Please Note: To view the Figures and Images contained within this education activity in color, access the electronic version of the reading.

CASE HISTORY

This peripheral blood smear is from a 51-year-old female who had a prior splenectomy for a 30-year history of polycythemia vera. A routine CBC showed a significant change in blood counts from previous exams. Laboratory data include: WBC = 33.5 x 10^9/L; RBC = 2.28 x 10^12/L; HGB = 7.1 g/dL; HCT = 20.0%; MCV = 88 fL; RDW = 16.1; PLT = 25 x 10^9/L.

ACUTE MYELOID LEUKEMIA (AML)

Overview of AML

AML is a heterogeneous group of malignant disorders characterized by an abnormal proliferation of immature cells of the myeloid lineage. These cells accumulate in the bone marrow and replace normal marrow elements leading to anemia, leukopenia, and thrombocytopenia, which cause many of the clinical complications of AML.

Morphologically, AML is defined by the presence of 20% or more myeloid blasts in the peripheral blood leukocyte differential or bone marrow aspirate differential count. Exceptions to this rule of 20% are discussed below (AML with recurrent genetic abnormalities on page 7). In the blood, it is recommended that the blast percentage be derived from a 200 cell leukocyte differential; in the bone marrow a 500 cell differential is recommended. Myeloid blasts include myeloblasts, monoblasts, and megakaryoblasts. Promonocytes are considered as “blast equivalents” for determining the blast percentage in cases of AML with monocytic differentiation. In addition, abnormal promyelocytes seen in acute promyelocytic leukemia are also considered “blast equivalents.”

Myeloblasts

Myeloblasts are typically medium to large cells (15 - 20 µm in diameter) with a high nuclear to cytoplasmic ratio. The nucleus is often round but may be irregular or folded and has finely reticulated chromatin with one or more distinct nucleoli. The cytoplasm is basophilic with few or no granules. Auer rods may be seen, which are pink to red, rod-shaped cytoplasmic inclusions that represent a crystallization of azurophilic (primary) granules. Auer rods define a cell as belonging to the myeloid lineage (Figure 1. on the following page). In the absence of Auer rods, it is not possible to definitively identify the lineage of a blast cell using routine Wright-Giemsa staining. Thus, lineage determination typically relies on flow cytometric immunophenotyping or cytochemistry. Flow cytometry can identify common myeloid antigens such as CD13, CD33, and CD14, as well as determine myeloperoxidase expression (Figure 2. on the following page). Cytochemical staining (e.g., myeloperoxidase or Sudan black B) may also be performed to help identify the lineage, but is used less commonly if flow cytometry is available.
Clinical Features of AML

Patients with acute myeloid leukemia are predominantly adults with a median age at diagnosis of 70 years. Signs and symptoms at the time of diagnosis often include fatigue and pallor (due to anemia), bleeding or bruising (due to thrombocytopenia), and fever or infection (due to neutropenia). Patients are treated with chemotherapy to induce remission. Once remission has been achieved, additional therapy is required to prevent relapse. These regimens are typically consolidation chemotherapy and, for some patients, allogeneic peripheral blood stem cell transplant or bone marrow transplant.
Risk Factors for AML

The current World Health Organization (WHO) Classification of AML (Table 1. below) is primarily based on the presence of certain genetic abnormalities in the myeloblasts as well as clinical data, such as a history of Down syndrome, history of previous cytotoxic therapy, or history of a previous myelodysplastic or myeloproliferative syndrome. This classification scheme is useful because it divides AML into reproducible diagnostic categories that provide important prognostic information, which helps guide therapy.

Most cases of AML have no identifiable cause (i.e., de novo AML), although several risk factors and predisposing conditions are known. Risk factors include certain genetic disorders, previous radiation therapy or chemotherapy, and certain environmental or occupational exposures (e.g., benzene), although the risk of the latter is not well-defined. AML can also arise due to progression of an underlying hematopoietic neoplasm such as a myeloproliferative neoplasm or myelodysplastic syndrome. The various risk factors for AML are summarized in Figure 3. on the following page and are further discussed below.

Table 1. World Health Organization Classification of AML (2008)

<table>
<thead>
<tr>
<th>AML with Recurrent Genetic Abnormalities</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AML with Balanced Translocations/Inversions</td>
<td></td>
</tr>
<tr>
<td>AML with t(8;21)(q22;q22)</td>
<td></td>
</tr>
<tr>
<td>AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)</td>
<td></td>
</tr>
<tr>
<td>Acute promyelocytic leukemia with t(15;17)(q22;q12)</td>
<td></td>
</tr>
<tr>
<td>AML with t(9;11)(p22;q23)</td>
<td></td>
</tr>
<tr>
<td>AML with t(6;9)(p23;q34)</td>
<td></td>
</tr>
<tr>
<td>AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2)</td>
<td></td>
</tr>
<tr>
<td>AML with t(1;22)(p13;q13)</td>
<td></td>
</tr>
<tr>
<td>AML with Gene Mutations*</td>
<td></td>
</tr>
<tr>
<td>AML with mutated NPM1*</td>
<td></td>
</tr>
<tr>
<td>AML with mutated CEBPA*</td>
<td></td>
</tr>
</tbody>
</table>

Myeloid Proliferations Related to Down Syndrome

AML associated with Down Syndrome
Transient abnormal myelopoiesis

Therapy-Related AML

AML with Myelodysplasia-Related Changes

AML, Not Otherwise Specified

*Provisional entities
Risk Factors for AML: Genetic Susceptibility

Certain rare genetic disorders are known to predispose to AML. These disorders include Fanconi anemia, Bloom syndrome, Shwachman-Diamond syndrome, and severe congenital neutropenia (Kostmann syndrome), among others. Of note, some of these genetic disorders also predispose to development of a myelodysplastic syndrome, which can subsequently progress to AML.

The most common genetic abnormality that predisposes to AML is Down syndrome. Individuals with Down syndrome have a 10- to 100-fold increased risk of both acute lymphoblastic leukemia and acute myeloid leukemia. Cases of AML in these patients frequently demonstrate megakaryoblastic differentiation and have unique clinical and molecular characteristics that warrant their recognition as a separate category within the WHO Classification. In addition, approximately 10% of Down syndrome neonates have a unique hematologic disorder referred to as transient abnormal myelopoiesis that has clinical and morphologic findings indistinguishable from AML (Figure 4. below). In most cases, this disorder resolves spontaneously over a period of weeks to months. However, in approximately 25% of cases non-transient AML develops within 1 to 3 years.

Figure 4.

Myeloid leukemia associated with Down syndrome shows a blast in the center of the image below a nucleated red blood cell.
Risk Factors for AML: Previous Cytotoxic Therapy
AML can occur in patients who have previously received cytotoxic therapy (either chemotherapy or radiation therapy) for a different disease. These cases are classified as “Therapy-Related AML” in the WHO Classification. The preceding disease may be a hematologic malignancy, non-hematologic solid tumor, or a non-neoplastic disease. The most common form of t-AML occurs 5 to 10 years after exposure to alkylating agent chemotherapy or radiation therapy. A second form of t-AML occurs 1 to 5 years after topoisomerase II inhibitor chemotherapy. These two forms of t-AML have different clinical, morphologic, and genetic features, although their separation may not always be clear cut (Figure 5. below). The prognosis of t-AML is generally poor.

Figure 5.

Therapy-related acute myeloid leukemia. A promonocyte is present at left with a myeloblast at right and the background is notable for many hypogranular platelets.

Risk Factors for AML: Certain Hematopoietic Neoplasms
The natural history of myelodysplastic syndromes, myeloproliferative neoplasms, and “overlap” myelodysplastic/myeloproliferative neoplasms is progression to acute leukemia, often AML.

Myelodysplastic Syndromes
Myelodysplastic syndromes are a group of clonal (neoplastic) hematopoietic stem cell disorders characterized by ineffective hematopoiesis involving one or more of the major myeloid lineages (granulocytic, erythroid, and megakaryocytic). Ineffective hematopoiesis is manifest as morphologic dysplasia involving any of these lineages with resultant peripheral blood cytopenias and, usually, a hypercellular bone marrow. Myelodysplastic syndromes are classified primarily according to the lineages involved and the percentage of blasts in the blood or bone marrow. The percentage of blasts is a strong predictor of the risk for progression to AML, with cases having 5 - 19% blasts in the blood or 10 - 19% blasts in the bone marrow having the highest risk. In the 2008 WHO Classification, these cases are included in the category “AML with Myelodysplasia-Related Changes.” These patients generally have a poor prognosis.
Myeloproliferative Neoplasms

Myeloproliferative neoplasms are clonal hematopoietic stem cell disorders characterized by proliferation of one or more of the myeloid lineages (granulocytic, erythroid, megakaryocytic, and mast cell). The prototypical neoplasm in this category is chronic myelogenous leukemia (CML), which is consistently associated with an abnormal fusion gene between the *BCR* gene on chromosome 22 and the *ABL1* gene on chromosome 9. The natural history of this disorder is from an initial “chronic phase” in which there is marked leukocytosis with absolute basophilia and usually < 2% blasts in the peripheral blood, to an “accelerated phase,” which may have up to 19% blasts in the blood, to a “blast phase” (AML) that is diagnosed when 20% or more myeloblasts are found in the blood or bone marrow (Figure 6. below). Interestingly, because the abnormal *BCR-ABL1* fusion gene is present in an early stem cell, in approximately 20 - 30% of cases of “blast phase” CML the blasts will be lymphoblasts instead of myeloblasts. Several other myeloproliferative neoplasms can progress to AML, most commonly polycythemia vera and primary myelofibrosis.

Figure 6. Chronic myelogenous leukemia, BCR-ABL1 positive, myeloid blast crisis. The blasts showed a megakaryoblastic phenotype by flow cytometry. In this image, 5 blasts are present and a neutrophil. The blasts are large with abundant basophilic cytoplasm. The blast in the center of the image shows cytoplasmic blebbing; while this feature is not specific for megakaryoblasts, it is a feature often associated with blasts of this lineage.

The Blood Cell Identification Case presented in this Survey is an example of AML occurring in association with underlying polycythemia vera. Polycythemia vera (PV) is a chronic myeloproliferative neoplasm characterized by uncontrolled production of red blood cells with resultant erythrocytosis. Virtually all patients have a mutation involving the *JAK2* gene on chromosome 9 that drives the proliferation. The *JAK2* mutation can be detected by molecular diagnostic studies; however, this mutation is not specific to polycythemia vera as it is also found in a significant proportion of cases of primary myelofibrosis and essential thrombocythemia. Patients with PV often have headache, dizziness, visual disturbances, and
hypertension due to the increased red cell mass. They are at significantly increased risk for deep vein thrombosis, myocardial ischemia, and stroke. Findings in the peripheral blood vary depending on the stage of the disease. In the early stages there is often trilineage proliferation (panmyelosis) and the diagnosis may be difficult to distinguish from other myeloproliferative neoplasms. Later stages of the disease are characterized by marrow fibrosis with resultant peripheral blood leukoerythroblastosis (i.e., immature granulocytes and nucleated red blood cells). The median survival of patients with PV is greater than 10 years. Most patients die from thrombosis or hemorrhage, although up to 20% of deaths are due to progression to a myelodysplastic syndrome or AML (Figure 7. below).

**Figure 7.**

![Image of blood cells](image)

Acute myeloid leukemia arising from polycythemia vera. A: Two myeloblasts are adjacent to a small lymphocyte in this field. B: In this image a myeloblast is adjacent to a giant hypogranular platelet.

**Myelodysplastic/Myeloproliferative Neoplasms**

Myelodysplastic/myeloproliferative neoplasms are rare clonal hematopoietic stem cell disorders characterized by features of both a myelodysplastic syndrome and a myeloproliferative neoplasm. The most common of these disorders is chronic myelomonocytic leukemia (CMML), which demonstrates dysplasia involving one or more of the myeloid lineages and persistent monocytosis.

**De novo AML**

The majority of cases of AML arise in patients with no known predisposing risk factors (the phrase “de novo” is from Latin and means “from the beginning”). Some of these patients may have a predisposing environmental or occupational exposure, such as benzene, but the mechanism of AML development in these patients is poorly understood. In the current WHO Classification, de novo AML is categorized as either “AML with Recurrent Genetic Abnormalities” or as “AML, Not Otherwise Specified.”

**AML with Recurrent Genetic Abnormalities**

AML with recurrent genetic abnormalities includes cases of AML with certain structural chromosomal rearrangements that can be detected by routine cytogenetic studies (karyotyping) or fluorescence in situ
hybridization (FISH). The chromosomal rearrangements associated with AML are typically balanced translocations or chromosome inversions. These rearrangements juxtapose two functionally normal genes, resulting in a new abnormal fusion gene. The fusion gene encodes for a functional chimeric protein that ultimately promotes leukemia development. Several of these chromosomal rearrangements are specific to AML (i.e., AML with t(8;21)(q22;q22), inv(16)(p13.1q22), t(16;16)(p13.1;q22), or t(15;17)(q22;q12)), permitting these cases to be classified as AML even when the blast percentage is less than 20%.

AML, Not Otherwise Specified
AML, not otherwise specified includes all cases of AML that do not fulfill criteria for inclusion in one of the other AML categories in the WHO Classification. As summarized above, this category excludes cases with a recurring genetic abnormality and cases occurring in patients with a history of Down syndrome, previous cytotoxic therapy, or a preceding myelodysplastic syndrome, myeloproliferative neoplasm, or myelodysplastic/myeloproliferative neoplasm. Thus, knowledge of the patient’s clinical history is essential for accurate AML classification.
References

Education Activity Authors

Tracy I. George, MD, FCAP: Tracy George, MD, is Director of Hematology for Stanford University Medical Center Clinical Laboratories, which serves Stanford Hospital and Clinics and Lucile Salter Packard Children’s Hospital. She is an assistant professor of Pathology at the Stanford University School of Medicine in Stanford, CA. Dr. George has written over 70 papers, book chapters, books, educational activities and abstracts in the areas of hematology, hematopathology and surgical pathology. She teaches medical students, residents, and fellows, participates in clinical service work in hematopathology, and performs translational research in the areas of myeloproliferative neoplasms and laboratory hematology. Dr. George is currently Chair of the Hematology and Clinical Microscopy Resource Committee and a member of the Council on Scientific Affairs for the College of American Pathologists (CAP).

Kyle T. Bradley, MD, MS, FCAP: Kyle T. Bradley, MD, is an Assistant Professor in the Department of Pathology & Laboratory Medicine at Emory University Hospital in Atlanta, GA. He is board certified in anatomic pathology, clinical pathology, and hematology by the American Board of Pathology. His primary responsibilities are in clinical service work and resident/fellow teaching in the areas of surgical pathology and hematopathology. Dr. Bradley has authored a number of original articles, abstracts, and educational activities in the fields of hematopathology and anatomic pathology and is a member of the Hematology and Clinical Microscopy Resource Committee for the College of American Pathologists (CAP).
**CASE HISTORY**
This blood film is from an 82-year-old male with fatigue, drenching sweats, recent weight loss, and large lymph nodes. Laboratory data include: WBC = 160.8 x 10^9/L; RBC = 2.60 x 10^12/L; HGB = 9.3 g/dL; MCV = 112 fl; and PLT = 89 x 10^9/L.

**DISCUSSION**
This Case History represents a patient with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) with increased numbers of prolymphocytes. It is important to recognize the large cells with prominent nucleoli as prolymphocytes rather than other neoplastic cells, such as blasts, reactive lymphocytes, or circulating lymphoma cells. One of the most helpful features for identification of prolymphocytes in this case is the recognition of the typical CLL/SLL lymphocytes in the background. CLL/SLL cells resemble normal small lymphocytes but tend to be slightly larger in size. These cells usually have a round or slightly indented nucleus and a mature, chromatin pattern. They typically lack clear-cut nucleoli although small chromocenters may be present. CLL/SLL cells often have scant to a moderate amount of pale, agranular cytoplasm, with most cases having scanty cytoplasm. The CLL/SLL cells within an individual case tend to closely resemble each other rather than having a spectrum of morphologic appearance that is more typical in a patient with non-neoplastic reactive lymphocytes (Figure 1. below).

---

**Figure 1.**

These images depict peripheral blood smears from four patients with CLL. Note the present of smudged cells (arrowheads). In the upper images the CLL cell population is comprised of small lymphocytes (slightly larger than red blood cells) and a prolymphocyte is present (arrow). In the lower images, the CLL population is somewhat larger lymphoid cells with more abundant cytoplasm although the lymphoid cells are still uniform in appearance. Although these neoplastic lymphoid cells are larger, they do not contain a prominent nucleus that is characteristic of a prolymphocyte.
Often times when CLL/SLL is present, there is a marked lymphocytosis, however the ability to identify these cells by their aberrant surface protein expression, referred to as their aberrant phenotype (to be discussed below), allows diagnosis of CLL/SLL without marked increases in lymphocyte numbers. As implied by the name of CLL/SLL, many patients will have leukemic and tissue manifestations including enlarged lymph nodes and spleen. Most patients have blood and bone marrow involvement, but in some patients lymph node involvement may be the predominant presentation. In the blood, most patients will have leukocyte counts that are < 30 x 10^9/L. In the current World Health Organization (WHO) Classification, the threshold count for a diagnosis for CLL is defined as > 5.0 x 10^9 per liter monoclonal lymphocytes (Table 1. below). International workshops on CLL have also required that this lymphocytosis be present for at least three months and will allow for a diagnosis of CLL if there are accompanying cytopenias or disease-related symptoms when lymphocyte counts are lower.

Table 1. Diagnostic Features of Chronic Lymphocytic Leukemia (CLL).

- Absolute mature lymphocytosis of ≥ 5 x 10^9/L sustained for at least 3 months
- Monoclonal B cell with mature phenotype, CD5 coexpression, weak CD20, weak surface immunoglobulin, CD23
- Antigens typically not expressed include CD10 and FMC7

Because CLL/SLL usually involves the bone marrow, other cytopenias are common. Many patients will have a normochromic, normocytic anemia, and this may also be accompanied by an immune-based hemolytic anemia, resulting in spherocytes on the peripheral blood smear. Thrombocytopenia is seen less frequently, in about ten to twenty percent of patients, and tends to manifest later in the course of disease. Clinical staging of CLL/SLL includes the presence or absence of these other cytopenias (Table 2. below), parameters that are important in determining the patient’s overall prognosis and need for therapeutic intervention.

Table 2. Staging Systems for Chronic Lymphocytic Leukemia (CLL).

<table>
<thead>
<tr>
<th>Rai Clinical Staging System for CLL</th>
<th>Binet Staging System for CLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>Lymphocytosis in blood and bone marrow only</td>
</tr>
<tr>
<td>Stage I</td>
<td>Lymphocytosis plus enlarged lymph nodes</td>
</tr>
<tr>
<td>Stage II</td>
<td>Lymphocytosis plus enlarged liver and/or spleen; lymphadenopathy may be present</td>
</tr>
<tr>
<td>Stage III</td>
<td>Lymphocytosis plus anemia (hemoglobin &lt; 11 g/dL [110 g/L]); lymph nodes, spleen, or liver may be enlarged</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Lymphocytosis and thrombocytopenia (platelet count &lt; 100 x 10^9/mm^3 [100 x 10^10/L]); anemia and organomegaly may be present</td>
</tr>
</tbody>
</table>

| Stage A | Hemoglobin ≥ 10 g/dL; platelets ≥ 100 x 10^9/mm^3 (100 x 10^10/L); < 3 anatomic sites* involved |
| Stage B | Hemoglobin ≥ 10 g/dL; platelets ≥ 100 x 10^9/mm^3 (100 x 10^10/L); ≥ 3 anatomic sites* involved |
| Stage C | Hemoglobin < 10 g/dL; platelets < 100 x 10^9/mm^3 (100 x 10^10/L) |

*Anatomic sites = inguinal nodes, axillary nodes, cervical nodes, liver or spleen
In examination of the blood smear of a patient with CLL, it is not uncommon to see smudged or disrupted cells due to the increased fragility of the neoplastic cells. This is often an indication that the lymphocytes are neoplastic and can be quite helpful in identification in early cases of CLL/SLL where there is not a marked increase in lymphocytes. Unfortunately, the presence of smudged cells is not entirely specific for CLL/SLL since they can also be seen with some acute leukemias, lymphomas, and a subset of reactive lymphocytosis. In addition to small neoplastic lymphocytes, most cases of CLL/SLL have occasional cells that are larger and have a single prominent nucleolus that are prolymphocytes. Prolymphocytes are not detected in normal blood smears, but are often seen in CLL/SLL where they make up <10% of the neoplastic population. Morphologically, prolymphocytes are round to oval in shape and have a centrally placed nucleus. The prolymphocyte nucleus contains condensed mature appearing chromatin with a single prominent nucleolus. There tends to be a moderate amount of pale agranular cytoplasm that is more abundant than is typically seen in small lymphocytes or blasts but less than in reactive lymphocytes (Figure 2, below).

Figure 2. Prolymphocytes

The feature of the prominent nucleolus often brings to mind the possibility of blasts but recognition of the condensed chromatin, moderately abundant cytoplasm and the context of the background of CLL cells is helpful in identifying these cells as prolymphocytes. Rarely, prolymphocytes may have more than one nucleolus and the nuclear contours may appear slightly irregular.

When evaluating a case of CLL/SLL, the number of prolymphocytes and more mature appearing B-cells should be evaluated. When prolymphocytes are the predominant form, >55% of the cells, a diagnosis of B prolymphocytic leukemia (PLL) will be made. These cases tend to have a much more aggressive clinical course and often have marked leukocytosis with cytopenias, massive splenic enlargement, and patients tend to lack lymphadenopathy. These patients often have rapid clinical progression with a poor outcome and require treatment with aggressive chemotherapy, although this is often relatively ineffective.
In some cases of CLL, there will be increased numbers of prolymphocytes that are >10% but <55%. In the past, these were referred to as mixed CLL or CLL/PLL. These cases that have increasing numbers of prolymphocytes are associated with a more aggressive disease course and increasingly severe cytopenias. The 2008 WHO Classification of Hematopoietic Neoplasms does not recognize the terminology of mixed CLL or CLL/PLL, and these cases with increased prolymphocytes may be given a diagnosis of atypical CLL (morphology) to indicate to clinicians the possibility of a more aggressive disease course.

In addition to morphology, examination of cell surface proteins (immunophenotyping) and cytogenetics/molecular studies are very useful in the diagnosis and assignment of prognosis for CLL/SLL. Most cases of CLL will be characterized by expression of low antigen density (weak) monotypic (either kappa or lambda light chain) surface immunoglobulin, weak expression of CD20, with expression of CD19 and CD23. The tumor cells are characterized by the aberrant co-expression of the T-cell marker CD5. Usually, the cases will be negative for FMC7 and CD10 (Table 1 on Page 2). Two other immunophenotypic markers, CD38 and ZAP-70, have also been shown to have prognostic importance. Those patients who have high expression of CD38 and overexpression of ZAP-70 are thought to have a more aggressive disease course. ZAP-70 and CD38 are independent prognostic factors. ZAP-70 expression appears to be linked to the presence of patients who have unmutated immunoglobulin heavy chains. These unmutated subtypes of CLL are associated with an unfavorable (more aggressive) clinical course.

Other genetic and molecular features that are important in prognosis include specific cytogenetic findings. Cytogenetic abnormalities are present in more than 80% of CLL/SLL cases (Table 3, on the following page). These are often detected by fluorescence in situ hybridization (FISH) of interphase nuclei, a technique in which fluorescently labeled probes of nucleic acid are used to detect chromosomal abnormalities. Those specific abnormalities that are associated with prognosis include deletion of chromosome 13q14, which is the most common cytogenetic abnormality associated with CLL (seen in >50% of cases that have abnormal FISH results). Chromosome 13q14 deletion appears to impart a better prognosis than those patients who have a normal karyotype or other cytogenetic abnormalities. Trisomy (3 copies) of chromosome 12 occurs in <20% of cases with abnormal karyotypes by FISH and is often associated with atypical morphologic features (slightly larger than normal cells with more irregular nuclear contours and more abundant cytoplasm). There does not appear to be prognostic implications associated with trisomy 12. Deletions of chromosome 11q22 - 23 are seen in approximately 20% of patients with abnormal cytogenetics and this particular genetic finding tends to be associated with increased significant lymphadenopathy. Deletions of chromosome 17p13 are seen in 5 - 10% of CLL patients and are associated with mutations in the TP53 gene. Deletion of chromosome 17p13 is associated with a very short survival in patients with CLL and is often associated with disease progression. In addition, deletion of chromosome 17p13 in CLL is associated with resistance to the chemotherapeutic drug fludaribine, an agent commonly used to treat CLL.
Table 3. Cytogenetic Findings in Chronic Lymphocytic Leukemia (CLL).

<table>
<thead>
<tr>
<th>Cytogenetic Finding</th>
<th>Incidence</th>
<th>Prognostic Implication</th>
<th>Clinical Associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal cytogenetics</td>
<td>20%</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Abnormal cytogenetics</td>
<td>80%</td>
<td>See below</td>
<td>See below</td>
</tr>
<tr>
<td>deletion 13q14</td>
<td>~40% of all cases</td>
<td>Good prognosis</td>
<td>None</td>
</tr>
<tr>
<td>trisomy 12</td>
<td>~15% of all cases</td>
<td>None</td>
<td>Atypical morphology</td>
</tr>
<tr>
<td>deletion 11q22-23</td>
<td>~15% of all cases</td>
<td>None</td>
<td>Often increased lymph node involvement</td>
</tr>
<tr>
<td>deletion 17p13</td>
<td>~3-10% of all cases</td>
<td>Poor prognosis</td>
<td>Associated with disease progression</td>
</tr>
</tbody>
</table>

Immunophenotypic analysis of prolymphocytes often shows a slightly different immunophenotype by flow cytometry. Prolymphocytes may have negative or weaker expression of CD5 and stronger expression of cell surface immunoglobulins and CD20 than classic cases of CLL. In addition, patients with increasing numbers of prolymphocytes may acquire additional genetic or cytogenetic abnormalities that are associated with progression of disease.

Identification of prolymphocytes clearly has an important effect on prognosis. It is important to identify prolymphocytes and distinguish them from potential morphologic look-alikes including blasts, malignant lymphoma cells and reactive lymphocytes (Table 4. on the following page and Figure 3. below).

Figure 3.

Upper left: Blood smear from a patient with B-lymphoblastic leukemia. Note the dispersed chromatin and scant cytoplasm in the blasts. A few cells also contain a nucleolus.

Upper right: Blood smear from a patient with follicular lymphoma. The lymphoma cells have condensed to somewhat smudgy chromatin, scant cytoplasm and prominent nuclear irregularities but nucleoli are lacking. A normal lymphocyte is also present in the lower center of the field.

Lower left: Blood smear from a patient with B-prolymphocytic leukemia. Prolymphocytes represented >55% of lymphocytes on this smear and showed characteristic features, including larger size with relatively condensed chromatin, prominent nucleolus, and moderate to abundant cytoplasm.

Lower right: Blood smear from a patient with Burkitt leukemia/lymphoma. Circulating Burkitt lymphoma cells will have a round to oval nucleus with moderately coarse chromatin and may have prominent nucleoli. However, the cytoplasm is dark blue in color and often contains numerous small vacuoles as seen in this case.
Table 4. Helpful Morphologic Features in Distinguishing Prolymphocytes.

<table>
<thead>
<tr>
<th></th>
<th>Size</th>
<th>N:C Ratio</th>
<th>Nuclear Contour</th>
<th>Chromatin</th>
<th>Nucleolus</th>
<th>Cytoplasm Amount</th>
<th>Cytoplasmic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolymphocyte</td>
<td>&gt;2RBC</td>
<td>High</td>
<td>Usually regular, occasionally slightly irregular</td>
<td>Mature clumped</td>
<td>Single, prominent</td>
<td>Moderate</td>
<td>None</td>
</tr>
<tr>
<td>Lymphoblast</td>
<td>1 to &gt;2RBC</td>
<td>High</td>
<td>Round to irregular</td>
<td>Immature</td>
<td>Absent to inconspicuous</td>
<td>Slight</td>
<td>None</td>
</tr>
<tr>
<td>Myeloblast</td>
<td>&gt;2RBC</td>
<td>High</td>
<td>Usually slightly irregular to moderately irregular</td>
<td>Immature</td>
<td>Variably prominent, may be multiple</td>
<td>Slight to moderate</td>
<td>May contain granules, vacuoles</td>
</tr>
</tbody>
</table>

**Lymphoma Cells**

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular Lymphoma</td>
<td>1-2RBC</td>
<td>Moderate</td>
<td>Often clefted, reniform</td>
<td>Mature, coarse</td>
<td>Absent or inconspicuous</td>
<td>Scant</td>
<td>None</td>
</tr>
<tr>
<td>Burkitt Lymphoma</td>
<td>1-2RBC</td>
<td>High</td>
<td>Round</td>
<td>Mature</td>
<td>May be absent or single conspicuous</td>
<td>Moderate</td>
<td>Basophilic cytoplasm with prominent vacuoles</td>
</tr>
<tr>
<td>Large Cell Lymphoma</td>
<td>&gt;2RBC</td>
<td>Moderate</td>
<td>Smooth to clefted</td>
<td>Mature</td>
<td>May have prominent single or multiple nucleoli</td>
<td>Moderate</td>
<td>May have vacuoles</td>
</tr>
</tbody>
</table>

**Reactive Lymphocytes**

|                          | 1->2RBC       | Low | Oval and usually smooth          | Mature    | Usually inconspicuous    | Abundant         | May have granules                     |
Key features for identifying prolymphocytes include their larger size with the centrally placed nucleus, prominent nucleolus, and a relatively high nuclear to cytoplasmic ratio. The relatively more abundant cytoplasm is a key feature in helping to differentiate prolymphocytes from lymphoid blasts and most myeloblasts where cytoplasm is typically less. In addition, the chromatin is typically more condensed and mature-appearing in prolymphocytes than in either myeloid or lymphoid blasts. The central placement of the nucleus and the single prominent nucleolus are helpful to differentiate prolymphocytes from reactive lymphocytes. Reactive lymphocytes tend to have eccentrically placed nuclei and abundant amounts of cytoplasm that may contain a few coarse azurophilic granules. Typically, reactive lymphocytes lack the prominent nucleolus that is seen in a prolymphocyte and may have more oval to round nuclei. Another potential differential diagnostic consideration would be a circulating lymphoma cell. Follicular lymphoma cells typically will have more prominent irregularities of the nuclear contours and usually lack nucleoli. The chromatin will be coarse to smudgy and cytoplasm is often scant. Circulating Burkitt lymphoma cells will have a round to oval nucleus with moderately coarse chromatin and may have prominent nucleoli. However, the cytoplasm is dark blue in color and often contains numerous small vacuoles. Large cell lymphomas that are present in the blood often appear blast-like and are large with variable nuclear contours. They may have one or more prominent nucleoli but often times the nucleus is eccentrically placed. Occasionally, vacuoles are seen in the cytoplasm. In cases of circulating lymphomas, the context is extremely important in helping to differentiate prolymphocytes from other lymphoma cells. Seeing the background of the more typical CLL/SLL cells and smudge cells indicate the large cells with single nucleoli are likely prolymphocytes. Albumin may be added to peripheral blood with many smudge cells (i.e., albumin preparation) in order to visualize the leukocytes.

The appearance of the bone marrow in patients with CLL/SLL may be variable. There may be formation of non-paratrabecular (not adjacent to bone trabeculae) lymphoid nodules composed of small, mature appearing lymphocytes, as well as an increase in an interstitial infiltration of neoplastic cells. In later stages of disease, there may be a diffuse effacement of the marrow and this is more often associated with significant cytopenias. Increased prolymphocytes may also be seen in the marrow and are usually associated with diffuse or interstitial infiltration rather than the nodular pattern of involvement.

CLL/SLL is generally considered to be an indolent disorder with slow progression and survivals greater than 10 years. The clinical course is often associated with the clinical stage of disease, as determined by either the Rai or Binet staging systems (see Table 2 on page 2), as well as prognostic factors such as expression of CD38 and ZAP-70 by flow cytometry, mutational status of the immunoglobulin heavy chain, as well as cytogenetic abnormalities. Transformation of CLL into more biologically aggressive disease is well recognized and seen in five to fifteen percent of patients. The most common pattern of transformation is characterized by the progressive increase in the number of prolymphocytes in the blood and bone marrow. Those patients with increasing prolymphocytes typically have progressive cytopenias and become refractory to therapy with chemotherapeutic agents. Other transformations may be a Richter’s transformation, which is development of an aggressive diffuse large B-cell lymphoma, requiring a more aggressive chemotherapeutic regimen. Rare cases may also transform to Hodgkin lymphoma.
References


Education Activity Authors

**Joan Etzell, MD, FCAP:** Joan Etzell, MD, is a Professor of Clinical Laboratory Medicine and the Director of the Clinical Hematology Laboratory at the University of California, San Francisco (UCSF). She is AP/CP and Hematology Board certified by the American Board of Pathology. Dr. Etzell is actively involved in the education of medical technologists, medical students, residents, and fellows in hematology / hematopathology. She serves as the Hematopathology Fellowship Director and Associate Residency Program Director in Laboratory Medicine in UCSF. Dr. Etzell has authored over 50 papers, book chapters, educational activities and abstracts in the areas of hematology and hematopathology. Dr. Etzell currently serves as the Vice-Chair of the Hematology and Clinical Microscopy Resource Committee for the College of American Pathologists (CAP).

**Sherrie L. Perkins, MD, PhD, FCAP:** Sherrie L. Perkins, MD, PhD, is a professor of Pathology at the University of Utah Health Sciences Center and the Chief Medical Officer for ARUP Laboratories in Salt Lake City, UT. She is the Director of Hematopathology for ARUP Laboratories and has responsibilities in teaching, resident training, clinical service and research. Dr. Perkins has written over 140 peer-reviewed papers and 70 book chapters in the areas of hematology and hematopathology. Dr. Perkins is currently a member of the College of American Pathologists (CAP) Hematology and Clinical Microscopy Resource Committee.