The arrowed cells are blasts as correctly identified by 83.2% of participants. Myeloblasts are the most immature cells in the myeloid series. They are normally confined to the bone marrow, where they constitute less than 3% of the nucleated cells. Myeloblasts are large, 15-20 μm in diameter, with high nuclear–cytoplasmic ratio (N:C). The nucleus may be round, oval, irregular, or folded and typically contains a distinct nucleoli. The nuclear chromatin is smooth and the cytoplasm is basophilic and may contain a few delicate granules and/or Auer rods. Blasts may be seen in the peripheral blood in cases of acute myeloid leukemia, myelodysplastic syndrome or myeloproliferative neoplasms. Blasts may also be seen in reactive conditions such as leukemoid reaction or after administration of granulocyte colony-stimulating factor (GCSF). Immunophenotyping (via flow cytometry, immunohistochemistry) and less commonly cytochemical analysis is used in differentiating myeloblasts from lymphoblasts.

The arrowed cell was incorrectly identified by 11.1% of the participants as a reactive lymphocyte. Immunoblasts and immunoblastic-like reactive lymphocytes are medium to larger cells (15 to 20 μm) with round to oval nuclei. They have finely to moderately dispersed chromatin with abundant parachromatin and one or more prominent nucleoli. These may resemble lymphoma cells or blasts. However, their cytoplasm is moderately abundant and often stains deeply basophilic and may show very prominent peripheral basophilia. These reactive lymphocytes correspond to Downey type III cells. The arrowed cell lacks the clumped chromatin, deep and peripheral basophilia and the more abundant cytoplasm seen commonly in reactive lymphocytes.

The arrowed cell is large to medium in size with a high N:C ratio. The nuclear chromatin is smooth and fine appearing, with distinct multiple nucleoli. The cytoplasm is basophilic and agranular, but is relatively modest in amount when compared to the size of the nucleus. The arrowed cell lacks the more clumped chromatin or parachromatin that is characteristic of a reactive lymphocyte. In addition, the cytoplasm is scant and lacks the deep or peripheral basophilia as typically seen in a reactive lymphocyte. Close attention to these nuclear and cytoplasmic features will aid in distinguishing blasts from reactive lymphocytes.
The arrowed cell is a red blood cell with a Howell-Jolly body as correctly identified by 99.5% of participants. Howell-Jolly bodies are about 1 µm in diameter and composed of DNA (nuclear remnant). They may be seen as single or multiple inclusions inside red cells and appear larger than Pappenheimer bodies. They are formed in the process of karyorrhexis, or an aberrant chromosome that is separated from the mitotic spindle. Normally, the spleen is efficient in removing these inclusions from the red cells. If the spleen is atrophied or removed, Howell-Jolly bodies can be found in the peripheral blood. Howell-Jolly bodies are also seen in megaloblastic anemia due to increased bone marrow karyorrhexis. Howell-Jolly bodies can be evaluated with a Wright-Giemsa or supravital stain.
<table>
<thead>
<tr>
<th>Identification</th>
<th>Participants</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte</td>
<td>904</td>
<td>99.2</td>
</tr>
</tbody>
</table>

The arrowed cells are lymphocytes as correctly identified by 99.2% of participants. Lymphocytes are small with high N:C ratios and typically contain round-oval nuclei with or without indentation. The chromatin is diffusely dense or coarsely clumped, and nucleoli are not visible. Some cells may exhibit a pale chromocenter that may be mistaken for a nucleoli. Mature lymphocytes typically have scant, lightly to moderately basophilic cytoplasm.
<table>
<thead>
<tr>
<th>Identification</th>
<th>Participants</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target cell</td>
<td>903</td>
<td>99.2</td>
</tr>
</tbody>
</table>

The arrowed cell is a target cell as correctly identified by 99.2% of participants. Target cells are red blood cells with excessive cell membrane. When the cell is flattened on a glass smear it appears as a central hemoglobinized area. This denser center is surrounded by an area of pallor which in turn is surrounded by the remainder of the red cell. Target cells are seen in patients following splenectomy, liver disease, hemoglobinopathies such as Hemoglobin C, SC, H, or E. Artifactual target cells are seen in humid, slow drying conditions.
<table>
<thead>
<tr>
<th>Identification</th>
<th>Participants</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet, giant</td>
<td>789</td>
<td>86.7%</td>
</tr>
<tr>
<td>Megakaryocytic cell</td>
<td>56</td>
<td>6.2%</td>
</tr>
<tr>
<td>Basket cell/smudge cell</td>
<td>45</td>
<td>5.0%</td>
</tr>
</tbody>
</table>

The arrowed blood component is a giant platelet as correctly identified by 86.7% of participants. Normal platelets are 1.5-3 µm in diameter, large platelets are 4-7 µm in diameter and giant platelets are larger than 7 µm in diameter. Morphologically the term giant platelet is used when its size is greater than a normal size red blood cell. Normal, large or giant platelets contain fine azurophilic granules. Giant platelets may be seen in reactive conditions such as leukemoid reaction or idiopathic thrombocytopenic purpura (ITP) or inherited conditions like May-Hegglin anomaly or Bernard-Soulier syndrome. Giant platelets are also seen in neoplastic conditions such as myeloproliferative neoplasms, or myelodysplastic syndromes.
Case Discussion

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is the most common leukemia of the western world. The median age of onset is 65 years; however there is increasing incidence in younger patients. Patients typically present with weakness, fatigue and enlarged lymph nodes or may be asymptomatic. The disease is diagnosed as CLL when there is primarily involvement of the bone marrow and peripheral blood, and SLL when disease is primarily nodal based, but CLL and SLL are grouped together because they are considered the same disease. In order to diagnose CLL, there must be > 5 x 10^9/L monoclonal lymphocytosis with the appropriate immunophenotype. The diagnosis may also be made on lymph node or tissue biopsy. CLL/SLL is a B-cell neoplasm which expresses the B cell markers CD19 and CD20 and also expresses CD5 and CD23.

CBC analysis shows a leukocytosis composed of an absolute lymphocytosis, with or without anemia and/or thrombocytopenia. Lymphocytosis is composed predominantly of atypical lymphoid cells, slightly larger than a mature small lymphocyte, with round to occasionally irregular nuclei, coarse chromatin, absent or indistinct nucleoli, and scant basophilic cytoplasm. Occasionally prolymphocytes may be present, which are larger cells with very distinct nucleoli, although these are usually less than 2% of lymphoid cells in typical CLL.

CLL/SLL is considered an indolent lymphoma with a variable clinical course. Prognosis is based on clinical staging of the patient, chromosomal aberrations and immunoglobulin gene mutational status. Chromosomal aberrations are seen in over 80% of cases. Del (17p) or mutated p53 genes, are seen in 5-10% of cases and portends to the poorest prognosis with a median survival of 2-3 years. Currently first line therapy is determined by the presence or absence of this aberration in conjunction with tumor stage (burden) and fitness level of the patient. While many cases of CLL/SLL are not treated, patients with significant symptoms, aggressive disease or poor prognosis cytogenetics may receive chemotherapy. Chemotherapeutic agents commonly used in the treatment of CLL/SLL include fludarabine, cyclophosphamide and rituximab.

One of the potential side effects of cyclophosphamide and/or fludarabine therapy is the development of a therapy-related myeloid neoplasm. Therapy-related myeloid neoplasms incorporate acute myeloid leukemias, myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms occurring in patients with previous exposure to cytotoxic therapy. Cytotoxic therapies include ionizing radiation therapy and certain chemotherapeutic drugs including alkylating agents, topoisomerase II inhibitors, antimetabolites or antitubulin agents. Depending on the type of cytotoxic therapy used, therapy-related myeloid neoplasms may develop anywhere from 1 to 10 years after treatment.

In our present case, the patient has a history of treated CLL. Two possible agents responsible for the development of the therapy-related myeloid neoplasm include cyclophosphamide (alkylating agent) and fludarabine (antimetabolite). Therapy-related myeloid neoplasms associated with alkylating agents and/or radiation will typically present 5 to 10 years after therapy, and these represent a majority of the cases. Most patients will present similar to de novo myelodysplastic syndromes. Cytopenias are present, denoting bone marrow failure. Peripheral blood smear analysis may also demonstrate myeloid dysplasia including pseudo-Pelger Huet nuclei and cytoplasmic hypogranularity. Multilineage dysplasia is evident in bone marrow aspirate smears, and in a smaller percentage of cases overt acute myeloid leukemia is seen at presentation.

The current case involves a patient with a long-standing history of treated CLL presenting with recent onset of cytopenias.

In this setting, the concerns are either progression of CLL, transformation of CLL into a more aggressive disease form, or the development of a therapy-related myeloid neoplasm. In our current case, there is a marked increase in the WBC associated with anemia and thrombocytopenia. CLL is typically composed of small mature appearing lymphocytes with a few scattered larger prolymphocytes. Unlike typical CLL, the leukocytosis in this case is secondary to an increase of cells in the myeloid lineage. Neutrophils are numerous and occasionally show
decreased cytoplasmic granularity. Additionally, there is an atypical mononuclear cell population present that may represent either prolymphocytes (figure 1A) or myeloblasts (figure 1B). Morphologically, both prolymphocytes and myeloid blasts have similar size, N/C ratios, irregular to round nuclei, distinct nucleoli and basophilic cytoplasm. However, whereas prolymphocytes appear as part of a continuum of small to larger lymphoid cells in CLL, myeloid blast cells are morphologically distinct and appear frankly immature. Prolymphocytes tend to have more abundant cytoplasm than their smaller lymphoid counterparts, but the cytoplasm has similar characteristics.

Prolymphocyte nuclear chromatin retains a similar coarseness to that of the more mature CLL cells, but is just subtly more open. Myeloblasts will tend to have more even chromatin and differing cytoplasmic characteristics with increased basophilia and occasional cytoplasmic vacuoles/granules. Auer rods may be present. When myeloblasts are present, they may be seen in a background of myelodysplasia and occasional giant platelets, especially in the setting of a therapy-related myeloid neoplasm. Definitive immunophenotypic analysis of the atypical mononuclear population via flow cytometric analysis can be performed on the peripheral blood specimen to classify the atypical cell population.

Overall prognosis of patients with therapy-related myeloid neoplasms is poor, as patients tend to be resistant to therapy and have poor bone marrow recovery following therapy, leading to prolonged cytopenias. Cytogenetic analysis is typically performed and often demonstrates cytogenetic aberrations in a majority of the cases. Patients with unbalanced chromosomal aberrations, including chromosomes 5 and 7, are typically associated with a complex karyotype. These changes are seen in cases presenting as a myelodysplastic phase or acute myeloid leukemia with dysplasia and are associated with alkylating agents or radiation therapy. In 20 to 30% of cases, patients will have a chromosomal translocation as seen in acute myeloid leukemia with recurrent genetic abnormalities including translocations of 11q23, t(8;21)(q22;q22), t(15;17)(q22;q12) and inv(16)(p13q22). These balanced translocations typically present as an overt acute myeloid leukemia and are associated with topoisomerase II inhibitor therapy.

FIGURE 1. A. This is a peripheral blood smear from a CLL/SLL patient. Prolymphocytes are seen in the center of the field and are surrounded by smaller CLL lymphoid cells. The larger lymphoid cells contain distinct nucleoli; the prolymphocyte chromatin is slightly more open than that of the neighboring CLL cells, but not as immature as that seen in blast cells. B. This is a peripheral blood smear from a patient with an acute myeloid leukemia. The blast contains smooth and open chromatin and deeply basophilic cytoplasm. Compare the fine chromatin pattern of this blast to that of the CLL cells and prolymphocytes in A.
REFERENCES:


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