

# Waldenstrom's Macroglobulinemia

## Medical Tests



SUPPORT • EDUCATION • RESEARCH

# IWMF

International Waldenstrom's  
Macroglobulinemia Foundation



## **WALDENSTROM'S MACROGLOBULINEMIA MEDICAL TESTS**

### **The IWMF Vision Statement**

*Support everyone affected by Waldenstrom's macroglobulinemia while advancing the search for a cure.*

### **The IWMF Mission Statement**

*To offer mutual support and encouragement to the Waldenstrom's macroglobulinemia community and others with an interest in the disease.*

*To provide information and educational programs that address patients' concerns.*

*To promote and support research leading to better treatments and ultimately, a cure.*

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Revised 2016

## **PREFACE & ACKNOWLEDGMENT**

This booklet was prepared for the individual who has an interest in Waldenstrom's macroglobulinemia (WM). Its general objective is to provide a simple reference booklet of medical tests commonly used to diagnose and monitor the disease status of WM patients, with particular attention to the Complete Blood Count (CBC), the White Blood Cell Differential, and Immunoglobulins.

Grateful acknowledgment is made to Robert Kyle, MD, of the Mayo Clinic for his medical review of the 2016 revision.

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## INTRODUCTION

Talking with your doctor and sharing your health history are usually the first parts of the health care process. The physical examination performed by your doctor identifies bodily changes and pinpoints physical problems or abnormalities. Multiple tentative or differential diagnoses are generated from this information.

Medical tests are then usually ordered to help your doctor narrow the search for the correct diagnosis and reach tentative agreement on therapeutic goals. Medical tests, in and of themselves, do not make the diagnosis nor do they alone dictate therapy; rather, they are pieces of the whole puzzle and should be looked at in that way.

The following medical tests are paired with information about typical signs or symptoms that can occur in WM. However, it is important to realize that patients with similar medical test results can have markedly different types and degrees of symptoms. Patients should be aware that several of these signs and symptoms, as well as the results of medical tests, can be associated with other conditions, and they should not necessarily assume that WM is the cause.

The following sections, where applicable, include “normal results” listed in metric units. The metric system is in virtually universal use in health care systems worldwide, with the major differences between countries being the concentration nomenclature used. The “normal results” for each test listed below are approximate only, as each laboratory establishes its own “normal” or reference ranges that are listed along with your results. Your laboratory’s reference ranges may differ somewhat from those listed below.

## BLOOD & SERUM TESTS FOR WM PATIENTS

Blood is a fluid tissue that performs many important vital functions in the human body. The most important of these functions is the transport and delivery of oxygen from the lungs to the body’s tissues and the subsequent transport of waste gases, predominantly carbon dioxide, from the body’s tissues to the lungs for elimination. The blood performs other vital functions such as transport and delivery of immune system cells, coagulation (clotting), participation in the acid-base and fluid balance systems of the body, regulation of body temperature, transport of nutrients and hormones to body tissues, and the transport to, and ultimate disposal of, wastes by the kidneys, lungs, and skin.

Blood is approximately three times thicker than water, is slightly salty in taste, and is very slightly alkaline, or basic (pH 7.4). The arteries carry bright red oxygen-rich blood from the lungs to the tissues, and the veins carry dark red oxygen-poor blood from the tissues back to the lungs.

The blood has two major components: the plasma, which is the clear, straw-colored fluid portion that contains proteins, enzymes, nutrients, and other dissolved molecules; and the formed elements that are the red blood cells, white blood cells, and platelets.

Some of the following blood tests are performed on whole blood and others on the serum portion of the blood. Serum is plasma minus the clotting factors. If whole blood is collected for serum testing, it is allowed to clot and the serum is then drawn off for further study.

### Complete Blood Count (CBC) & White Blood Cell Differential

The Complete Blood Count (CBC) is a commonly ordered group of tests that evaluate the red blood cells, white blood cells, and platelets. The CBC is an automated test that can be done rapidly and occasionally requires definitive evaluation by a pathologist or a hematologist under direct microscopy. The CBC measures hematocrit, hemoglobin, the volume (MCV) of each red blood cell (RBC), hemoglobin per RBC (MCH), concentration of hemoglobin in the red blood cell (MCHC), and the number of RBCs, white blood cells (WBCs), and platelets. A White Blood Cell Differential or “Diff” counts the individual types of WBCs, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils. If the automated differential is abnormal, a manual differential is performed to verify the results. The manual differential is labor intensive and involves the preparation of a microscope slide and visually counting the cells and examining their morphology under a microscope.

This section will review in some detail:

1. The Red Blood Cells: including the Red Blood Cell Count, Hematocrit, Hemoglobin, and Red Cell Indices (MCV, MCH, and MCHC).
2. The White Blood Cells: including the White Blood Cell Count and the White Blood Cell Differential.
3. The Platelets: Platelet Count and Mean Platelet Volume.

## 1. The Red Blood Cells

### Red Blood Cell Count (RBC)

The most vital role of the red blood cell is to carry oxygen from the lungs to the tissues and to subsequently transport waste gases from the tissues for expulsion in the lungs.

#### ***Why is this test done?***

1. To evaluate the number and size(s) of red blood cells.
2. To determine the hemoglobin content and condition of red blood cells.
3. To assist in diagnosing health problems related to blood.

#### ***What are the normal results?***

1. **Men:** 4.2 to 5.4 million RBCs per microliter of blood ( $4.2$  to  $5.4 \times 10^{12}$  /L). **Women:** 3.6 to 5.0 million RBCs per microliter of blood ( $3.6$  to  $5 \times 10^{12}$  /L).

#### ***What is the significance of an abnormal result?***

1. A decreased red blood cell count may indicate anemia, fluid overload, or severe bleeding.
2. An elevated count may indicate polycythemia (a disease state characterized by an increase in RBCs).
3. Further tests are required to determine the exact diagnosis.

### Hematocrit (Hct)

This test simply measures the percentage (%) of red blood cells in a blood sample. The results vary by sex and age of the patient, with younger individuals such as infants and pre-teens having lower values.

#### ***Why is this test done?***

1. To help diagnose blood disorders.
2. To help calculate the volume and concentration of blood cells.

#### ***What are the normal results?***

1. **Men:** 42% to 54%. **Women:** 38% to 46%.

#### ***What is the significance of an abnormal result?***

1. A low hematocrit % may indicate anemia, fluid overload, or massive blood loss.
2. A high hematocrit % may indicate polycythemia, dehydration, or other conditions.

### Hemoglobin (Hb or Hgb)

The hemoglobin molecule is the iron-containing metallo-protein in red blood cells. Its purpose is to carry oxygen in the blood. Hemoglobin makes up 97% of the dry weight of the RBC. The hemoglobin portion of the CBC measures the amount of hemoglobin per volume of whole blood.

#### ***Why is this test done?***

1. To detect anemia or polycythemia or to assess response to various therapies.

2. To assist in the calculation of additional information for a Complete Blood Count.

***What are the normal results?***

1. The hemoglobin concentration varies somewhat depending on the type of sample obtained (capillary finger sample, central circulation via a central line, or most commonly peripheral veins). Hgb values also depend on the person's sex, and the maximum value declines with the person's age.

2. **Men:** 14 to 18 g/dL or 140 to 180 g/L. **Women:** 12 to 16 g/dL or 120 to 160 g/L.

***What is the significance of an abnormal result?***

1. Low hemoglobin values may be indicative of anemia, recent blood loss, or fluid overload.

2. Elevated hemoglobin values are commonly seen in polycythemia or dehydration.

**Red Cell Indices**

The red cell indices provide important information about the volume or size (MCV), hemoglobin weight (MCH), and hemoglobin percentage (MCHC) of the red cells in a sample.

***Why is this test done?***

1. The information is of importance when diagnosing and evaluating anemia.

***What are the normal results?***

1. MCV – mean corpuscular volume – the ratio of the hematocrit to the red blood cell count: 80 to 100 fL.

2. MCH – mean corpuscular hemoglobin – the weight of the hemoglobin in an average red blood cell: 26 to 32 pg.

3. MCHC – mean corpuscular hemoglobin concentration – the % level of hemoglobin in a given volume of red blood cells: 30% to 60% g/dL.

***What is the significance of an abnormal result?***

1. MCV calculates the average size of the RBCs and notes if they are microcytic (small), macrocytic (big), or normocytic (normal). Small RBCs are commonly seen in iron deficiency anemia, whereas large RBCs are typical of some vitamin deficiency states. MCV is a key value in evaluating anemia.

2. MCH will often yield clues to the state of hemoglobin synthesis. Low values are seen in states of chronic diseases, iron deficiency anemia, etc.

3. MCHC values are helpful in distinguishing normochromic (normally colored) red cells from hypochromic (paler) and hyperchromic (darker) red blood cells, which can occur in certain disease states.

**Red Cell Distribution Width (RDW)**

The RDW provides a quantitative measure of variation in size of the circulating RBCs.

***Why is this test done?***

1. A rather advanced test used to grossly evaluate the age distribution of the red blood cell population, as younger RBCs are larger than older RBCs.

***What are the normal results?***

1. 13.5% to 15.5%.

***What is the significance of an abnormal result?***

1. An increased result can often indicate a response to therapy for anemia, as young RBCs (which are larger) are being produced at a more rapid rate than usual.

2. A low value will suggest a static population of RBCs, with not much variability in size (and age).

## 2. The White Blood Cells

### White Blood Cell Count (WBC)

Also known as the leukocyte count, the white blood cell count (WBC) determines how many white blood cells are in a given volume of blood. On any given day, the WBC may vary by as much as 2,000, be it due to strenuous exercise, stress, or infection. The number of white blood cells may increase or decrease significantly in certain diseases, but as a diagnostic tool, the WBC is most useful when a patient's white cell differential and health status are also considered.

#### **Why is this test done?**

1. To detect infection, inflammation, or certain hematological malignancies.
2. To determine the requirement for further evaluations, such as the white blood cell differential or a bone marrow aspirate and biopsy.
3. To monitor a patient's response to cancer therapy.

#### **What are the normal results?**

1. Normal white blood cell counts range from 4,000 to 10,000 WBCs per cubic milliliter ( $\text{mm}^3$ ) of whole blood (4 to  $10 \times 10^9/\text{L}$ ).

#### **What is the significance of an abnormal result?**

1. An elevated white blood cell count, referred to as leukocytosis, often indicates infection. Stressful events such as trauma, stroke, or a heart attack can also transiently elevate the WBC. Certain hematological malignancies are characterized by elevated white blood cell counts.
2. A low white blood cell count, referred to as leukopenia, often indicates bone marrow problems. Toxic chemicals and viral infections can cause a reduction in WBCs. Influenza or other viral infections, typhoid fever, measles, infectious mononucleosis, hepatitis, and rubella can characteristically cause leukopenia.

### White Blood Cell Differential

The white blood cell differential is used to evaluate the distribution of the five major types of white blood cells, or leukocytes: neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

#### **Why is this test done?**

1. To evaluate the body's capacity to resist and overcome infection.
2. To determine the stage and severity of an infection.
3. To detect parasitic infections.
4. To detect and assess allergic reactions.
5. To detect and identify various types of leukemia and lymphoma.

#### **What are the normal results?**

The following chart provides relative levels for the five types of white blood cells classified in the white blood cell differential. In some laboratories, one may see the term "granulocytes" – this refers to the combined number and/or percentage of white blood cells that have granules in their cytoplasm. These are neutrophils, eosinophils, and basophils.

Cell Type	% of Cells	Absolute Numbers
Neutrophils	48% to 77%	$1.9-8.0 \times 10^3/\text{mm}^3$ ( $\times 10^9/\text{liter}$ )
Lymphocytes	16% to 43%	$0.9-5.0 \times 10^3/\text{mm}^3$ ( $\times 10^9/\text{liter}$ )
Monocytes	0.6% to 9.6%	$0.16-1.0 \times 10^3/\text{mm}^3$ ( $\times 10^9/\text{liter}$ )
Eosinophils	0.3% to 7%	$0.0-0.8 \times 10^3/\text{mm}^3$ ( $\times 10^9/\text{liter}$ )
Basophils	0.3% to 2%	$0.0-0.2 \times 10^3/\text{mm}^3$ ( $\times 10^9/\text{liter}$ )



### **What is the significance of an abnormal result?**

The following table summarizes how abnormal white blood cell differential patterns provide evidence for a wide range of diseases and conditions:

	<b>Increased By:</b>	<b>Decreased By:</b>
Neutrophils	<ul style="list-style-type: none"><li>• Infections</li><li>• Trauma</li><li>• Metabolic disorders</li><li>• Stress response</li><li>• Inflammatory disease</li><li>• Leukemias</li></ul>	<ul style="list-style-type: none"><li>• Marrow suppression</li><li>• Infections</li><li>• Liver &amp; spleen disorders</li><li>• Collagen vascular diseases</li><li>• Vitamin deficiency</li></ul>
Eosinophils	<ul style="list-style-type: none"><li>• Allergic disorders</li><li>• Parasitic infections</li><li>• Skin diseases</li><li>• Malignancy</li><li>• Various other mechanisms</li></ul>	<ul style="list-style-type: none"><li>• Stress response</li><li>• Trauma</li><li>• Cushing's syndrome</li></ul>
Basophils	<ul style="list-style-type: none"><li>• Leukemias</li><li>• Hemolytic anemias</li><li>• Hodgkin's lymphoma</li><li>• Chronic inflammatory disease</li><li>• Kidney disease</li></ul>	<ul style="list-style-type: none"><li>• Hyperthyroidism</li><li>• Ovulation</li><li>• Pregnancy</li><li>• Stress</li></ul>
Lymphocytes	<ul style="list-style-type: none"><li>• Infections</li><li>• Endocrine disorders</li><li>• Immune disorders</li><li>• Chronic lymphocytic leukemia</li><li>• Inflammatory diseases</li></ul>	<ul style="list-style-type: none"><li>• Severe illness</li><li>• Corticosteroid therapy</li><li>• Immunosuppression</li><li>• Chemotherapy</li><li>• Malignancy</li></ul>
Monocytes	<ul style="list-style-type: none"><li>• Infections</li><li>• Collagen vascular disease</li><li>• Leukemias</li><li>• Lymphomas</li></ul>	<ul style="list-style-type: none"><li>• Immunosuppression</li></ul>

### **3. The Platelets**

#### **Platelet Count (Plt)**

Platelets, or thrombocytes, are the smallest formed elements in blood. They promote blood clotting after an injury.

#### **Why is this test done?**

1. To help determine if blood clots normally.
2. To evaluate platelet function.
3. To assess the effects of chemotherapy or radiation therapy on platelet production.
4. To diagnose and monitor a severe increase or decrease in platelet count.

#### **What are the normal results?**

1. Normal platelet counts range from 130,000 to 400,000 platelets per mm<sup>3</sup> of whole blood (130 to 400 x 10<sup>9</sup>/L).

#### **What is the significance of an abnormal result?**

1. Low platelet counts (thrombocytopenia) can be caused by bone marrow suppression due to cancer or infection; folic acid or vitamin B12 deficiency; trapping of platelets in an enlarged spleen; increased platelet destruction due to immune

disorders; or mechanical injury to platelets. A platelet count that falls below 20,000 can sometimes cause spontaneous bleeding. When it drops below 5,000, fatal central nervous system bleeding or massive gastrointestinal hemorrhage is possible.

2. A high platelet count (thrombocytosis) can be caused by severe bleeding, infection, cancer, iron-deficiency anemia, and recent surgery, pregnancy, or spleen removal. A high count can also be caused by inflammatory disorders.

### **Mean Platelet Volume (MPV)**

Additional information on platelet function is obtained from the mean platelet volume (MPV).

#### ***Why is this test done?***

1. Measurement of the mean platelet size can often provide indirect evidence for the pathogenesis of thrombocytopenia (low platelet count).

#### ***What are the normal results?***

1. Normal platelet size is 7.2 to 11.1 fL.

#### ***What is the significance of an abnormal result?***

1. Large platelets are present when there is thrombocytopenia stemming from increased platelet destruction.

2. Mean platelet volume rises because larger, newly-made platelets will make up a higher proportion of the circulating platelets.

3. An MPV that is the same as the MPV for a non-thrombocytopenic person is not a normal finding in a thrombocytopenic patient and suggests that failure of platelet production has contributed significantly to the pathogenesis of the thrombocytopenia.

### **Reticulocyte Count**

Reticulocytes are immature red blood cells. They are generally larger than mature red blood cells. One can assess effective RBC production by measuring the reticulocyte count. The number of reticulocytes is expressed as a percentage of the total red cell count. Generally, the higher the reticulocyte count, the higher the red cell distribution width (RDW).

#### ***Why is this test done?***

1. To detect anemia or monitor its treatment.

2. To distinguish between different types of anemias.

3. To help assess blood loss or the bone marrow's response to anemia.

#### ***What are the normal results?***

1. Reticulocytes compose 0.5% to 2.0% of the red blood cell count.

#### ***What is the significance of an abnormal result?***

1. A low reticulocyte count indicates low production of new red blood cells such as occurs in hypoplastic or pernicious anemia. Bone marrow failure, aplastic anemia, and myelodysplastic syndrome also produce low reticulocyte counts.

2. A high reticulocyte count demonstrates response to therapy for anemia or the healthy bone marrow's response to anemia or blood loss.

### **Erythrocyte Sedimentation Rate (Sed Rate, ESR)**

This test measures the rate at which erythrocytes (red blood cells) settle to the bottom of a special blood sample test tube during a specific time period. The ESR is a sensitive but nonspecific test that is frequently the earliest indicator of disease when other tests or physical signs are normal. The ESR commonly increases greatly in multiple myeloma, WM and widespread inflammatory disorders.

### ***Why is this test done?***

1. To evaluate the condition of red blood cells.
2. To monitor inflammatory or malignant disease.
3. To aid in the detection and diagnosis of such diseases as rheumatoid arthritis, lupus erythematosus, or other connective tissue diseases.

### ***What are the normal results?***

1. Normal sedimentation rates range from 0 to 20 millimeters per hour.
2. The rates gradually increase with age; a reading of 30 to 40 may not be unusual for a person > 65 years of age.

### ***What is the significance of an abnormal result?***

1. The erythrocyte sedimentation rate increases in multiple myeloma, WM, other cancers, pregnancy, anemia and acute or chronic inflammation.
2. Increased serum viscosity in WM, caused by elevated IgM levels, can significantly increase the ESR.

## **Serum Immunoglobulins**

The immunoglobulin M (IgM) concentration and its increase or decrease are one of the key indicators of the malignant WM B-cell's activity. Physicians utilize IgM levels as part of the criteria for the diagnosis of WM and as a key marker for disease progression and treatment efficacy. The IgM levels are an indicator of disease remission or relapse, and many will use the IgM levels and, more importantly, the trend of IgM values over time as laboratory indication of when symptoms may appear and warrant therapy.

Monoclonal IgM is the immunoglobulin produced by the monoclonal WM B-cells. The monoclonal IgM molecules all have the same molecular composition. In very rare cases a patient with WM can have two types of monoclonal IgM proteins from two different clones of malignant WM B-cells. Some patients may even have monoclonal IgM from one cancerous WM cell line and a second monoclonal immunoglobulin from a different class (usually IgG) from another cancerous cell line. Again, this is rare, and a full discussion of biconality is beyond the scope of this booklet. The polyclonal IgM produced by normal immune system B-cells has different molecular compositions based on which antigen the IgM targets. Much of the polyclonal IgM is produced in response to foreign pathogens; whereas the monoclonal IgM of WM B-cells is secreted by the tumor cells without antigen stimulation.

Patients and physicians should be alert to the possibility that the presence of a circulating monoclonal IgM may interfere with one or more laboratory tests performed on liquid-based automated analyzers, either by precipitating during the analysis, or by virtue of IgM's specific binding properties.

The most common artifacts in the above situation are a low value for HDL cholesterol, a high value for bilirubin, as well as altered measurement of inorganic phosphate. Infrequently other potential examples include interference with measurement of LDL cholesterol, C-reactive protein, antistreptolysin-O, creatinine, glucose, sodium, chloride, bicarbonate, urea nitrogen, albumin, iron, and inorganic calcium.

Re-analysis of these specimens using a different test method or a dilution of the sample can be employed for obtaining accurate measurements. These events may occur in patients whose clinicians are unaware of the presence of the underlying monoclonal protein and might result in the mismanagement of patients with monoclonal gammopathy, especially as regards measurement of HDL and LDL cholesterol and estimation of cardiovascular risk.

The two most common laboratory methods for measuring IgM concentration in a patient's serum are nephelometry and electrophoresis.

### **1. Measurement by Nephelometry**

Nephelometry is a technique for measuring the "cloudiness" of a liquid such as drinking water. A more common term is "turbidity," which is analyzed by a nephelometer. The nephelometer measures the amount of light that is lost by

scattering while passing through the solution. Particles or large molecules such as IgM protein suspended in the serum cause the light to be scattered.

A nephelometer reads the light scattered from the particles, not the directed beam's intensity once it has passed through the sample. Therefore, a sample without particles will not scatter light and have a reading of zero. If particles are introduced or created by precipitation of the sample, then light will be scattered and the intensity of the scattered light can be measured with a light sensor or detector. The intensity of the light scatter is in direct proportion to the amount of suspended particles in the sample.

The nephelometer is used to measure the immunoglobulins (IgG, IgA, IgM and IgD) in the serum. IgE must be measured by a more sensitive technique such as an enzyme-linked immunoassay. The techniques vary somewhat, but generally the serum sample is added to a quantity of distilled water and a specific antigen is subsequently added that will cause the desired immunoglobulin to precipitate in the solution and form tiny particles. These Ig/antigen particles will scatter light. The amount of light from the Ig/antigen precipitate can be compared to standardized values of known Ig concentration.

Both monoclonal and polyclonal IgM will react with the antigen and precipitate to scatter light. Nephelometry cannot distinguish how much of the total IgM is monoclonal. However, we know that under normal conditions, the polyclonal IgM of a normal immune system varies from 50 to 300 mg/dL; we can therefore infer that a reading of 1000 mg/dL corresponds to a monoclonal IgM of 800 mg/dL (if we average a "normal" IgM value at 200 mg/dL). This is of course a gross oversimplification but is nonetheless satisfactory in most instances, as a static IgM value is not as important as a measurement of the IgM value trend over time. One must keep in mind, however, that the IgG and IgA values are often reduced in patients with WM.

***Why is this test done?***

1. To provide a rapid and accurate measurement of the serum concentration of IgA, IgG, and IgM.

***What are the normal results?***

1. IgA: 60 to 400 mg/dL.
2. IgG: 750 to 1600 mg/dL.
3. IgM: 50 to 300 mg mg/dL.

***What is the significance of an abnormal result?***

Abnormal IgA results:

1. Increased levels of IgA: Chronic infections (especially involving the gastrointestinal tract), inflammatory bowel disease, rheumatic fever.
2. Decreased levels of IgA: Hereditary IgA deficiency, agammaglobulinemia, hypogammaglobulinemia, protein-losing gastroenteropathy, chemotherapy and/or immunotherapy.

Abnormal IgG results:

1. Increased levels of IgG: IgG multiple myeloma, IgG MGUS, chronic infections, hyperimmunization, liver disease, rheumatoid arthritis (and other connective tissue diseases), rheumatic fever.
2. Decreased levels of IgG: Agammaglobulinemia, hypogammaglobulinemia, lymphoma, leukemia, preeclampsia, chemotherapy and/or immunotherapy.

Abnormal IgM results:

1. Increased levels of IgM: Infectious mononucleosis, lymphosarcoma, WM, IgM MGUS, IgM multiple myeloma, rheumatoid arthritis (and other connective tissue diseases).
2. Decreased levels of IgM: Agammaglobulinemia, hypogammaglobulinemia, leukemia, chemotherapy, and/or immunotherapy.

## 2. Measurement by Electrophoresis

Serum protein electrophoresis (SPE, SPEP) is a laboratory technique very commonly used to evaluate patients with high serum protein, such as WM and multiple myeloma. In certain instances, despite normal serum protein levels, an SPE is performed nonetheless for such conditions as unexplained neurological disorders and other diseases.

The basic SPE separates serum proteins based on their physical properties: the net charge (positive or negative) of the protein molecules and the size and shape of the proteins. There exist additional specialized SPE tests used on occasion when warranted: examples include zone electrophoresis and immunofluorescence/immunofixation.

The resultant pattern of a typical SPE depends on the concentration of the two major types of serum proteins: albumin and globulins. Albumin, produced by the liver, is the major protein constituent in normal serum. Globulins, on the other hand, usually represent a much smaller fraction of serum proteins. The identification of subsets of globulins and the determination of their relative quantity are the primary objective of SPE.

Albumin, the largest component of normal serum proteins, is also correspondingly the largest peak on the electrophoretic pattern, and lies closest to the positive electrode. The next five protein components represent the globulin subsets: alpha1, alpha2, beta, and gamma. The globulin peaks migrate towards the negative electrode, with the gamma fraction closest to the negative electrode.

### Indications for Serum Protein Electrophoresis

- Suspected multiple myeloma, Waldenstrom's macroglobulinemia, primary amyloidosis, or related disorders.
- Unexplained peripheral neuropathy (not attributed to longstanding diabetes mellitus, toxin exposure, chemotherapy, etc.).
- New-onset anemia associated with renal failure/insufficiency and bone pain.
- Back pain in which multiple myeloma is suspected.
- Hypercalcemia (high blood calcium), weight loss, fatigue, bone pain, abnormal bleeding.
- Rouleaux formations (stacks and aggregations of RBCs) noted on peripheral blood smear.
- Renal insufficiency with associated serum protein elevation.
- Unexplained pathologic fracture or lytic lesion identified on X-ray.
- Bence Jones proteinuria (protein in the urine).

### Components of Serum Proteins

Albumin comprises approximately 60% of the total serum proteins. It is responsible for much of the plasma's colloidal osmotic pressure and serves as the major transport protein for large molecules such as fatty acids, bilirubin, many drugs, and certain hormones.

The alpha1 globulin fraction includes alpha1-antitrypsin, thyroid-binding globulin, and transcortin. The alpha2 globulins are composed of ceruloplasmin, alpha2-macroglobulin, and haptoglobin.

The beta globulin fraction is composed mostly of transferrin and beta-lipoproteins. Infrequently, IgA, IgM, and sometimes IgG, along with complement, can be identified in the beta fraction.

The gamma globulin fraction is of particular interest to WM patients and their physicians, as immunoglobulins migrate in this region of the electrophoretic pattern. Although immunoglobulins can be found throughout the entire electrophoretic spectrum, IgM is typically well represented in the gamma region. The inflammatory marker C-reactive protein (CRP) is located in the area between the beta and gamma fractions. Figure 1 below shows a typical SPE pattern.

### Interpretation of SPE Results

The levels of serum proteins vary in response to physiologic changes in the body secondary to acute inflammation, malignancy, traumatic injury, necrosis of tissues, burns, and chemical injuries.

As previously stated, the interpretation of SPE is primarily focused on the gamma region, which contains the immunoglobulins. Although many conditions can cause an increase in the gamma region, several disease states can cause a homogeneous spike-like peak. Monoclonal gammopathies, such as WM, MGUS, and multiple myeloma,

constitute a group of diseases that are characterized by proliferation of a single clone of immature B-cells or plasma cells that produce a homogeneous M protein. The characteristic M-spike is a classic feature on SPE. See Figure 2.

**Why is this test done?**

1. Monoclonal gammopathies are characteristic of a clonal process that is malignant or potentially malignant. Polyclonal gammopathies, on the other hand, may be the result of any number of reactive or inflammatory processes such as infections, connective tissue diseases, liver diseases, malignancies, hematologic and lymphoproliferative disorders, and other inflammatory conditions.

**What are the normal results?**

1. Albumin: 3.3 to 5.7 g/dL

Alpha1: 0.1 to 0.4 g/dL

Alpha2: 0.3 to 0.9 g/dL

Beta: 0.7 to 1.5 g/dL

Gamma: 0.5 to 1.7 g/dL

2. An M protein (monoclonal protein) is characterized by the presence of a sharp, well-defined peak in the gamma region. A polyclonal gammopathy is characterized by a broad peak in the gamma region.

3. It is important to note that in some patients, the SPE may appear normal because the complete monoclonal immunoglobulin is absent or is present at a level below the sensitivity of the test.

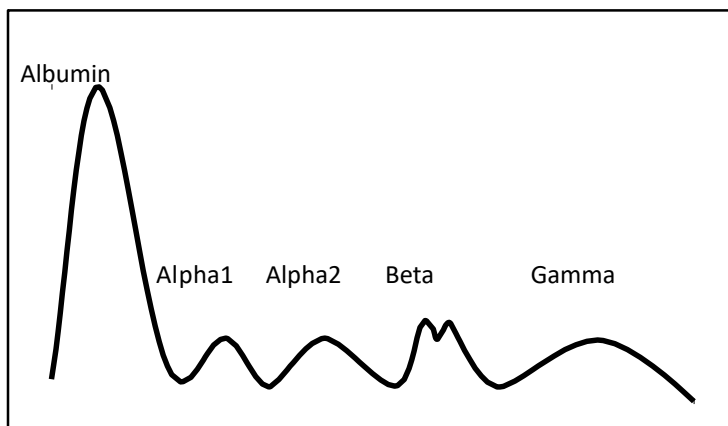
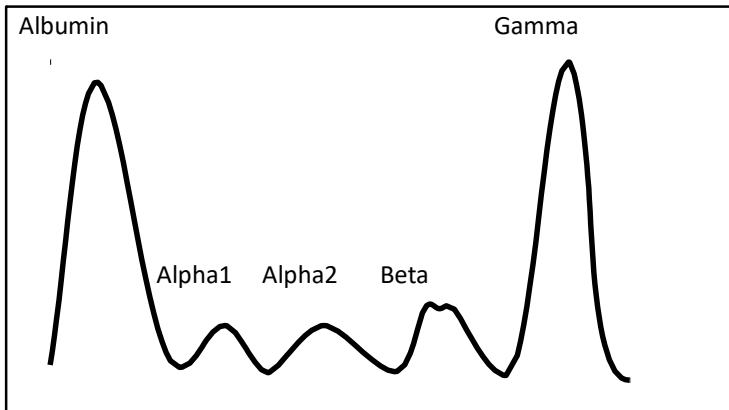


Figure 1: Typical normal serum protein electrophoresis pattern.

**What is the significance of an abnormal result?**

Once SPE has determined the presence of a localized protein band (M-spike) usually in the gamma region, the type of gammopathy must be identified by immunofixation electrophoresis (IFE). The presence of an abnormality is seldom diagnostic by itself. Instead, it provides a clue. Follow-up testing is then usually performed to identify the nature of the underlying disease. This testing may include bone marrow biopsy, flow cytometry, urine testing, genetic testing for specific mutations, etc. The most common causes of monoclonal gammopathy are monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, WM, other lymphomas, and amyloidosis.



**Figure 2: Abnormal SPE pattern in a patient with a monoclonal gammopathy.**

The following table summarizes SPE protein patterns and several associated conditions or disorders:

<p><b>Increased albumin</b> Dehydration</p> <p><b>Decreased albumin</b> Chronic cachectic or wasting disease Chronic infections Hemorrhage, burns, or protein-losing enteropathies Impaired liver function resulting from decreased synthesis of albumin Malnutrition Nephrotic syndrome Pregnancy</p> <p><b>Increased alpha1 globulins</b> Pregnancy</p> <p><b>Decreased alpha1 globulins</b> Alpha1-antitrypsin deficiency</p> <p><b>Increased alpha2 globulins</b> Adrenal insufficiency Adenocorticosteroid therapy Advanced diabetes mellitus Nephrotic syndrome</p> <p><b>Decreased alpha2 globulins</b> Malnutrition Megaloblastic anemia Protein-Losing enteropathies Severe liver disease Wilson's disease</p>	<p><b>Increased beta globulins</b> Biliary cirrhosis Carcinoma Cushing's disease Diabetes mellitus Hypothyroidism Iron deficiency anemia Malignant hypertension Nephrosis Polyarteritis nodosa Obstructive jaundice Third-trimester pregnancy</p> <p><b>Decreased beta globulins</b> Malnutrition</p> <p><b>Increased gamma globulins</b> Amyloidosis Chronic infections Chronic lymphocytic leukemia Cirrhosis Hodgkin's lymphoma WM Other B-cell lymphomas Multiple myeloma Rheumatoid and collagen diseases (connective tissue disorders)</p> <p><b>Decreased gamma globulins</b> Agammaglobulinemia Hypogammaglobulinemia</p>
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## Serum Viscosity (SV)

Serum viscosity is the property of the serum portion of the blood that resists its flow. It is compared to the viscosity of distilled water at room temperature and is a function of the concentration of protein in the serum.

High serum viscosity (hyperviscosity) can be caused by an overabundance of the immunoglobulin proteins, such as IgM in WM. Plasmapheresis is the treatment of choice for initial management of hyperviscosity. Optimal treatment for hyperviscosity consists of treatment for the underlying disorder (with chemotherapy, immunotherapy, etc.). If the underlying disease process is left untreated, the hyperviscosity will recur after plasmapheresis.

During the test, serum is allowed to flow through a narrow tube (viscometer), and its rate of flow is timed and compared to the flow rate for distilled water. The higher the serum viscosity value, the more viscous (thick) the serum is.

### ***Why is this test done?***

1. Serum viscosity testing is performed to monitor a patient at risk for developing hyperviscosity syndrome.

### ***What are the normal results?***

1. Normal serum viscosity is 1.0 to 1.8.

2. No exact diagnostic cut off exists for hyperviscosity, as different patients will have symptoms at different values.

## Serum Free Light Chains - (sFLC, Kappa & Lambda Free Light Chains)

This test is ordered to help detect, diagnose, and monitor plasma cell disorders, including WM, multiple myeloma, and amyloidosis, and to monitor the effectiveness of treatment. It is more sensitive in detecting low levels of free light chains in the blood than the traditional serum protein tests (serum protein electrophoresis and immunofixation electrophoresis).

Immunoglobulins are made of two light chains and two heavy chains. The heavy chains in each immunoglobulin molecule are made up of one of five types, designated by the Greek letters mu ( $\mu$ )-(IgM); gamma ( $\gamma$ )-(IgG); alpha ( $\alpha$ )-(IgA); delta ( $\delta$ )-(IgD); or epsilon ( $\epsilon$ )-(IgE). The light chains in each immunoglobulin molecule can be either one of two types designated by the Greek letters kappa ( $\kappa$ ) or lambda ( $\lambda$ ). Plasma cells normally produce more light chains than heavy chains and more  $\kappa$  light chains than  $\lambda$  light chains. These excess light chains are not joined to heavy chains and they circulate in the blood as free light chains (FLCs).

With plasma cell disorders, such as WM, multiple myeloma, or amyloidosis, the malignant cells divide uncontrollably and produce large amounts of an abnormal monoclonal immunoglobulin (M protein). This protein may take the form of an intact immunoglobulin or one of its component parts – a kappa or lambda light chain, or rarely, a heavy chain.

Serum free light chain testing can be performed along with a serum protein electrophoresis test to detect abnormal monoclonal protein (M protein) production and to calculate a kappa/lambda free light chain ratio. If the protein electrophoresis test is abnormal, then an immunofixation electrophoresis test is performed to determine which immunoglobulin is present in excess. If a plasma disorder is detected, then the free light chain test may be ordered periodically to monitor the condition and to evaluate the effectiveness of treatment.

### ***Why is this test done?***

1. sFLC testing is often performed at the time of initial diagnostic evaluation to establish a baseline for future reference. At present, however, it is not considered necessary for determining response to therapy or for routine monitoring for most patients with WM. It could be useful for selected patients who are being evaluated for light chain amyloidosis or light chain deposition disease as well as those who already have either of those conditions.

### ***What are the normal results?***

1. Kappa ( $\kappa$ ): 3.3 to 19.4 mg/L. Lambda ( $\lambda$ ): 5.7 to 26.3 mg/L.

2. Free light chains will normally be present in the blood at low levels, with a kappa/lambda ratio of approximately 0.26 to 1.65.



### ***What is the significance of an abnormal result?***

1. A rising involved (monoclonal) individual kappa or lambda light chain level well above the normal range associated with a rising ratio of the involved FLC/uninvolved FLC suggests that the WM cells are producing more monoclonal protein and are proliferating. The reverse suggests that a patient may be responding to treatment. These values may precede changes in IgM by several weeks. It is the trend that is important.
2. Test results need to be correlated over time with the level of the M protein by SPEP, the IgM by nephelometry, hemoglobin, platelet count, white count, kidney function tests, bone marrow findings, the patient's general level of energy for daily tasks, the status of other illnesses that the patient may have, and the oncologist's impression of the status of the disease.

### **Serum Beta-2-Microglobulin**

Beta-2-microglobulin (beta-2-M) is a small membrane protein associated with the heavy chains of class I major histocompatibility complex proteins and is on the surface of all nucleated cells. Serum beta-2-M levels are elevated in diseases associated with increased cell turnover.

Levels are elevated in several benign conditions such as chronic inflammation, liver disease, renal dysfunction, some acute viral infections, and a number of malignancies, especially hematologic malignancies associated with the B-cell lineage, such as WM and multiple myeloma.

### ***Why is this test done?***

Since it is a non-specific marker that can be present in several conditions, an elevated beta-2-microglobulin level alone cannot be used to establish a diagnosis, but it is often part of a panel used to determine prognosis and treatment.

### ***What are the normal results?***

1. 0 to 3 µg/mL

### ***What is the significance of an abnormal result?***

1. Beta-2-microglobulin may be elevated in proportion to tumor mass and is important, when used with other tests, in determining prognosis. Low serum beta-2-microglobulin levels, when used with other tests, may indicate decreased disease activity or the absence of the disease. Likewise, increasing levels may reflect increasing disease burden.

## **OTHER SELECTED TESTS FOR WM PATIENTS**

### **Basic Metabolic and Comprehensive Metabolic Panels**

A Basic Metabolic Panel (BMP) is a blood test consisting of a set of seven or eight biochemical tests and is one of the most common lab tests ordered by health care providers. The BMP provides key information regarding fluid and electrolyte status (calcium, potassium, and sodium); kidney function (blood urea nitrogen, creatinine); and blood sugar (glucose) levels. It is frequently employed as a screening tool during a physical exam because it provides an overall view of the body's metabolic function. The Comprehensive Metabolic Panel (CMP) includes the additional analytes total protein, globulin, albumin, magnesium, and tests for liver function such as ALT (alanine amino transferase), AST (aspartate amino transferase), and total bilirubin. A discussion of each test is beyond the scope of this booklet, except to note that as WM disease progression occurs, the levels of several of these analytes may be affected. In particular, as IgM increases, total protein and globulin will increase and albumin may decrease. Rarely, WM patients may have kidney complications associated with the disease, which can result in abnormal levels of blood urea nitrogen, creatinine, and certain electrolytes.

## Urine Tests

**Bence Jones proteins:** These are abnormal two-unit (dimers) complexes of immunoglobulin light chains found in the urine of some patients, particularly those with multiple myeloma and WM. Protein electrophoresis is performed on a urine sample collected over a 24-hour period, followed by immunofixation.

**Urinalysis:** A low-cost test in which a single urine specimen is examined in order to screen for various diseases. The test determines the presence or absence of the following: specific gravity (concentration of urine), pH (acidity), protein (mainly albumin), glucose (sugar), ketones (products of fat metabolism), red blood cells, white blood cells (leukocytes), bilirubin (suggestive of liver disease or red cell destruction), and urobilinogen (suggestive of liver disease). A microscopic analysis of the urine can also detect the presence of cells, bacteria or yeast, and crystals. If abnormalities are detected in these results, additional testing may be required.

**Uric acid:** A small breakdown product of purines, which are part of DNA, uric acid is excreted primarily by the kidneys, as well as by the gastrointestinal tract. Increased uric acid crystals are deposited in various tissues: knee, elbow, ankle, and particularly the “big toe” joint are favored areas. “Gout” is the general term used for the inflammatory and very painful condition caused by uric acid crystal deposition in joints. Uric acid increases with rapid cell turnover, such as cancer (particularly during chemotherapy for cancer where there is large amount of cell death), as a result of drug therapy (diuretics and certain chemotherapies in particular), and in many other conditions.

## Bone Marrow Biopsy (BMB)

Bone marrow is the soft tissue found in the middle of certain bones (hip and pelvis, sternum, spinal vertebrae, among others) and is the site where red blood cells, white blood cells, and platelets are produced.

Bone marrow aspiration and biopsy are obtained when there is a need to explain an abnormal finding relating to an individual’s red blood cells, white blood cells, or platelets. This procedure can also be performed when a physician wishes to assess the status of a known hematological disease; determine if treatment is required; determine if a treatment for a known disorder requires further modification; or to assess the result of a particular treatment (e.g. after chemotherapy).

The bone marrow biopsy can also provide important information about whether normal blood cell production is occurring in the marrow or whether there are problems in producing certain lineages of blood cells.

The procedure is performed with either a local anesthesia or light sedation. Bone marrow biopsies are usually obtained from the posterosuperior iliac spine (posterior upper aspect of the hip bone). The skin is cleaned with an antiseptic solution, and the area is anesthetized using local anesthetic. A large-bore needle is inserted, and for the aspiration, a fluid specimen in which bone marrow is suspended is removed. For a biopsy specimen, a solid core of marrow cells accompanied by bone is removed. The resulting specimens are evaluated by a pathologist, and a diagnosis is rendered thereafter. Specialized tests can be performed on the aspiration and biopsy specimens, such as flow cytometry (see below) and staining of marrow cells to detect surface antigens (Immunophenotyping). Discomfort from the procedure varies among patients; most individuals will note deep aching bone pain during the procedure and for a few days afterwards – hence the popularity of having the procedure done under light sedation if available.

## Flow Cytometry

This is a test that measures the percentages of cells in a sample of bone marrow and includes information about the cells’ characteristics; size, shape, granularity, and presence of tumor markers/antigens.

These surface antigens on cells can be identified with antibodies that are tagged with fluorescent dyes. The antibody-labeled cells are suspended in a stream of fluid. This stream passes through an instrument called a flow cytometer, which is an electronic laser-based detection instrument capable of analyzing thousands of cells per second, identifying and sorting them according to the characteristics listed above.

Flow cytometry can be used in the diagnosis of WM. If there are no WM cells found in the bone marrow, but the MYD88 mutation is detected along with elevated IgM levels or an IgM M-spike, the diagnosis of MGUS (Monoclonal Gammopathy of Undetermined Significance) is made. A high number of WM cells is a sign of active WM or smoldering WM (without symptoms) that will likely progress to WM.

## MEDICAL GENETICS TESTS

### Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) assays can detect and identify pathogenic organisms; help diagnose genetic diseases; detect biological relationships, such as identifying parents of children; and identify and characterize genetic mutations and rearrangements found in certain cancers.

PCR is a technique in molecular genetics that is used to amplify selected sections of DNA or RNA for analysis. PCR is done in a test tube and relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with an enzyme called DNA polymerase, are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. The amplified segments are then compared to other nucleotide segments from a known source to see if that particular DNA segment in the patient sample is present.

The MYD88 L265P mutation that is expressed in > 90% of WM patients can be detected by using PCR. The presence or absence of this mutation is increasingly considered to be of importance in the diagnosis and treatment of WM patients, and PCR testing for the mutation is now being included in recommendations for the diagnostic workup of WM.

### Genome Sequencing

Whole genome sequencing (WGS) is a method to determine the complete DNA sequence (whole genome) of an individual organism, by establishing the order of the DNA nucleotides or bases (Adenine, Thymine, Guanine, and Cytosine) that make up the organism's DNA. Almost any biological human sample containing a full copy of DNA can provide the genetic material necessary for complete genome sequencing. Such samples may include saliva, epithelial cells, bone marrow, hair (as long as the hair contains a hair follicle), etc.

Today, whole genome sequencing is performed by automated processing in special instruments. In principle, whole genome sequencing can provide raw data on all six billion nucleotides in an individual human's DNA. Because sequencing generates a lot of data, its output is stored electronically and requires a large amount of computing power and storage capacity.

Whole genome sequencing of WM cells in the bone marrow, along with other confirmatory tests, was used to demonstrate the widely expressed mutation MYD88 L265P in WM patients. The presence or absence of this mutation is helpful in the diagnosis of WM.

Other genome sequencing variations are available, including whole exome sequencing, which is a technique for sequencing only the subset of DNA that encodes for proteins (the exome). Humans have about 180,000 such genes, constituting about 1% of the human genome, or approximately 30 million base pairs. The goal of this approach is to identify genetic variation that is responsible for certain common diseases without the high costs currently associated with whole genome sequencing.

Although genome sequencing is not currently used in the everyday clinical setting of WM, it has become a widely used research tool responsible for many recent discoveries about the disease and is being incorporated as part of testing in certain clinical trials. As genome sequencing becomes less expensive, we may one day expect to see it achieve more widespread clinical use.

## TESTS FOR CERTAIN CONDITIONS IN WM

### Amyloidosis

Amyloidosis is a group of conditions of diverse etiologies characterized by the accumulation of insoluble fibrillar proteins (amyloid) in various organs and tissues of the body so that vital function is compromised. The associated disease states may be inflammatory, hereditary, or neoplastic, and the deposition can be local or systemic. Amyloidosis in WM is usually caused by fragments of light chains and affects predominantly the kidneys and heart.

Many tests may be used to diagnose amyloidosis and determine which parts of the body are affected. A biopsy is the only way to make a definitive diagnosis of amyloidosis. This biopsy consists of a small amount of tissue, usually abdominal fat, which is stained for the presence of amyloid and examined under a microscope. An alternative method is a fine needle aspiration biopsy (FNAB) from subcutaneous fat. Imaging tests may be used to find out whether organs such as the heart or kidneys are affected. Biopsies may also be taken from the liver, nerves, heart or kidneys. These are more invasive procedures.

The direct fluorescent antibody technique is used to detect the presence of immune complex depositions in tissues such as the kidneys and in the histological diagnosis of amyloidosis. In this method, a molecule of interest is detected directly, using an antibody labeled or tagged with a fluorescent molecule.

Once diagnosed with light chain amyloidosis, patients may be monitored for the status of their disease activity by means of serum free light chain testing, described in a preceding section.

### Anemia

The simplest definition of anemia is a condition that occurs when one doesn't have enough healthy red blood cells. It is the most common disorder of the blood, and it is the most common manifestation of lymphoplasmacytic cell infiltration in the bone marrow of WM patients. Symptoms of anemia often initiate the process leading to a WM diagnosis. This may include pallor, weakness, fatigue, lightheadedness, palpitations of the heart, and shortness of breath.

To diagnose anemia, a physician may ask about medical and family history, perform a physical exam, and order a Complete Blood Count (CBC), described earlier in this booklet. Of particular interest are the number of red blood cells, the hematocrit, the hemoglobin, and the red cell indices. Red blood cells may also be examined under the microscope for unusual size, shape, and color.

A doctor may order additional tests to determine the underlying cause. These can include looking for a source of chronic blood loss (colonoscopy, endoscopy), iron deficiency (iron studies of the blood), vitamin deficiencies, the presence of cold agglutinins, direct antiglobulin test, urinalysis, serum chemistries, etc. With WM, it is often necessary to study a sample of bone marrow to fully diagnose the cause of anemia.

There are more than 400 types of anemia, which are divided into three major groups by general cause:

**Anemia caused by blood loss** – Red blood cells can be lost through bleeding, which often can occur slowly over a long period of time and go undetected. This kind of chronic bleeding commonly results from the following:

- Gastrointestinal conditions such as ulcers, hemorrhoids, gastritis (inflammation of the stomach), and cancer.
- Use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin or ibuprofen, which can cause ulcers and gastritis.
- Other health conditions, such as multiple pregnancies and heavy menstrual cycles.

**Anemia caused by decreased or faulty red blood cell production** – With this type of anemia, the body may produce too few blood cells or the blood cells may not function correctly. Red blood cells may be faulty or decreased due to abnormal shape, hemoglobin abnormalities, a lack of minerals and vitamins needed for proper red blood cell production, or chronic conditions in the bone marrow. Conditions associated with these causes of anemia include the following:

- Hereditary conditions – sickle cell anemia, thalassemia

- Iron-deficiency anemia
- Vitamin deficiency – e.g. vitamin B-12 and folate
- Bone marrow and stem cell problems – aplastic anemia, myelodysplasia
- Other chronic health conditions such as leukemia or lymphoma – The tumor cells increase and crowd the normal blood-forming cells in the marrow so that they cannot adequately supply the body's need for RBCs. Some cancers may produce chemokines, or substances that may interfere with the normal mechanisms for red blood cell formation. Chronic kidney disease can lead to anemia because the kidneys cannot produce enough erythropoietin, a hormone that stimulates red blood cell production.

**Anemia caused by destruction of red blood cells** - When red blood cells become fragile and rupture prematurely (hemolysis), this is known as hemolytic anemia. Hemolytic anemia can be present at birth or develop later. Sometimes there is no known cause. Known causes of hemolytic anemia may include:

- Stressors such as infections, drugs, snake or spider venom, or certain foods, such as fava beans, if one has glucose 6 PD deficiency
- Toxins from advanced liver or kidney disease
- Vascular grafts, prosthetic heart valves, tumors, severe burns, exposure to certain chemicals, severe hypertension, and clotting disorders
- An enlarged spleen can trap red blood cells and destroy them before they are programmed for normal cell death
- Inappropriate attack by the immune system - In rare cases of WM, the cancerous B-cells produce monoclonal IgM that initiates an antibody attack on the body's own red blood cells at cold temperatures, causing them to break down. This condition is called cold agglutinin disease or cold hemolytic anemia. See **Cold Agglutinin Disease** below.

## Cold Agglutinin Disease (CAD)

Cold agglutinin disease is an autoimmune hemolytic anemia caused by auto-antibodies called cold agglutinins that bind to red blood cells at the cooler temperatures present in the capillaries of the skin and subcutaneous tissues, causing red blood cell destruction (hemolysis). If these antibodies are present in large amounts, they can trigger significant red blood cell destruction that leads to anemia. Cold agglutinins are detectable in low levels in normal patients but are usually insignificant. The presence of cold agglutinins is used to help diagnose certain types of atypical pneumonias and certain hemolytic anemias.

Several tests are used to diagnose cold agglutinin disease and determine any possible associated condition. These include a complete blood count, reticulocyte count, urinalysis, cold agglutinin titer, serum chemistries, and direct antiglobulin test (also called DAT or direct Coombs test). Further testing can involve serology for infectious and autoimmune diseases, serum protein electrophoresis, bone marrow aspiration and biopsy, and flow cytometry. Some blood tests are affected by performing them at room temperature in the laboratory and may have to be repeated after warming the samples.

## Cryoglobulinemia

Cryoglobulins literally mean “cold antibodies in the blood,” referring to the fact that the antibodies involved precipitate at a temperature below 37°C (body temperature) and then re-dissolve upon warming. Unlike the auto-antibodies of cold agglutinins, cryoglobulins do not bind to red blood cells. Cryoglobulins can produce a variety of symptoms because the precipitated antibody physically obstructs smaller blood vessels. WM patients with cryoglobulinemia can experience pain, cyanosis, and numbing of fingers and toes when exposed to cold. Clinical disease can also be associated with the formation of immune complex antigen-antibody (cryoprecipitable immunocomplex) deposits in the kidneys and other tissues.

Three types of cryoglobulinemia have been described: Type I (monoclonal cryoglobulins); Type II (mixed monoclonal IgM-polyclonal IgG immunocomplexes) can be seen as well in autoimmune diseases; Type III (mixed polyclonal IgM-polyclonal IgG immunocomplexes) can be seen with autoimmune diseases, infections, and other diseases.

WM patients should be tested for cryoglobulinemia at diagnosis, since it can not only complicate treatment, but can also affect the results of other laboratory testing used in the management of the disease. For the cryoglobulin test, a blood specimen is obtained in warm (37°C) tubes and kept warm while the serum is removed by centrifugation. The serum sample is then incubated at 4°C and observed for the development of a precipitate. Type I tends to precipitate within 24 hours, while Type III can take up to 7 days. Other tests can help to characterize the type and severity of cryoglobulinemia present. These include rheumatoid factor and other auto-antibodies, serum titers for viral and other infections, urinalysis, complement evaluation, serum protein electrophoresis, serum viscosity, liver function studies, and tissue biopsy.

## Peripheral Neuropathy (PN)

Peripheral neuropathy is a clinical condition in which there is a permanent or transient problem with the functioning of the nerves outside the spinal cord. In the case of WM, the neuropathy may be caused by the monoclonal IgM binding to and attacking components of the nerve, such as the myelin sheath, or as a result of certain treatments for WM that are neurotoxic. The symptoms of a neuropathy may include numbness, weakness, burning pain, and loss of reflexes. The pain may be mild or severe and disabling.

Several tests may be performed to determine the cause and extent of the neuropathy and are used in context with the patient's clinical picture:

**Electromyography (EMG)** – Electromyography measures minute electrical discharges produced in skeletal muscle both at rest and during voluntary contraction and is used in the diagnosis of neuromuscular disease. The electrode for EMG is inserted through the skin, and the resulting electrical discharge or motor unit potential is recorded. Nerve conduction studies, which constitute a separate diagnostic protocol, can be performed simultaneously. Nerve conduction studies measure the speed at which nerves carry electrical signals and are often used to diagnose peripheral neuropathy, as well as carpal tunnel syndrome.

**Nerve biopsy** – This type of biopsy is used to evaluate primary or secondary neuropathies or systemic diseases like amyloidosis. Various special studies can be done on a nerve biopsy, including biochemical analysis, electron microscopy, histology, immunochemistry, various molecular studies, and virology. The site selected for the nerve biopsy is critical and should come from an area of active disease. The sural nerve at the lower part of the leg is the preferred site for the peripheral neuropathy associated with WM.

**Nerve conduction studies** – These studies are a noninvasive method for assessing a nerve's ability to carry a neural impulse and its speed of transmission. Larger peripheral motor and sensory nerves are electrically stimulated at various intervals along a motor nerve. Typically, two metal plates are placed on the skin at a distance from each other. An electrical stimulus passes through one plate and causes the nerve to fire, resulting in a "compound" muscle action potential that can be measured and recorded.

## Visual Disorders

WM can affect vision, particularly if a patient is exhibiting signs of hyperviscosity due to elevated IgM levels. Some tests used for monitoring visual disorders include the following:

**Fluorescein angiography** – This is a technique used to diagnose chorioretinal disease based on the enhancement of anatomic and vascular details in the retina after intravenous injection of fluorescein. The distribution pattern of the fluorescein dye can be viewed and photographed with a fundus camera as a function of its intrinsic fluorescence.

**Fundoscopy (Fundoscopic Examination, Ophthalmoscopy)** – This refers to the examination of the back of the eye (fundus) with an ophthalmoscope, which is part of the routine examination of a patient. Ophthalmoscopy allows a magnified evaluation of the blood vessels, nerves, and retina. Retinal hemorrhages, retinal detachments, and swollen blood vessels, as can occur in patients with elevated serum viscosity and other abnormalities, can be easily viewed using this technique.

**Tonometry** – This technique measures intraocular pressure (in mm Hg) by direct pressure or a puff of air on the eyeball. Tonometry is used to diagnose and manage glaucoma and ocular hypertension and in routine ocular examination. Normal intraocular pressure ranges from 15 to 20 mm Hg.

## IWMF Vision Statement

*Support everyone affected by Waldenstrom's macroglobulinemia while advancing the search for a cure.*

## IWMF Mission Statement

*To offer mutual support and encouragement to the Waldenstrom's macroglobulinemia community and others with an interest in the disease.*

*To provide information and educational programs that address patients' concerns.*

*To promote and support research leading to better treatments and ultimately, a cure.*

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Published by the International Waldenstrom's Macroglobulinemia Foundation (IWMF)

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