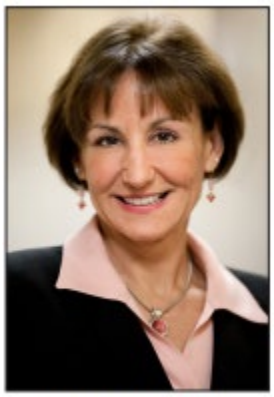
LABORATORY DIAGNOSIS AND MONITORING OF WALDENSTROM'S MACROGLOBULINEMIA

BY JANIS ATKINSON, MD



Dr. Janis Atkinson

Dr. Janis Atkinson graduated from Rush Medical College in Chicago, IL, in 1986. She completed an internal medicine internship and a residency in pathology at Northwestern University, Chicago, from 1986 through 1992 and was appointed to the medical staff of Saint Francis Hospital, Evanston, IL, in 1992.

At Saint Francis Hospital (now known as AMITA Health Saint Francis Hospital), she has served as chair of the Department of Pathology and medical director of the Laboratory since 2000. She is also an assistant clinical professor at the University of Illinois and teaches medical students and residents.

She has served in several leadership roles for her hospital and health care system, including member of the Board of Directors, president of the Medical Staff, president of the Medical Executive Committee, president of a multi-hospital pathology group, and vice president of medical affairs for Alverno Laboratories, which serves the multi-hospital system.

She lives in Wilmette, IL, with her husband Jeff. They have two children, Grant and Kelsey.

My experience with Waldenstrom's macroglobulinemia (WM) is both professional and personal. As a pathologist, one of my jobs is to recognize and diagnose this disease through analysis of biopsy material and lab test results. On a personal level, my husband was diagnosed with WM in February of 2011. He has been treated with three different monoclonal antibody and chemotherapy regimens and, after a good response to bendamustine and Rituxan, is currently in a "watch and wait" mode.

This article is for those who were just diagnosed with WM and for those who are medium- or long-term survivors. It is sub-divided into three parts. The first part will introduce what the medical profession knows about the causes of WM. This will help to form the foundation for the second and third parts, which will cover diagnosis and monitoring.

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 the mutation can be discovered by blood testing, the cause of the

WM mutation cannot. WM is usually not inherited, and most people affected have no history of the disorder in their family.

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 IgM—make the blood thicker than normal and can cause the symptoms that are collectively referred to as hyperviscosity syndrome. About 30% of patients will experience this syndrome.

The cells that are associated with WM are abnormal 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, 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 things: the abnormal lymphocytes and plasma cells and the IgM proteins made by the cells.

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 or lymph node for this purpose. Special methods beyond simple microscopy are used to establish the diagnosis, which is not always straightforward. Other diseases, like multiple myeloma, can look very similar to WM, and the two cannot always be differentiated without additional testing.

Identifying the Proteins, Including 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 constituent pieces—are produced by the tumor cells and serve as unique signatures of the disease. The IgM protein is a large molecule composed of different pieces or chains. 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 the 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 proteins with one specific charge are seen, it produces a "spike" pattern on the tracing of the protein's electrical charges, and, in combination with the tissue sample findings, a diagnosis of WM can be made. Once the diagnosis is established, that molecule can be identified as either IgM kappa or IgM lambda and will be used to monitor disease regression or activation.

Monitoring the Disease

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 IgM goes up. Conversely, as the disease responds to treatment, the IgM proteins decline. Normal ranges may differ at various institutions; one example of normal range from a large national lab is 40-230 mg/dL.

Normal lab values (or "reference values") provided in this article are from the Mayo Clinic, a large Midwest reference laboratory. 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 lab report, you should rely on your lab report.

SPEP: The SPEP may be used in disease monitoring as well, since the abnormal proteins will produce a characteristic pattern on the tracing.

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.

24-hour Urine Testing: A 24-hour urine collection is a simple lab test that measures what's 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. 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: Probably the single most important test to check after establishing the diagnosis is serum viscosity. This test measures the "thickness" or "sludginess" of the blood. The IgM proteins can cause red blood cells to stick or clump together, which makes blood thicker (like espresso compared to coffee). If the serum if very viscous, blood flow to the organs such as brain and kidney can be affected. This is why some patients have symptoms of blurry vision, for

example. If the blood is too thick, additional therapies might be used, including plasmapheresis, to remove the unwanted protein. A plasma exchange can normalize viscosity. The normal range for serum viscosity is given as a ratio relative to water (which is 1.0): 1.4-1.8.

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. Normal ranges for beta-2 microglobulin are 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. Normal levels of LDH are 122-222 U/L.

Amyloid: Rarely, the kappa and 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 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. A Congo Red stain is applied to the fat sample in the laboratory, and, if an apple-green refractive index is seen under polarized light, the presence of amyloid is confirmed.

IgG Levels: IgG is the most common immunoglobulin in the body, and, as such, is very important to 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 487-1,327 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, and platelet count. Infection, anemia, and bleeding may be complications of the disease.

> *WBC:* Reduction of WBCs can be seen during active disease or with treatment. Increased WBCs can be seen in the case of infection. Low WBC count is a common side-effect of therapy that targets the white cells, like bendamustine and Rituxan. These therapies usually diminish the lymphocyte portion of the WBCs. The normal range for the WBC count is 3.4 to 9.6 $x10^9$/L.
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> *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, 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. 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 fragments of cells that are important for proper blood clotting. Low platelets can lead to bleeding problems. Transfusion may be necessary if they drop below 50,000 x 10^9/L.

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 1500 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/AST: These enzymes are increased if a patient has liver disease (like hepatitis). This is not a typical complication of WM, but an increase in ALT/AST can be caused by some of the therapies.

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 deposit in the joints.

Cholesterol: Serum cholesterol has been known to drop in WM. There is some speculation that this may be because the disease increases cholesterol metabolism.

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 trend their lab tests to get a better picture of what may be going on.

Some tests may take longer to come back because they are sent to an outside laboratory, including the tests for SPEP, the 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.

Scoring System for WM

Lab test results provide valuable information for staging WM. The International Prognostic Scoring System for WM includes the following five features:

1) Age >65 years
2) Hemoglobin less than 11.5 g/dL
3) Platelets less than 100,000 x 10^9/L
4) Beta-2 microglobulin greater than 3 mcg/L
5) IgM concentration greater than 7 g/dL (or 7000 mg/dL).

This scoring system can help predict survival. Patients with lower levels of IgM and beta-2 microglobulin tend to do better than those with higher levels. Patients with WM who are older, are anemic (based on a low blood hemoglobin level), or have a low platelet count tend to have a poorer outcome.

Except for age, each of these factors is worth a single point. The points are added to make a score, which is used to divide patients into three risk groups:

Low Risk: Includes patients 65 or younger who have no more than one point.

Intermediate Risk: Includes patients who are older than 65 with two or fewer points, and those younger than 65 who have two points.

High Risk: Includes those of any age who have at least three points.

Conclusion

Laboratory testing provides valuable information used for diagnosis, monitoring, and prognostic scoring for 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.