Laboratory Diagnosis and Monitoring of Waldenstrom Macroglobulinemia Fact Sheet

This Fact Sheet is subdivided into three parts. The first part briefly introduces what the medical profession knows about the causes of WM. This will help to form the foundation for the second and third parts, which cover diagnosis and monitoring of Waldenstrom macroglobulinemia (WM) through the use of laboratory testing.

THE CAUSE

While much is still unknown as to why any individual person will get WM, it is known that there is an association with a gene mutation that occurs after birth—a "somatic mutation"—to a gene known as MYD88. The forces that cause gene mutations during our lifetimes are many and include smoking, radiation, viruses, chemicals (carcinogens), obesity, hormones, and chronic inflammatory conditions. While this WM mutation can be discovered by bone marrow or blood testing, the cause cannot. WM is not inherited, although approximately 20% of patients diagnosed with WM have a history of the disease or a related blood disorder within their families.

The impact of the disease on the patient can be understood by the symptoms and by lab test abnormalities that can be seen and measured. Jan Waldenström first identified the disease in the 1940s when he noticed that some of his patients were experiencing an unusual combination of symptoms, including bleeding and blurry vision. He was able to figure out that these symptoms were a side effect of the buildup of abnormal cells and the proteins that the abnormal cells secrete into the blood. These proteins—called immunoglobulin M or IgM—make the blood thicker than normal and can cause symptoms that are collectively referred to as hyperviscosity syndrome. About 30% of WM patients will experience this syndrome. See Page 3 for an explanation of serum viscosity.

The cells that are associated with WM are abnormal (mutated) B lymphocytes and plasma cells. While lymphocytes and plasma cells are part of our normal immune system, in WM their growth is unregulated. An overgrowth of these cells can cause tumors in the lymph glands, bone marrow, the brain, or other tissues in the body.

The term "macroglobulinemia" in the disease name is a reference to the IgM protein, because it is naturally a very large molecule. The term can be divided into its three constituent parts: "macro" means large, "globulin" is a type of protein, and "emia" means in the blood.

DIAGNOSIS

The diagnosis of WM, from a laboratory point of view, requires identification of two main findings: the abnormal lymphocytes and plasma cells in the bone marrow and the IgM proteins made by these cells circulating in the blood.

Identifying the Cells: Pathologists study a tissue sample using a microscope to visually identify the abnormal cells that are characteristic of WM. Usually, diagnostic tissue samples are taken from bone marrow for this purpose. Special stains and other methods beyond simple microscopy are used to establish the diagnosis, which is not always straightforward. One of these special methods looks for the presence of the MYD88 mutation, which is found in about 90-95% of WM patients. Diseases like multiple myeloma, marginal zone lymphoma, and others can look very similar to WM and cannot always be differentiated without additional testing.

Identifying the Proteins: IgM and Free Light Chains: Blood and urine tests must be performed to identify the presence of the abnormal proteins associated with WM. The abnormal proteins—IgM and its pieces or chains—are produced by the tumor cells and serve as unique signatures of the disease. Some of these chains are referred to as "light chains" because they are smaller than the others that make up the entire molecule.

We use the light chains to help determine whether the IgM molecules are normal or abnormal. Light chains normally come in two varieties, kappa or lambda. If an excess of one or the other type is present, that is indicative of disease, since normally we would see a mix of the two types in a predictable ratio.

We use serum and urine protein electrophoresis (SPEP and UPEP) and immuno-focusing electrophoresis (IFE) to detect these proteins. If too many IgM proteins with one specific charge are seen, it produces a "spike" pattern on the tracing of the protein's electrical charge, and, in combination with the tissue sample findings, a diagnosis of WM can be made. Once the diagnosis is established, that IgM protein can be identified as either IgM kappa or IgM lambda and will be used to monitor disease regression or activation.

MONITORING THE DISEASE

Normal lab values (or "reference values") provided in this Fact Sheet are from the Mayo Clinic, a large Midwest reference laboratory in the United States. The ranges of normal lab values vary between laboratories and will always be provided as part of your individual lab test report for comparison purposes. If a normal value provided here differs from your laboratory report, you

should rely on your laboratory report. When possible, it is preferable to have your tests performed at the same place/laboratory each time your physician orders them, so there will be no variation of the normal range. Your physician may not order every one of the tests described here.

IgM: Since the excess IgM proteins in WM come from the abnormal tumor cells, the quantity of IgM can be used to monitor disease activity. As the disease progresses, more protein is produced, and the amount of IgM in the blood goes up. Conversely, as the disease responds to treatment, the IgM proteins decline. The normal range is 37-287 mg/dL.

SPEP: The SPEP may be used in disease monitoring as well since the abnormal proteins will produce a characteristic pattern on the tracing (see page 2).

Kappa and Lambda Light Chains: The light chains are pieces of the IgM molecule, and these are often monitored. The ratio will be abnormal in the presence of disease. These can be measured in serum and urine (see page 2). The normal reference values for kappa free light chains are 0.33-1.94 mg/dL and for lambda free light chains are 0.57-2.63 mg/dL. The normal range for kappa/lambda free light chain ratio is 0.26-1.65.

24-hour Urine Testing: A 24-hour urine collection is a simple lab test that measures certain substances in the urine. The test is used to check kidney function and to look for the abnormal light chain proteins that can be found in WM. The entire IgM protein cannot be found in the urine because it is too large to pass through the kidney. Only the smaller pieces of the IgM protein—the light chains—can be found there. This urine test is also useful in the diagnosis of amyloidosis, a serious complication of WM. A 24-hour urine collection is done by collecting your urine in a special container over a full 24-hour period. The container must be kept cool until the urine is returned to the lab.

Serum Viscosity: If the IgM is above 3,000-4,000 mg/dL, one should consider testing the serum viscosity. This measures the "thickness" or "sludginess" of the serum. If the serum is very viscous (hyperviscous), blood flow to organs such as the retina, brain, and kidneys can become severely damaged (hyperviscosity syndrome). This is why some WM patients have symptoms of blurry vision, for example. If serum viscosity is left untreated, it can cause blindness. If the blood is too thick, additional therapies might be used, including plasmapheresis, to remove the unwanted protein. A plasma exchange can temporarily normalize viscosity, but subsequent treatment of the underlying WM is necessary to better control high IgM levels. The normal

range for serum viscosity is given as a ratio relative to water (which is 1.0): normal is less than 1.5 cp.

Beta-2 Microglobulin: This is a small protein in the blood that is shed by the tumor cells, and it increases as the tumor cells increase, so it is used to monitor disease burden. As such, it is considered an important prognostic tumor marker. It also can spike transiently during therapy as tumor cells break down. The normal range for beta-2 microglobulin is 1.21-2.70 mcg/mL.

Lactate Dehydrogenase (LDH): This is an enzyme in many of the cells of the body, including white blood cells. It is involved in tumor metabolism. Tumor cells produce more LDH than normal cells. Elevated LDH can serve as a prognostic marker and to monitor treatment response and recurrence. The normal range of LDH is 122-222 U/L.

Amyloid: In some cases, the kappa or lambda light chains that build up during WM can deposit in the tissues where they are known as amyloid. Amyloid buildup in the organs such as the liver, heart, or kidney can cause damage. Lambda light chains are more likely to form these deposits than kappa. Approximately 3% of WM patients will experience complications caused by amyloid deposits in organs. The most common method used to diagnose amyloid is to take a small needle aspiration sample of abdominal wall fat and stain it for the presence of amyloid.

IgG Levels: IgG is the most common type of immunoglobulin and, as such, is very important for normal immune function to fight off infection. One consequence of WM treatment can be that normal lymphocytes are damaged along with the abnormal ones that are targeted. When this happens, IgG levels can go down, and the risk of infection increases. To avoid this, intravenous immune globulin infusions (IVIG) may be necessary to maintain healthy IgG levels. Normal reference values are 767-1,590 mg/dL. Intravenous immunoglobulin is a safe product. The donors are screened, and the plasma is heat-treated (pasteurized) and filtered.

Complete Blood Count (CBC): Several components of the CBC are carefully monitored in patients with WM, including the white blood cells (WBC), hemoglobin (Hgb or Hb), and platelet (Plt) count. Infection, anemia, and bleeding may be complications of the disease.

> *White Blood Cells:* Reduction of WBCs can be seen during active disease or with treatment. Increased WBCs can be seen in the case of infection. The normal range for the WBC count is 3.4 to 9.6 $x10^9$/L.
>
> *Hemoglobin:* Hemoglobin is a measure of the protein that carries oxygen in red cells. Low hemoglobin can be seen in WM if the tumor cells are interfering with normal red

cell production in the bone marrow or if iron is low, which leads to anemia. Blood transfusion may be ordered if the hemoglobin is below 7 g/dL or if symptoms are present, such as shortness of breath. Because anemia can also be caused by vitamin or mineral deficiencies, physicians may follow up with testing of iron parameters, vitamin B12, and folic acid. Iron parameters include total iron, transferrin, ferritin, total iron binding capacity, and iron saturation percentage. Iron deficiency can be nutritional but can also be caused by a protein secreted by the WM cells themselves. The normal hemoglobin for males is 13.2-16.6 g/dL and for females is 11.6-15.0 g/dL.

Platelets: Platelets are important for proper blood clotting. Normal platelet counts range from 152,000 to 440,000 platelets per mm^3 of whole blood (152 to 440 K/uL). Low platelet counts can lead to bleeding problems. Platelet transfusion may be necessary if they drop below 50,000 per mm^3.

CD4 Counts: Some therapies (especially bendamustine) can produce a prolonged drop in subsets of immune cells, including CD4 positive lymphocytes. These important cells, which help fight infection, often are monitored to evaluate the strength of the immune system and, if low, may predict the need for supplemental therapies like antibiotics. Normal CD4 counts are between 500 and 1,500 cells/microliter.

Creatinine: Creatinine in the blood is increased when kidney function is impaired. The kidneys can be at risk if there are too many of the kappa or lambda light chains in the blood that are produced by the tumor cells. Normal creatinine is 0.6-1.2 mg/dL in males and 0.5-1.1 mg/dL in females.

ALT and AST: These enzymes are increased if a patient has liver damage or disease (like hepatitis). This is not a typical complication of WM, but an increase in ALT and/or AST can be caused by some of the therapies. The normal range for ALT is 7 to 55 units per liter (U/L) and for AST is 8-48 U/L.

Uric Acid: Uric acid can be increased in the blood following breakdown of tumor cells, so it can occur during treatment. Excess uric acid can lead to gout if the crystals are deposited in the joints. The normal range for uric acid is 3.7-8.0 mg/dL.

Cholesterol: Serum cholesterol has been known to drop in WM. There is some speculation that this may be because the disease increases cholesterol metabolism and IgM interferes with the measurement of HDL cholesterol in some test methods used.

A few general points to mention about interpreting lab tests may be helpful. If a lab value is flagged as abnormal but is only a little bit higher or lower than the normal range, that may or may not be significant. The important thing is to monitor the trend over time. Many WM patients find it helpful to keep track of their lab tests to get a better picture of what may be going on.

Some test results may take longer to come back because they are sent to an outside laboratory, including the SPEP, IFE, and serum viscosity.

Many laboratory tests rely on immunologic methods—meaning that immunoglobulins are used as part of the lab test—and the immunoglobulin M protein in the blood of WM patients can, occasionally, cause interference. The interference can result in falsely high or low results. A wide variety of laboratory tests can be affected, including blood counts, serum sodium, calcium, phosphorous, thyroid function tests, bilirubin, HDL, and more. Luckily, these types of interferences are the exception rather than the rule.

CONCLUSION

Laboratory testing provides valuable information used for diagnosis and monitoring patients with WM. It is worthwhile to become familiar with the tests in order to understand their purpose and meaning. Unusual or unexpected results should be discussed with your physicians.

ACKNOWLEDGEMENTS

The IWMF acknowledges Janis M. Atkinson MD, FCAP, System Administrative Laboratory Director, Ascension IL, Medical Laboratory Director, Ascension Saint Francis Hospital, Evanston, IL, for her primary authorship of this IWMF Fact Sheet. In 2019, Dr. Atkinson wrote an article for the *IWMF Torch* entitled *Laboratory Diagnosis and Monitoring of Waldenstrom's Macroglobulinemia.* That article was used as the basis of this IWMF Fact Sheet.

The IWMF also acknowledges the important contributions to our knowledge about WM that have been published by the International Workshops on Waldenstrom Macroglobulinemia (IWWM) and the National Comprehensive Cancer Network (NCCN®).

ABOUT THE IWMF

The International Waldenstrom's Macroglobulinemia Foundation (IWMF) is a patient-founded and volunteer-led, nonprofit 501(c)(3) organization with an important vision, "A World Without

WM," and a mission to "Support and educate everyone affected by WM to improve patient outcomes while advancing the search for a cure."

More information about Waldenstrom macroglobulinemia and the services and support offered by the IWMF and its affiliate organizations can be found on our website, www.iwmf.com.

The IWMF relies on donations to continue its mission, and we welcome your support. The Foundation maintains a Business Office at 6144 Clark Center Ave., Sarasota, FL 34238. The Office can be contacted by phone at 941-927-4963, by fax at 941-927-4467, or by email at info@iwmf.com.

This Fact Sheet was supported by Pharmacyclics, an AbbVie Company, and Janssen Biotech, Inc.