also known as *M protein* or *paraprotein*. Laboratory analysis of plasma cell dyscrasias depends on identification and quantitation of the M protein. This commentary will focus on the laboratory evaluation of plasma cell dyscrasias: multiple myeloma, Waldenstrom macroglobulinemia, heavy-chain disease, and monoclonal gammopathy of unknown significance (MGUS).

**Plasma Cells**

Plasma cells are normally found in the bone marrow in small quantities. They are rarely seen in the peripheral blood and are usually considered abnormal if present there. A plasma cell resembles a lymphocyte, but it is larger, with darker blue cytoplasm, and its nucleus is off center (eccentric). There may be a clear, non-staining area near the nucleus called a *perinuclear halo* (Figure 1). During development in the lymph nodes or bone marrow, T and B lymphocytes acquire specific receptors that give them antigen specificity. The contact and binding of specific antigens to receptors triggers a complex sequence of cellular events known as *blast transformation*. These transformed cells, called immunoblasts, may be either B or T cells, and differentiate into effector cells capable of mediating the immune response or long-lived memory cells. B immunoblasts continue to transform into plasma cells, which represent the most fully differentiated lymphocytes, and their main function is antibody production.

![Figure 1. Plasma cell showing deep blue cytoplasm, eccentric nucleus, and perinuclear halo. The red blood cells are showing rouleaux (stacking like coins).](image-url)
Plasma Cell Dyscrasias

Multiple Myeloma

Multiple myeloma, or plasma cell myeloma, is the most serious and common of the plasma cell dyscrasias. In multiple myeloma, the normal cellular elements of the bone marrow are gradually replaced by a malignant proliferation of plasma cells. The result is a large increase in serum immunoglobulins. The immunoglobulin is monoclonal and is visually represented as a tall, sharp peak on serum protein electrophoresis. The monoclonal peak is usually IgG or IgA but can rarely be IgD or IgE. Plasma cell proliferation frequently presents with bone lesions, or “punched-out” lesions, identified via radiography. The proliferation is diffuse and frequently occurs in the pelvis, spine, ribs, femur, humerus, and skull. The peripheral blood rarely exhibits neoplastic plasma cells, but rouleaux (stacking like coins) of red blood cells is usually present owing to the plasma imbalance of proteins interfering with the charge on the surface of the red blood cells (Figure 1). Anemia is common, as is decreased numbers of white blood cells and platelets. Patients usually present to their physician with symptoms of bone pain and/or pathologic fractures due to tumor infiltration. Complications include increased serum calcium due to bone lesions and kidney damage leading to renal failure due to increased plasma protein and calcium.

Waldenstrom Macroglobulinemia

Waldenstrom macroglobulinemia is a rare disease that results from a malignant proliferation of IgM-producing lymphocytes that leads to large amounts of IgM immunoglobulin in the plasma. It has been recently reclassified by the World Health Organization’s classification system as lymphoplasmacytic lymphoma because of the presence of a solid tumor in the lymphoid organs in addition to bone marrow involvement. The tumor cells may appear as small lymphocytes, plasmacytoid lymphocytes, or mature plasma cells. Usually one morphological type will predominate. Serum protein electrophoresis shows a monoclonal spike of IgM paraprotein migrating in the gamma (γ) region. The amount of IgM does not affect survival or correlate with symptoms. A decrease in numbers of white blood cells and platelets is common. Patients usually present with weakness, fatigue, anemia, bleeding, and, sometimes, hyperviscosity of serum, owing to the large IgM immunoglobulins. Hyperviscosity may lead to poor circulation in small blood vessels, visual impairment, headache, dizziness, and deafness.

Waldenstrom Macroglobulinemia vs. Multiple Myeloma

Differential diagnosis of these two diseases depends on serum protein electrophoresis followed by immunofixation to identify the type of monoclonal protein. Multiple myeloma is most commonly IgG and Waldenstrom is characterized by IgM. The presence of lytic bone lesions is characteristic of multiple myeloma and is not usually seen in Waldenstrom macroglobulinemia. The hyperviscosity frequently seen with Waldenstrom is not usually seen in multiple myeloma. The complete blood cell count values and
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Peripheral blood smear results may be similar, with both demonstrating anemia, leukopenia, and thrombocytopenia. Rouleaux of red blood cells may be seen in both disorders.

Heavy-Chain Disease
Heavy-chain disease is a rare disorder in which immunoglobulin heavy chains are produced without accompanying production and attachment to light chains, owing to genetic mutations. This disease more closely resembles a B-cell lymphoma because tumor cells are usually located in the mucosa of the small intestine. Classification is according to the type of heavy chain produced, alpha (α), gamma (γ), or mu (µ).

α-Heavy-chain disease is the most common in young adults who live in northern Africa and the Middle East. Symptoms are usually gastrointestinal, respiratory, or lymphomatous. Gastrointestinal symptoms are most common, and patients usually present with intestinal malabsorption with diarrhea, abdominal pain, and weight loss. γ-Chain disease is a very rare disorder usually appearing in persons aged between 60 and 70 years. Twenty-five percent also have an autoimmune disease such as rheumatoid arthritis. µ-Heavy-chain disease is the rarest form of heavy-chain disease; it has been diagnosed in fewer than 50 people worldwide as of 2014.

Monoclonal Gammopathy of Unknown Significance
Individuals with a monoclonal gammopathy of unknown significance (MGUS) produce a monoclonal immunoglobulin but do not have symptoms of organ damage or other laboratory findings associated with multiple myeloma or the other plasma cell dyscrasias. Monoclonal gammopathy of unknown significance is considered premalignant and may be found in 3.5% of people aged 50 and older. The International Myeloma Working Group has identified three criteria that define the presence of MGUS: a serum monoclonal protein concentration of less than 3 g/dL, a plasma cell count of less than 10% of the total cells in the bone marrow, and the absence of signs or symptoms associated with multiple myeloma, such as increased serum calcium, renal failure, anemia, and lytic bone lesions.

Laboratory Evaluation of Immunoproliferative Disorders
Serum Protein Electrophoresis
Serum protein electrophoresis (SPE) is a procedure that separates serum proteins based on their size and electric charge, resulting in five regions: albumin, α1, α2, beta (β), and γ globulins. (Figures 2-4). Figure 2 shows the distribution of proteins in normal serum. Figure 3 shows a polyclonal increase in serum proteins. There is still a broad distribution of proteins along with an increased height in the γ globulin region. An increase in total immunoglobulin without an increase in any one specific class is characteristic of nonmalignant conditions such as infections or autoimmune disease. Figure 4 shows a
monoclonal spike, indicating an increase in one specific class of immunoglobulin. This is produced by an identical clone of plasma cells and indicates malignancy.

Figure 2. Normal serum protein electrophoresis pattern. The lower band represents the distribution of proteins as they are separated on the agarose gel. The darker the band, the larger the concentration of that protein. The top part of the figure represents a densitometer tracing from the gel strip. Figure reproduced with permission of Helena Laboratories.
Figure 3. Polyclonal electrophoresis pattern showing increased height in the gamma (γ) globulin region. This type pattern is usually seen in nonmalignant conditions. Figure reproduced with permission of Helena Laboratories.
Immunofixation Electrophoresis

If an abnormality is detected on SPE, such as a monoclonal spike or a quantitative abnormality of serum immunoglobulins, or if the clinical picture suggests one of the plasma cell dyscrasias, the next step is typically immunofixation electrophoresis (IFE). Immunofixation electrophoresis is the assay of choice to identify the type of monoclonal protein present in the sample. In IFE, the patient serum is electrophoresed as in serum protein electrophoresis in six different parallel lanes on agarose gel. It is then incubated with the antiserum to IgG, IgA, IgM, and kappa (κ) and lambda (λ) light chains. The sixth lane contains whole human serum. Complexes are formed by interaction of the antisera and proteins in the patient serum. These are then stained and visualized as areas of diffuse staining that indicate polyclonal immunoglobulins and narrow intense bands that indicate monoclonal immunoglobulins (Figure 5).
Figure 5. On the left, a serum protein electrophoresis (SPEP) of a patient with a monoclonal band in the gamma region is shown. Subsequent immunofixation electrophoresis identified the monoclonal protein as an IgG antibody (paraprotein) with a lambda (λ) light chain (arrow).

**Immunoglobulin Quantitation**

Quantitation of the amount of immunoglobulin in each peak on SPE is accomplished by densitometry. The usual percentages are as follows: albumin 58% to 70%; α-1, 2% to 5%; α-2, 6% to 11%; β, 8% to 14%; and γ, 9% to 18%. The total serum protein is determined by various other methods. The reference range for total protein concentration is 6 to 8 g/dL.
Immunoglobulin quantitation of bands in IFE is usually determined by turbidometry or nephelometry. Smaller laboratories may use radial immunodiffusion. The IgG concentration range in healthy adults is 0.548 to 17.68 g/dL, IgA is 0.078 to 0.322 g/dL, and IgM is 0.045 to 0.153 g/dL.¹

**Urine Protein Electrophoresis and Immunofixation Electrophoresis**
Some patients with plasma cell dyscrasias produce an excessive amount of free monoclonal immunoglobulin light chains (Bence Jones proteins). These are rapidly cleared from the serum and may not be detectable by SPE or IFE on serum. These light chains are excreted in the urine and may be detected by similar testing on urine samples. Urine samples are collected over a 24-hour period, total protein is measured, the sample is filtered or centrifuged to remove sediment, and then it is concentrated so enough protein is present to produce visible bands on the gel. After this preparation, the procedures are like those described for serum. These free light chains may contribute to kidney damage by depositing in the glomeruli and tubules of the kidneys and by their involvement in the formation of renal casts. The International Myeloma Working Group recommends that patients with plasma cell dyscrasias be routinely monitored by urine protein electrophoresis (UPE) and urine IFE.³

**Serum Free Light-Chain Analysis**
Automated testing methods for serum free light chains (sFLC) are now available and have advantages over the traditional UPE and urine IFE described above. Collection of a 24-hour urine sample takes time and may not detect small amounts of light chains. Automated assays are highly sensitive and can detect very low concentrations of free light chains. This sensitivity allows detection of monoclonal immunoglobulins in serum or urine of patients deemed negative by traditional methods. These tests are latex-enhanced immunoassays that use polyclonal antibody reagents that recognize a diverse range of free light chains that may be normally bound to heavy chains and hidden. This allows a quantitative measurement of free κ and free λ chains as well as calculation of a κ/λ ratio. An abnormal κ/λ ratio along with an increase of either κ or λ chains is a sensitive indicator for the presence of a malignant plasma cell clone. The International Myeloma Working Group recommends the use of the sFLC assay along with SPE and serum IFE to screen for multiple myeloma and other plasma cell dyscrasias. This group also stated that the sFLC assay can replace the 24-hour urine IFE to screen for most plasma cell dyscrasias.³

**Immunophenotyping by Flow Cytometry**
As stated earlier, lymphomas and leukemias are classified by morphological features, cytochemical staining, and immunophenotype determined with flow cytometry and cytogenetics. **Immunophenotyping** is the analysis of cell surface markers to determine their lineage and stage of maturation. The presence of CD antigens on the surface of hematopoietic cells is usually detected by flow cytometry. In this procedure, panels of fluorescent-labelled antibodies are incubated with cells thought to be malignant.
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The panels are chosen to correlate with the specific antigens usually present on the cells in the suspected disease. Flow cytometry analyzes the fluorescence emitted by the cell along with the cell size and other characteristics to produce the immunophenotype of the cell population. This is compared with charts of known CD markers to determine the cell lineage.

**Cytogenetic and Molecular Testing**

Cytogenetics may also be helpful in the diagnosis of various hematologic malignant neoplasms. Chromosome translocations and other abnormalities are known to be characteristic of specific leukemias and lymphomas. Traditional cytogenetic testing by karyotyping may be enhanced by fluorescence in situ hybridization (FISH), a method designed to directly identify a specific region of DNA in a cell. A short sequence of single-stranded DNA called a *probe* is used. Probes are complementary to the region of DNA to be studied. Advantages of FISH are that it is quick and very sensitive and does not require cell culture.

Some lymphoid hematologic malignant neoplasms can also be evaluated by molecular techniques to detect subtle abnormalities not detected by karyotyping or FISH. Polymerase chain reaction is the most common procedure used to detect the exact gene rearrangements typical of some diseases. Polymerase chain reaction is an efficient and cost-effective way to copy, or *amplify*, small segments of DNA or RNA. Millions of copies of these small segments result in enough sample to analyze accurately.

Molecular analysis can detect gene rearrangements of immunoglobulin heavy-chain genes, immunoglobulin and/or light-chain genes, and T-cell receptor genes. These gene rearrangements occur before cellular expression of the immunoglobulin. Rearrangement of T-cell receptor genes is an early sign of T-cell lineage that may be helpful in distinguishing the origin of malignant cells in a leukemia.4

**Summary**

Immunoproliferative disorders stemming from plasma cell dyscrasias display an overproduction of a single immunoglobulin component, resulting in the presence of M protein. Laboratory evaluation generally begins with serum protein electrophoresis, with abnormalities analyzed via immunofixation electrophoresis. Peaks in the SPE for each immunoglobulin can be quantitated by densitometry. Some patients produce an excessive amount of free monoclonal immunoglobulin light chains which are cleared from serum rapidly; urine protein electrophoresis and urine IFE can be used to monitor light chain levels. More recently, the adoption of serum free light chain analysis, flow cytometry immunophenotyping, cytogenetics, and molecular testing have allowed a degree of automation in the testing of patient samples as well as more specific classification of immunoproliferative disorders.
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References


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