CLL SOCIETY
Resource Library

BLOOD TESTING

Regular blood testing for those with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) is an important part of managing the disease, as it can provide valuable insights into your overall health. These laboratory tests assist you and your healthcare team in assessing prognosis, determining the most suitable treatment options, monitoring response to treatment, and the treatment's effectiveness, as well as observing overall health.

WHAT BLOOD TESTS ARE USUALLY PERFORMED?

The blood tests most frequently used to diagnose and manage CLL and SLL include a complete blood count (CBC) with differential and flow cytometry (FC). Cytogenetic testing, which are tests looking for genetic changes, are also performed. These include fluorescence in situ hybridization (FISH), TP53 mutation, immunoglobulin heavy-chain variable region (IGHV) mutation, karyotyping, and next-generation sequencing (NGS).

WHAT DO THE TEST RESULTS MEAN?

Within any one blood test, there can be many components with a range of results. For those with CLL and SLL, it is important to understand that **a single abnormal laboratory result is usually not the basis for deciding if it is time for treatment.** Instead, blood tests will be repeated often so that you and your healthcare team can closely monitor the disease and watch for any unusual trends over time.

COMPLETE BLOOD COUNT WITH DIFFERENTIAL

This common and inexpensive blood test looks at the different blood cells produced by the bone marrow. It is used to diagnose the disease and is checked on a routine schedule that is specific to each person's needs. The following blood components are tested as part of the CBC with differential:

- **Hemoglobin (Hgb)** – This blood test measures the amount of hemoglobin that is present. Hemoglobin is a molecule present that is responsible for transporting oxygen to various tissues throughout the body.
- **Red Blood Cells (RBC)** – Also called erythrocytes, red blood cells contain hemoglobin and play a vital role in the transport of oxygen throughout the body. If the number of red blood cells decreases, this will also lead to a drop in hemoglobin levels and anemia can occur. Individuals with anemia may experience fatigue, weakness, shortness of breath, pale skin, lightheadedness, or other symptoms.
- **White Blood Cells (WBC)** – Also called leukocytes, white blood cells are a crucial part of the immune system because they are responsible for fighting infections, cancers, and inflammation in the body. There are many distinct types of white blood cells including neutrophils, eosinophils, basophils, monocytes, and lymphocytes. While CLL and SLL specifically involve the cancerous (massive reproduction) of abnormal B-lymphocytes, neutrophils, T lymphocytes, and natural killer cells, may also be impacted.
- **Neutrophils** – As a type of white blood cell, neutrophils act as one of the first lines of defense against infection. When the neutrophil count is too low, there is an increased risk of bacterial infection.
- **Lymphocytes** – As a type of white blood cell, lymphocytes are responsible for antibody production and fighting off infection. The lymphocyte count is usually elevated in those with active CLL and SLL but may be abnormally low in people who are on certain types of treatment.
- **Absolute Lymphocyte Count (ALC)** – The absolute lymphocyte count reflects the number of white blood cells that are lymphocytes (rather than the percentage) and when followed over time it can show if the cancer is progressing or if it is responding to therapy. There is no ALC value that demands treatment. ALC Counts can exceed 500,000 or more. But if the person otherwise feels good with no other symptoms, then continuing to monitor the disease without starting treatment is appropriate.
- **Platelets** – Also called thrombocytes, platelets are tiny cell fragments that help control bleeding. Whenever an injury occurs, platelets come together to form a clot at the site of injury. When there aren't enough platelets in the body, it can lead to increased bruising or bleeding.

FLOW CYTOMETRY (FC)

While FC is usually performed using a blood sample, this specialized test can examine cells from the bone marrow and lymph nodes as well. This test analyzes biomarkers on the surface of cells to identify their unique immune fingerprint. The process, called immunophenotyping, provides a definitive diagnosis of CLL and SLL. FC has been used in clinical trials to monitor an individual's response to treatment, predict relapse, and inform decisions about further treatment strategies. FC can also be used to assess measurable residual disease (MRD) and detect as little as one single

BLOOD TESTING (continued)

cancer cell that exists among 10,000 cells. When no cancer cells are found among 10,000 cells, the result is said to be undetectable MRD (uMRD) to the level of one in 10,000 (10^{-4}) cells.

CYTOGENETIC TESTING

This type of testing looks for changes in the chromosomes of the cells. Understanding genetic characteristics can help determine if the risk level of the cancer is high, intermediate, or low. Cytogenetic testing can also predict how the disease may progress over time and how it may respond to therapy. The appropriate treatments should then be offered based on the results of these tests using established guidelines. Cytogenetic testing is usually conducted on a blood sample, but it may also be performed using a sample of bone marrow. The most important cytogenetic tests for those with CLL and SLL include:

- **Fluorescence-In-Situ-hybridization** – FISH testing is a laboratory technique to detect chromosomal abnormalities within cancer cells. Common abnormalities that are associated with CLL and SLL include deletions (missing parts) of chromosome numbers 11, 13, and 17. If missing parts are found on these particular chromosomes, they are referred to as deletion 11q, deletion 13q, or deletion 17p. An additional chromosomal abnormality FISH testing can detect is an extra copy of chromosome number 12. If this is detected, it is referred to as trisomy 12. The results of FISH testing can help your healthcare team understand how aggressively the cancer might behave and can predict how the disease will respond to certain therapies. The chromosomal changes detected by FISH testing can change over time, so the test should be performed at the time of diagnosis (or minimally before the first treatment is started), and again before each new subsequent treatment.
- **TP53 Mutation** – TP53 is a gene that helps to slow the growth of cancer cells. The TP stands for tumor protein. TP53 mutation status can change over time, so it must be evaluated at diagnosis (or minimally before the first treatment is started), and again before each subsequent new treatment.
  - » *TP53 mutation* means that the gene has changed and become ineffective at slowing cancer growth. It is associated with an adverse type of CLL and SLL that is more difficult to treat. Those with TP53 mutation should not receive traditional chemoimmunotherapy since it will not be effective in treating the disease.
  - » *TP53 unmutated* means that the gene is unchanged and can still suppress cancer growth.
- **Immunoglobulin Heavy-chain Variable region Mutation** – The IGHV gene is responsible for fighting off cancer cells. This test looks to see if the IGHV gene has mutated or not. IGHV status does not usually change over time, so it only needs to be tested once, typically at the time of diagnosis or before the first treatment is started. IGHV status can help your healthcare team understand how the disease might behave in the future and the prognosis (outlook).
  - » *Mutated IGHV* status is associated with better responses to certain treatments, longer periods of remission, prolonged survival, and these individuals may do very well with time-limited treatment options (such as BCL2 inhibitors).
  - » *Unmutated IGHV* status is associated with those who may require earlier treatment. Those with unmutated IGHV should not receive traditional chemoimmunotherapy since it will not be effective in treating the disease.
- **Karyotyping** – This test makes a map of all the chromosomes or genetic material inside the CLL and SLL cells. The presence of certain changes may predict how well individuals will respond to certain treatments. When three or more chromosomal abnormalities are found, the person is said to have a complex karyotype (CK), which is generally associated with a poorer prognosis.
- **Next Generation Sequencing** – NGS can be used for two distinct purposes. It can look for genetic mutations (including TP53, Notch1, C481 and many others) that help to determine prognosis and predict responses to therapy. NGS can also be used as another methodology for MRD testing. However, when NGS is used for MRD testing, it requires a blood sample that was collected early in the disease so that the sample can later be compared to a blood or bone marrow sample that is collected when the cancer becomes more active. As mentioned above, MRD testing using flow cytometry can detect as little as one single cancer cell that exists among 10,000 cells (10^{-4}). But MRD testing using NGS is much more sensitive and can detect as little as one single cancer cell that exists among one million cells (10^{-6}). So MRD testing utilizing NGS can be utilized to detect even deeper remissions, monitor the effectiveness of treatments, and to watch for signs of relapse. While NGS testing has not yet a become a standard of care for CLL and SLL, it is frequently used in other blood cancers after the completion of time-limited therapy or after a specified duration of continuous therapy has been completed.