

Immune fixation electrophoresis

Electrophoresis is used to identify the presence of abnormal proteins, to identify the absence of normal proteins, and to determine when different groups of proteins are increased or decreased in serum. It is frequently ordered to detect and identify monoclonal proteins – an excessive production of one specific immunoglobulin. Protein and immunofixation electrophoresis are ordered to help detect, diagnose, and monitor the course and treatment of conditions associated with these abnormal proteins, including multiple myeloma and a few other related diseases.

Protein is usually excreted in the urine in minute amounts. When it is present in moderate to large amounts, it often indicates some degree of kidney dysfunction and/or abnormal protein production. The primary reason that urine protein and immunofixation electrophoresis are ordered is to look for monoclonal protein production. This protein may show up in both the serum and urine, or it may be seen only in the urine.

Urine protein electrophoresis may also be ordered to help diagnose the cause and estimate the severity of protein excretion due to kidney damage or disease. This damage or disease may be due to diabetes, chronic inflammation, an autoimmune condition, or a malignancy.

Electrophoresis is not usually necessary to assess the loss of small to moderate amounts of protein due to temporary conditions, such as a urinary tract infection or an acute inflammation.

Immunofixation permits the detection and typing of monoclonal antibodies or immunoglobulins in serum or urine. It is of great importance for the diagnosis and monitoring of certain blood related diseases such as myeloma.

The method detects by precipitation: when a soluble antigen (Ag) is brought in contact with the corresponding antibody, precipitation occurs, which may be visible with the naked eye or microscope.

Immunofixation identifies (antibodies) in a mixture in function of their specific electrophoretic mobility. For the identification antigens are used that are specific for the targeted antibody.

Specifically, immunofixation allows the detection of monoclonal antibodies representative of diseases such as myeloma or Waldenström macroglobulinemia.

Protein electrophoresis may be ordered when a doctor is investigating symptoms that suggest multiple myeloma, such as bone pain, anemia, fatigue, unexplained fractures, and recurrent infections. It may also be ordered as a follow-up to other laboratory tests, such as an abnormal total protein and/or albumin level, elevated urine protein levels, elevated calcium levels, and low white or red blood cell counts. Immunofixation electrophoresis is usually ordered when the protein electrophoresis test shows the presence of an abnormal protein band that may be an immunoglobulin.

Once a disease or condition has been diagnosed, electrophoresis may be ordered at regular intervals to monitor the course of the disease and the effectiveness of treatment.

Monoclonal protein production may be due to a monoclonal gammopathy of undetermined significance (MGUS). Most patients with MGUS have a benign course, but they must continue to be monitored regularly as some may develop multiple myeloma after a number of years.

Serum protein electrophoresis may also be ordered when symptoms suggest an inflammatory condition, an autoimmune disease, an acute or chronic infection, a kidney or liver disorder, or a protein-losing condition. Urine protein electrophoresis may be ordered when there is protein detected in the urine or when the doctor suspects a monoclonal protein may be present.

This test is most often used to check the levels of certain antibodies associated with multiple myeloma and Waldenström's macroglobulinemia.

Those antibodies include IgG, IgM, IgA, lambda light chain, and kappa light chain.

Immunofixation has also been used to study changes in protein structure (for example, glucose-6-phosphate dehydrogenase), and in the genetic typing of alpha-1 antitrypsin.

Normal Results

Monoclonal immunoglobulins are not present.

What Abnormal Results Mean

The presence of monoclonal proteins may indicate:

- Immune system disorders such as multiple myeloma or Waldenstrom's macroglobulinemia.
- Cancer

Macroglobulinemia of Waldenstrom is a cancer of the B lymphocytes (a type of white blood cell). It is associated with the overproduction of proteins called IgM antibodies. Waldenstrom's macroglobulinemia is a result of a condition called lymphoplasmacytic lymphoma. The cause of the overproduction of the IgM antibody is unknown, but researchers believe it is made by lymphoma cells. Overproduction of IgM causes the blood to become too thick. This is called hyperviscosity. It occasionally makes it harder for blood to flow through small blood vessels.

Most people with this condition are over age 65, however, it may occur in younger people.

Protein and immunofixation electrophoresis tests give your doctor a rough estimate of how much of each protein is present. The value of protein electrophoresis lies in the proportions of proteins and in the patterns they create on the electrophoresis graph. The value of immunofixation electrophoresis is in the identification of the presence of a particular type of immunoglobulin.

Certain conditions or diseases may be associated with decreases or increases in various serum proteins, as reflected below.

Albumin

Decreased:

- Malnutrition and malabsorption
- Pregnancy
- Kidney disease (especially nephrotic syndrome)
- Liver disease
- Inflammatory conditions
- Protein-losing syndromes

Increased:

- Dehydration

Alpha1 globulin

Decreased:

- congenital emphysema (a1-antitrypsin deficiency, a rare genetic disease)
- severe liver disease

Increased:

Acute or chronic inflammatory diseases

Alpha2 globulin

Decreased:

- hyperthyroidism
- severe liver disease
- hemolysis

Increased:

- Kidney disease (nephrotic syndrome)
- Acute or chronic inflammatory disease

Beta globulin

Decreased:

- Malnutrition
- Cirrhosis

Increased:

- hypercholesterolemia
- iron deficiency anemia
- some cases of multiple myeloma or MGUS

Gamma globulin

Decreased:

- variety of genetic immune disorders
- secondary immune deficiency

Increased:

- Polyclonal: - chronic inflammatory disease

- rheumatoid arthritis
 - systemic lupus erythematosus
 - cirrhosis
 - chronic liver disease
 - acute and chronic infection
 - recent immunization
- Monoclonal: - Waldenstrom's macroglobulinemia
 - multiple myeloma
 - monoclonal gammopathies of undetermined significance (MGUS)

Multiple myeloma:

Multiple myeloma is a prototype of a group of conditions known as plasma cell neoplasms. Plasma cell neoplasms are a group of related disorders each of which is associated with proliferation and accumulation of immunoglobulin-secreting cells that are derived from the B-cell series of immunocytes. Monoclonal components occur in both the malignant plasma cell disorders, that is say, multiple myeloma, Waldenstrom's macroglobulinemia, solitary bone plasmacytoma, extra medullary plasmacytoma, osteosclerotic myeloma (POEMS Syndrome), amyloidosis, and heavy chain disease, as well as in the clinically unclear monoclonal gammopathy of undetermined significance/smoldering multiple myeloma.

Signs and Symptoms

The clinical features of multiple myeloma develop from tissue damage by multiple bone tumors, complications from the monoclonal component, and an increased vulnerability to infections due to depressed normal immunoglobulin levels. These complications provide the first clues to the diagnosis and form the basis for defining the stage and prognosis.

Subjective:

1) Skeletal System

- a) Bone pain is the most common symptom resulting from pathologic fractures.
- b) Compression fractures of the thoracic and lumbar vertebral bodies usually result in severe spasms and back pains. Multiple compression fractures may culminate in painless dorsal kyphosis and loss of as much as six inches of height.
- c) Pleuritic pain from pathologic rib and clavicular fractures is also common and is associated with marked local tenderness.
- d) Destruction of the proximal bones of the extremities is less frequent and distal bones of the extremities are rarely affected.
- e) Band-like or radicular pain should alert the clinician to impending spinal cord impression and is a serious complication representing an emergency requiring immediate diagnosis and treatment.

2) Hypercalcemia

- a) Nausea, confusion, polyuria and constipation are common symptoms secondary to hypercalcemia.

3) Anemia

- a) Easy fatigability or dyspnea on exertion is usually secondary to anemia.

4) Hyperviscosity

- a) When immunoglobulins are present at concentrations greater than 5 gm/dl, some IgG or IgA multiple myeloma globulin's can produce features of hyperviscosity syndrome. Lassitude, confusion, headache, transient disturbances of vision and increased bleeding tendency could be related to this syndrome.

5) Infections

a) Recurrent bacterial infections are a major cause of illness and are the most common cause of death in-patients with advanced myeloma.

6) Amyloid

a) Systemic amyloidosis with or without multiple myeloma could present with weakness, weight loss, ankle edema, dyspnea, paraesthesias, lightheadedness or loss of consciousness due to lack of blood flow to the brain (syncope).

b) Aching in the hands, particularly at night, can signify median nerve compression associated with Carpal Tunnel Syndrome, caused by amyloid infiltration of the transverse carpal ligament.

Objective:

No specific physical abnormalities may be detected.

1) Skeletal System

a) Most patients with symptomatic myeloma will have tenderness on pressure over an involved bone, kyphosis or a pathologic fracture to indicate the sight of bone lesions.

b) In approximately 15% of patients, firm plasma cell tumors arise from areas of underlying bone destruction and may be palpated on the skull, sternum, clavicles and ribs, where the affected bone is close to the skin.

c) Spinal examination could reveal kyphosis and on palpation, tenderness at the area of fracture or plasmacytoma, also palpation could trigger radiculopathy.

d) Signs of imminent cord compression. Upper extremities could demonstrate signs of carpal tunnel syndrome.

2) Hyperviscosity

a) Fundal examination, especially in patients with suspected hyperviscosity syndrome, could reveal segmental dilatation of the retinal veins with retinal hemorrhages.

3) Plasmacytomas

a) Mouth, throat and neck examination might reveal extra medullary plasmacytoma, which usually develop in the nasopharyngeal area or paranasal sinuses.

b) Neurologic signs could be caused by spinal cord or nerve root compression, sensory motor peripheral neuropathy or myelomatous meningitis.

c) In the rare instances where pleural effusion may develop from plasmacytoma and plasmacytosis, pleural effusion could be detected clinically and radiologically

d) Spinal examination could reveal kyphosis and on palpation, tenderness at the area of fracture or plasmacytoma, also palpation could trigger radiculopathy, which could help in localizing the site of imminent cord compression. Upper extremities could demonstrate signs of carpal tunnel syndrome.

4) Infections

a) Signs of lobular pneumonia could be detected on auscultation and palpation of the chest.

5) Amyloid

a) Cardiac examination could reveal ventricular gallop as a sign of failure secondary to severe anemia, hypercalcemia or amyloid heart disease.

b) Skin plaques secondary to amyloid deposit, also joint infusions may be presenting features. Generalized edema secondary to

nephrotic syndrome and/or congestive heart failure could be elicited from the physical examination.

Prognosis:

The median survival of multiple myeloma without any treatment is seven months. Since the introduction of chemotherapy, the median survival has improved to 36-48 months. Cure is rare in this disease. Several clinical and laboratory parameters provide important prognostic information that is extremely valuable in evaluating disease progression and different treatment regimens.

Prognostic factors

- a) The staging system of Durie and Salmon which is based on hemoglobin, serum calcium, and monoclonal protein concentration, as well as the characteristics of the bone survey, classify patients into three stages which correlate with myeloma cell mass.
- b) Serum beta-2 microglobulin uncorrected for serum creatinine predicts survival remarkably well in myeloma patients.
- c) Karyotypic abnormalities occur in 30% of myeloma patients. Irrespective of the treatment status, patients with an abnormal karyotype have a significantly shorter median survival than those with a normal karyotype.
- d) The plasma cell labeling index powerfully and independently predicts survival. Also, it is a useful tool to differentiate between monoclonal gammopathy of undetermined significance and multiple myeloma.
- e) Other prognostic factors such as serum Interleukin-6, C-reactive protein, serum Interleukin-2, and serum IL-6 receptor appear to be other factors that could assist in predicting the course of the disease and/or response to therapy.

Complications:

a) Bone destruction - Approximately 20% of patients with multiple myeloma have bone demineralization only. Radiographs of the axial skeleton, which must include both femurs, will support the diagnosis of multiple myeloma in approximately 70% of patients. 10% of patients will have a normal skeletal survey, presumably because at least 30% of bone calcium must be lost before radiographic changes are evident. Rarely, in 1.4% of patients an osteoblastic reaction is present, which is suggestive of the POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal protein and skin changes).

b) Renal Failure - Renal failure occurs in approximately 25% of patients, more frequently in patients with more extensive disease. Most patients with mild azotemia have no symptoms, however easy fatigability, nausea, vomiting, and confusion occur with severe renal insufficiency. Pathogenesis is multi-factorial, but more than 90% of patients with renal failure have Bence-Jones proteinuria or hypercalcemia or both.

c) Infection - Recurrent bacterial infections are a major cause of illness and are the most frequent cause of death in patients with advanced myeloma. Infections result primarily from the marked depression of production of normal immunoglobulins that occur in more than 75% of patients. Streptococcus pneumoniae, and Hemophilus influenzae, is the most common pathogens in previously untreated myeloma patients and in non-neutropenic patients who respond to chemotherapy. However, in neutropenic patients and in those with refractory disease, staphylococcus aureus and gram-negative bacteria are the predominant organisms. Pneumococcal vaccination may be worth trying, however most multiple myeloma patients respond poorly to bacterial antigenic stimulation. Herpes zoster could be seen in patients with multiple myeloma more commonly in those complicated by renal failure.

d) Hyperviscosity syndrome - Symptoms and signs of this condition are generally not seen unless the relative serum viscosity is greater

than 4.0 units (normal range 1.4-1.8) and the full blown classic syndrome is usually not observed unless the viscosity is greater than 5.0 units. The signs and symptoms include lassitude, confusion, blurred vision, dizziness, vertigo, diplopia (double vision) and increase tendency to bleeding, especially oro-nasal bleeding.

e) Neurologic - Thoracic or lumbo-sacral radiculopathy is the most frequent neurologic complication. Root pain results from compression of the nerve by the vertebral lesion or by the collapsed bone itself. Spinal cord or cord compression from an extradural plasma cell tumor results in back pains with radicular features, weakness or paralysis where an immediate diagnosis and treatment is necessary. Occasionally, patients with multiple myeloma experience peripheral neuropathy, which may be severe. Electromyography studies suggest that this complication occur more frequently than clinically recognized. Although the pathogenesis is unclear, the neuropathy may be caused by associated amyloidosis in some patients. Severe motor neuropathy occurs more frequently in younger patients with localized or osteosclerotic myeloma.

f) Hypercalcemia - At diagnosis, one-fourth of patients have serum calcium concentrations greater than 11.5 mg/dl after correction for serum albumin. Some hypercalcemic patients may not show bone destruction on radiographs. Nausea, confusion, polyuria and constipation are common symptoms.

g) Secondary malignancies - Secondary acute leukemia develops in approximately 2% of patients who survive 2 years, which is 50-100 times more frequent than in normal individuals. Fewer than 5% of patients with multiple myeloma have acute leukemia diagnosis or acquire the disease within several months after starting chemotherapy. The frequency of solid tumors in multiple myeloma patients is no higher than in persons of similar age or sex.

Laboratory Procedures:

Changes Noted on Routine Screening Tests:

Routine laboratory screening, such as CBC, SMA-16, urinalysis and chest x-ray are usually nonspecific regarding reaching a diagnosis, however they are extremely helpful in pointing to specific diagnostic procedures.

a) CBC

i) Anemia is present in most patients and provides a major diagnostic clue. Several factors account for anemia, such as bone marrow infiltration by plasma cells, renal failure and chronic disease.

Low serum B-12 levels may occur without signs of functional B12 deficiency; however, evaluation of B12 deficiency should be sought.

High levels of IgA or IgG frequently increase the plasma volume and the hematocrit may be 6 points less than the value expected from the measured red cell volume.

ii) Thrombocytopenia is uncommon at the time of diagnosis and usually reflects a marked degree of bone marrow replacement by plasma cells.

iii) Mild granulocytopenia occurs frequently for reasons that are unclear and usually persist throughout the clinical course. Granulocytopenia does not have any impact on the outcome of the disease.

b) Immunoglobulins

i) Elevated total globulins, hypoalbuminemia or overall hypoproteinemia secondary to nephrotic syndrome could be observed.

c) Chemistry

i) Hypercalcemia, hyperuricemia, increased serum creatinine secondary to multiple myeloma and/or renal failure.

- ii) Increased LDH is noted in 10-15% of patients and usually signifies a poor prognosis.
- iii) The serum alkaline phosphatase is usually normal but may be elevated in-patients with healing pathologic fractures or osteosclerotic lesions.
- iv) Proteinuria is detected in approximately 65% of patients.

d) Radiological Studies

- i) Chest x-ray may reveal osteolytic lesions in the clavicles, scapulae or ribs, a sub pleural plasmacytoma attached to a rib or a pleural effusion.
- ii) Cardiomegaly may be seen in-patients with cardiac amyloidosis.

Diagnostic procedures and Tests:

When plasma cell dyscrasia is suspected the following tests should be performed to confirm the diagnosis, detect complications, assist in the staging of the disease and establish baseline values for following the treatment progress.

1) Myeloma Proteins: Serum and urine protein electrophoresis demonstrate a peak or localized band in 80% of patients, hypogammaglobulinemia in 10% and no apparent abnormalities in the remainder. In multiple myeloma patients with hypogammaglobulinemia almost always these patients have a monoclonal light chain protein in the urine.

Immunoelectrophoresis and immune fixation are rather expensive, however are very sensitive when compared to protein electrophoresis which could miss up to 15% of monoclonal gammopathies. In 99% of the cases, a monoclonal protein in the serum or the urine or both is detected and in 1% of the patients no monoclonal protein is noted indicating non-secretory multiple myeloma which is secondary to a defect in the synthesis or assembly of the light or heavy chains.

2) Bone Marrow Aspirate and Biopsy: This procedure is helpful in evaluating the different etiologies for the cytopenias and is also crucial for the diagnosis of multiple myeloma as well as evaluating cell morphology and smacytosis may be spotty but an increase in the bone marrow plasma cells is usually easy to demonstrate from most bone marrow sites. Reactive plasmacytosis secondary to connective tissue disorders, liver disease, viral and bacterial infections, and carcinoma could be differentiated from the monoclonal plasma cell proliferation of multiple myeloma or monoclonal gammopathy of unknown significance by performing immune staining on the bone marrow. If available, plasma cell labeling index on the bone marrow could add important prognostic information and possibly aid in structuring the management plans for the patient. A labeling index of less or equal to 0.8% usually indicates a good prognosis. Cytogenetic abnormalities occur in 30% of multiple myeloma patients and its presence usually indicates shorter median survival. Numeric anomalies occurred most often in chromosome 11 and structural aberrations occurred most often in chromosomes 1, 11 and 14.

The recognition of light chain protein (Bence-Jones protein)

3) Urinalysis: Proteinuria is detected in approximately 65% of patients depends on the demonstration of the monoclonal light chain by immune electrophoresis or immune fixation. 24 hour urine collection for protein quantization supplemented by urine protein electrophoresis is essential for following response to therapy.

4) Radiologic Procedures: A complete bone survey is an essential part of the evaluation of monoclonal gammopathies. The skeletal survey should include the complete spine, long bones of the arms and legs, skull, ribs, and pelvis. The order listed is the order of frequency of involvement. Approximately 20% of patients will have bone demineralization alone. Radiographs of the axial skeleton, which must include bone femurs, will support the diagnosis of multiple myeloma in approximately 70% of the patients. Punched out lesions are best seen on lateral skull radiographs. 10% of the patients will have a normal skeletal survey at the time of diagnosis.

a) Computed Tomography and Magnetic Resonance Imaging; May detect bone destruction more sensitively and especially useful in detecting the extent of extra medullary soft tissue lesions. MRI of the lumbar spine and pelvis may detect more advanced disease that may require chemotherapy, primarily in-patients with an apparently localized plasmacytoma or with asymptomatic indolent multiple myeloma.

Differential Diagnosis:

Lytic bone lesions could be related to metastatic carcinoma, connective tissue diseases, chronic infections or lymphoma. Patients with multiple myeloma must be differentiated from those with monoclonal gammopathy of undetermined significance and smoldering multiple myeloma.

Asymptomatic patients with an M-component of less than 3 gm/dl, fewer than 10% bone osteolytic lesions, anemia, hypercalcemia, or renal function impairment have monoclonal gammopathy of unknown significance.

Asymptomatic patients who have both an M-component higher than 3 gm/dl or more than 10% but less than 20% bone marrow plasma cells fulfill the criteria for smoldering multiple myeloma. These patients do not have anemia, renal failure, hypercalcemia, osteolytic bone lesions or other clinical manifestations related to the monoclonal protein. Clinically and biologically these patients with smoldering multiple myeloma are closer to monoclonal gammopathy of undetermined significance than to overt multiple myeloma. The recognition of these patients is extremely important because they should not be treated with chemotherapy until progression occurs.

There is no particular lab parameter or clinical factor that could differentiate patients with MGUS/SMM from overt multiple myeloma. The decrease levels of uninvolved immunoglobulins are not a useful criterion for differentiation because 30-40% of patients with MGUS also have a decrease in the uninvolved immunoglobulin. Although the

presence of Bence-Jones proteinuria is suggestive of multiple myeloma, it is not unusual to find small amounts of monoclonal light chains in the urine of patients with MGUS. Lytic bone lesions in the skeletal survey strongly suggest the diagnosis of multiple myeloma.

In-patients recently diagnosed with MGUS, serum electrophoresis should be repeated after three months to exclude an early myeloma and if results are stable the test should be repeated in six months. Patients should be aware that the evolution of MGUS to multiple myeloma can be abrupt and therefore they should be re-examined promptly if their clinical condition deteriorates.

Therapy for Multiple Myeloma

MM remains a disease for which cure is rare. Most patients succumb to their disease within 36-48 months from the time of diagnosis. Limitations of effective therapy for MM are primarily associated with the low cell proliferation rate and multi-drug resistance.

Therapy for multiple myeloma includes induction, maintenance, and supportive aspects. The induction portion of the treatment aims at reducing the tumor volume, and achieving a plateau phase. The standard maintenance approach is no treatment.

Different drugs and treatment modalities as bone marrow transplantation has been entertained, and used without a significant impact on the disease free or the overall survival.

Supportive care in multiple myeloma has advanced significantly over the past few years. Growth factor support with erythropoietin replacement, GM-CSF for stimulating the WBC is a safe and effective method to decrease or prevent the occurrence or the severity of neutropenia. Cell component support, has improved with a better chances at a transfusion match with lower complication rates. The different regimens used in the induction portion of the disease are overall comparable. The difference between the regimens is mainly related to the toxicity profile, as well as

the quickness of response, and the ability of the combination to reverse some of the combination side effects.

Symptoms

- Bleeding of the gums
- Blurred or decreased vision
- Dizziness
- Easy bruising of the skin
- Fatigue
- Headache
- Mental status changes
- Nosebleeds
- Numbness, tingling, or burning pain in the hands, feet, fingers, toes, ears, or nose
- Rash
- Unintentional weight loss
- Vision loss in one eye

Additional symptoms that may be associated with this disease:

- Bluish skin discoloration
- Fingers that change color upon pressure
- Flank pain
- Swollen glands

Exams and Tests

A physical examination may reveal a swollen spleen, liver, and lymph nodes. An eye exam may show enlarged veins in the retina or retinal bleeding (hemorrhages).

A CBC shows a low number of red blood cells and platelets. Blood chemistry shows evidence of kidney disease. A serum viscosity test can tell if the blood has become thick. Symptoms usually occur when the blood is four times thicker than normal.

A test called serum protein electrophoresis shows an increased amount of the IgM antibody. Levels seen in Waldenstrom's macroglobulinemia are generally greater than 3 g/dL.

Bone lesions are very rare. If they are present, a bone marrow examination will show cells that resemble both lymphocytes and plasma cells.

Additional tests that may be done:

- 24-hour urine protein
- Total protein
- Serum globulin electrophoresis
- Immunofixation in urine
- T (thymus derived) lymphocyte count

Treatment

Plasmapheresis (plasma exchange) removes unwanted substances from the blood. In macroglobulinemia, it removes or reduces the high level of IgM, and is used to quickly control the symptoms caused by blood thickening.

Drug therapy may include steroids, Leukeran, Alkeran, Cytosan, fludarabine, or rituximab, or combinations of chemotherapy drugs.

Patients who have a low number of red or white blood cells or platelets may need transfusions or antibiotics.

Prognosis

The average survival is about 5 years. In some people, the disorder may produce few symptoms and progress slowly.

Possible Complications

- Changes in mental function, possibly leading to coma
- Congestive heart failure

- Gastrointestinal bleeding
- Vision problems

Precaution

Immunizations within the previous six months can increase immunoglobulins as can drugs such as phenytoin (Dilantin), procainamide, oral contraceptives, methadone, and therapeutic gamma globulin.

Aspirin, bicarbonates, chlorpromazine (Thorazine), corticosteroids, and neomycin can affect protein electrophoresis results.

Alternative Names

Waldenstrom's macroglobulinemia;

Macroglobulinemia - primary; Lymphoplasmacytic lymphoma.

