PROFICIENCY TESTING SERVICE
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PARTICIPANT STATISTICS | **CELL IDENTIFICATION** | **SECOND QUADRIMESTER 2019**

Q2-2019 Acute Lymphoblastic Leukemia (ALL)

A 52 year old physical therapist presents with a 6 week history of increasing fatigue and a 1 week history of bruising and nosebleeds. Lab values are as follows: WBC-93.7, RBC-3.13, Hgb-9.7, Hct-29.8, normal RBC indices, Platelets-36,000.

The patient was initialized suspected of having leukemia. A bone marrow exam and immunophenotying were performed. The patient was diagnosed with B-cell lymphoblastic leukemia.

Normal lymphocytes consist primarily of two functional classes of cells. T lymphocytes (T-cells) and B lymphocytes (B cells) are the major cellular components of the adaptive immune response. T cells recognize antigens, combat microorganisms and effect the rejection of foreign tissues in case of transplant, etc. and this type of response is called the cell-mediated response. B cells recognize antigens and form antibodies against them and this type of response is known as the humoral immune response.

Lymphoid tissue consists of 1) the marrow stem cell pool, the source of the primitive precursors of both B and T cells, 2) the central lymphoid tissue which includes the thymus, where precursor T cells mature into immunologically competent T-cells, and the bone marrow, where precursor B-cells mature into immunologically competent B-cells, and 3) the peripheral lymphoid tissues, which are the sites to which the immunologically competent T and B-cells migrate. These tissues include the lymph nodes, the spleen, specialized sites in the oropharynx, accumulations in the gut, urinary and respiratory tracts and the bone marrow. The ratio of T cells to B cells is approximately 4:1.

T cells move back and forth between the lymphoid tissues and the blood, continuously monitoring antigens throughout the body. When they detect a foreign entity (i.e. infection) they come into action, the helper T cells send signals to the B cells, which produce the plasma cells. Plasma cells act instantly and produce the specialized antibodies for the particular infection. After that the B cells send signals again to the T cells which then produce killer T cells. Killer T cells destroy the foreign entity. Having complementary functions, T cells and B cells work together to develop and maintain immunity.

Acute lymphoblastic leukemia (ALL) is a systemic, neoplastic proliferation of lymphoblasts originating in lymphocyte progenitor cells of the bone marrow or thymus.
The National Cancer Institute (NCI) estimated 5,960 people would receive a diagnosis of ALL in the United States in 2018. They further estimated 1,470 people would die from the disease in 2018. From 1975 to 1976, the five-year survival rate for ALL for all ages was under 40%. As treatments have improved these numbers have steadily improved and currently the NCI reports the five-year survival rate in the United States is over 68%. In the United States ALL is the most common cause of cancer and death from cancer among children. Nearly 80% of cases of ALL occur in patients under 17, particularly those between the ages of two and five. In adults, ALL is the second most common acute leukemia. The percentage of Americans with ALL who pass away is highest for those who are between the ages of 65 and 74.

The first attempt at classifying ALL was the French American British (FAB) morphological criteria that divided ALL into 3 subtypes (L1, L2 and L3) based on cell size, cytoplasm, nucleoli, vacuolation and basophilia. With the advent of flow cytometric laboratory methods and the development of immunophenotying it became possible to use the cytogenetic profile of the lymphoblasts to identify different sub types of ALL. In 1997, the World Health Organization proposed a composite classification in attempt to account for morphology and cytogenetic profile of the leukemic blasts and identified three types of ALL: B lymphoblastic, T lymphoblastic and Burkitt-cell Leukemia. Later revised in 2008, Burkitt-cell Leukemia was eliminated as it is no longer seen as a separate entity from Burkitt Lymphoma, and B-lymphoblastic leukemia was divided into two subtypes: B-ALL with recurrent genetic abnormalities and B-ALL not otherwise specified. B-ALL with recurrent genetic abnormalities is further delineated based on the specific chromosomal rearrangement present .In 2016, two new provisional entities were added to the list of recurrent genetic abnormalities and the hypodiploid was redefined as either low hypodiploid or hypodiploid with TP53 mutations. In adults, B-cell ALL accounts for ~75% of cases. T-cell ALL comprises the remaining ~25%.

The hallmarks of ALL are chromosomal abnormalities and genetic alterations involved in differentiation and proliferation of lymphoid precursor cells. Although in most cases the cause is unknown, there is evidence that genetic risk factors may be a factor in a small group of patients. For example patients with Down syndrome, Klinfelter's syndrome, Fanconi anemia, Bloom syndrome or neurofibromatosis type 1 have a higher incidence of cancers, including ALL, than does the general population. Viral infections including with EBV and HTLV-1 (human T cell leukemia virus) have been associated with increases in ALL. Environmental risk factors that can result in chromosomal damage include significant chemical exposure (i.e. benzene), radiation exposure or having received prior chemotherapy. Other potential causes of chromosomal damage being investigated include excessive exposure to electromagnetic fields or prolonged, exposure to pesticides or diesel fumes.

Several characteristic genetic changes lead to the creation of a leukemic lymphoblast. These changes include chromosomal translocations, intrachromosomal rearrangements, changes in the number of chromosomes in leukemic cells, and additional mutations in individual genes. Chromosomal translocations involve moving a large region of DNA from one chromosome to another. This move can result in placing a gene from one chromosome that promotes cell division to a more actively transcribed area on another chromosome. The result is a cell that divides more often. Other large changes in chromosomal structure can result in placement of two genes directly next to each other. The result is the combination of two usually separate proteins into a new fusion protein. This protein can have a new function that promotes the development of cancer. Examples of this include the *BCR-ABL1* fusion gene of the Philadelphia chromosome. *BCR-ABL1* encodes an always-activated tyrosine kinase that causes frequent cell division. These mutations produce a cell that divides more often, even in the absence of growth factors.

ALL results when enough of these genetic changes are present in a single lymphoblast. In childhood ALL, for example, one fusion gene translocation is often found along with six to eight other ALL-related genetic

changes. The initial leukemic lymphoblast copies itself into an excessive number of new lymphoblasts, none of which can develop into functioning lymphocytes. These lymphoblasts build up in the bone marrow and may spread to other sites in the body, such as lymph nodes, the mediastinum, the spleen, the testicles, and the brain.

Clinical presentation consists of a combination of clinical symptoms caused by lymphocyte numbers building up in the bone marrow and these other organs. Diagnosis of ALL is established by the presence of 20% or more lymphoblasts in the bone marrow or peripheral blood. The anemia, thrombocytopenia and leukopenia that are the results of bone marrow failure cause the patients to experience any or all of the following, fever, weight loss, night sweats, easy bleeding or bruising, fatigue, dyspnea and infection. In about 20% of patients, the disease will be found to have progressed to involve extra-medullary sites and on PE these patients will also be found to have lymphadenopathy, splenomegaly and/or hepatomegaly. Additionally, at the time of initial diagnosis, a spinal tap will be positive for CNS involvement in a 5–8% of ALL patients. Development of a mediastinal mass is also a possible additional complication of T-cell ALL.

Treatment options and prognosis is central to the management of ALL and these are determined by evaluating numerous variables. Cytogenetic changes have a significant role in risk determination. The cytogenetic aberration with the greatest impact on prognosis and treatment is the presence of the Philadelphia chromosome. Cytogenetic testing, with FISH and/or PCR methodologies, is used to determine the presence or absence of the Philadelphia chromosome and to identify other possible chromosomal abnormalities.

Although most commonly thought of as a diagnostic test for the diagnosis of CML, Philadelphia chromosome (BCR-ABL1) is not restricted to CML. It is also present in 11%–29% of ALL patients. It is present in only about 1-3% of pediatric ALL patients but has been reported to be as high as 50% in patients 60 years of age or older.

Risk stratification allows the physician to determine the most appropriate initial treatment regimen as well as when to consider allogeneic stem cell transplantation (Allo-SCT). Until the last few years, risk stratification has been based on clinical factors such age at diagnosis, subtype of ALL, the peripheral WBC count at time of diagnosis, whether the disease has spread to nearby organs and whether the disease has involved the CNS. To these traditional prognostics factors clinicians now add individualized, detailed cytogentic data. They have now been able to dramatically refine individual prognosis and guide management with the result begin constantly improving patient outcomes.

Rather than stages, ALL is more often characterized by the "phases" of the disease. These phases include: 1) Untreated ALL, 2) ALL in remission, 3) MRD (minimal residual disease), 4) refractory ALL and 5) relapsed/recurrent ALL.

Frontline therapy is the same for B-cell ALL and T-cell ALL. Treatment of ALL can take several years. Despite advances in management, the backbone of therapy remains multi-agent chemotherapy with vincristine, corticosteroids and an anthracycline with allogeneic stem cell transplantation (ASCT) for eligible candidates. ALL is typically treated initially with induction treatment aimed at bringing about remission. This is then followed by consolidation chemotherapy. Intended to destroy any remaining leukemic cells and reduce the risk of relapse, consolidation chemotherapy protocols are typically administered in several separate several cycles that may be given over a period of years.

Once consolidation therapy is completed, maintenance therapy is initiated and the next goal becomes to further reduce the risk of relapse and maintain the long-term survival of the patient. If during any point of the treatment leukemia cells are found in the central nervous system, intrathecal chemotherapy is administered. Radiation therapy is an additional treatment option and can be utilized if the leukemia has spread to the brain, spinal cord, or skin.

ALL is notable for being the first disseminated cancer to be cured. Survival rates for children increased from five-year survivals rates of less than 10% in the 1960s to ten-year survivals rates of 90% in 2018. However, these stunning statistics are not seen in adult ALL patients. Despite a high rate of response to induction chemotherapy, adult patients are often unable to tolerate the dose-intensification consolidation regimens and only about only 30–40% of adult patients achieve long-term remission. Recent innovative treatment approaches aim to improve this situation.
Despite the ability of chemotherapy to prolong survival, until recently the best hope for long-term survival in these regimens has been allogenic bone marrow transplantation (Allo-SCT). Bone marrow transplantation may be used if the ALL recurs following standard treatment. Stem cell transplantation is not used as the first or primary treatment for ALL. It may be used as a treatment for high-risk ALL patients or for patients who do not respond to treatment. Allo-SCT is a difficult and dangerous treatment and can have serious, life threatening side effects. Because of this, it is not a treatment option for every ALL patient.

A stem cell transplant is usually not considered for a child unless 1) doctors have determined that the child’s type of ALL is not likely to respond well to chemotherapy, 2) chemotherapy has not worked well or 3) the patient has relapsed.

The upper age limit for an allogeneic transplant depends on the treatment center. Older patients are often excluded from Allo-SCT on the basis of performance status and medical comorbidities. Some older and sicker patients may be helped by reduced-intensity allogeneic transplant. This treatment uses lower doses of chemotherapy than a standard allogeneic transplant; it does not completely inactivate the patient’s immune system or treat the ALL as aggressively.

The goal of stem cell transplantation is to cure the patient’s cancer by destroying the cancer cells in the bone marrow with high doses of chemotherapy and then replacing them with new, healthy blood-forming stem cells. The healthy blood stem cells will grow and multiply forming new bone marrow and blood cells. There are two main types of stem cell transplantation. Allogenic, this is when the patient receives stem cells from a matched or a partially mismatched related donor or an unrelated donor and autologous, this when the patient receives their own stem cells.

Treatment modalities are continuing to advance and prognosis for the adult ALL patient continue to improve. For example, about 25 percent of adults and about 3 percent of children have an ALL subtype called “Ph-positive ALL” (also known as either “Ph+” or “Philadelphia chromosome-positive ALL”). In Ph+ ALL the Philadelphia chromosome contains the abnormal BCR-ABL fusion gene that makes an abnormal protein that helps leukemia cells to grow.

Prognosis of both adults and children with Ph+ ALL treated with standard chemotherapy has been very poor, with less than 5% of adults being cured. However, 2016 clinical studies performed at a leading cancer treatment center in the United States, have modified standard chemotherapy protocols and added TKIs (tyrosine kinase inhibitors) imatinib, dasatinib or ponatinib and achieved some dramatically improved outcomes. Although these TKIs have some unpleasant side effects such as low blood counts, abnormal bleeding and pain, nausea and vomiting, diarrhea, fatigue, rashes, headaches and muscle, bone and joint pain they have contributed to some remarkably improved outcomes. Some of these clinical studies have shown complete remission rates for ALL Ph+ patients of 95% and patient survival rates of 55% at 3 years. Tyrosine kinase inhibitors (TKIs) are used to treat Ph+ ALL by blocking (inhibiting) the BCR-ABL protein from sending signals that cause leukemia cells to form. TKIs are a type of targeted therapy. Targeted therapy uses drugs or other substances that target and attack specific cancer cells but are less likely to harm normal cells.

Additional advances in treatment have progressed in the area of cellular immunotherapy. CAR T is a personalized therapy using the patient's own immune cells, or T cells, to fight cancer. For this treatment, a patient's T cells are removed from their blood and sent to a lab where the cells are genetically modified to better enable them to identify and attack cancer cells. A chimeric antigen receptor (CAR) is added to each T cell. These modified CAR-T cells are then re-infused into the patient and these modified T cells then are able to locate and subsequently destroy the cancer cells. The research is still very new, and until more studies have been completed, researchers remain cautious. CAR T therapy is FDA approved for adults with aggressive B cell non-Hodgkin's lymphoma who are not responding to chemotherapy and pediatric and young adult patients (up to 25 years of age) with ALL who are not responding to chemotherapy. Investigators hope CAR T-cell therapy will one day become a standard therapy for certain B-cell malignancies such as ALL and chronic lymphocytic leukemia. Researchers working with CAR-T-cells have also identified this kind of therapy as a "bridge" to bone marrow transplant for ALL patients who stop responding to chemotherapy.

There are currently two FDA-approved CAR-T cell therapies available. One of which is Tisagenlecleucel (Kymriah™). This drug has been approved for the treatment of patients up to age 25 with B-cell precursor ALL that is refractory or in second or later relapse. Tisagenlecleucel is the first CAR-T cell approved by the FDA, and the first gene therapy approved in the United States. Tisagenlecleucel was approved based on a single study of 63 patients with relapsed or refractory pediatric precursor B-cell ALL. The remission rate was 83 percent.

Preventing relapses may be an additional treatment where CAR T cells may prove to be very important. Also, for those cases where the disease has spread to the CNS, there is very early evidence that CAR T cells may be and effective treatment to eradicate cancer cells that have escaped chemotherapy or radiation.

Cell Identification

Specimen 6

Result	No.	Flag
Platelet, normal	207	
Platelet, giant	3	***
Hypochromic	1	***
Erythrocyte, normal RBC	1	***
Abnormal Platelet, would refer	1	***

Total Population 213
Intended result: Platelet, normal

Specimen 7

Result	No.	Flag
Segmented Neutrophil (PMN, poly)	208	
PMN with Toxic Granulation/Vacuolizatio	3	***
Hypersegmentated Neutrophil	2	***

Total Population 213
Intended result: Segmented Neutrophil

Specimen 8

Result	No.	Flag
Eosinophil, any stage	211	
Basophil, any stage	1	***

Total Population 212
Intended result: Eosinophil

Specimen 9

Result	No.	Flag
Metamyelocyte	111	
Monocyte, any stage	64	
Immature WBC, would refer	15	
Immature Neutrophil	9	
Abnormal, would refer	7	
Abnormal Granulocyte, would refer	3	
PMN with Toxic Granulation/Vacuolization	2	
Abnormal Lymphocyte, would refer	1	
Blast, undifferentiated	1	

Total Population 213
Intended result: Metamyelocyte

*The intended result was Metamyelocyte 29 of 40 referees reported the intended result.

Specimen 10

Result	No.	Flag
Lymphocyte, normal	201	
Blast, undifferentiated	6	***
Abnormal, would refer	3	***
Abnormal Lymphocyte, would refer	3	***
Monocyte, normal/any stage	1	***
Myeloblast	1	***

Total Population 215
Intended result: Lymphocyte, normal

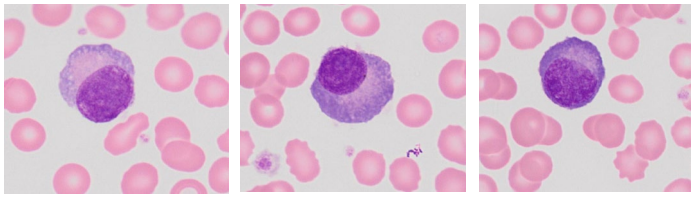
Correct responses are defined as those reflecting agreement among 80% or more of all participants or referees. Unacceptable responses are indicated by "*****" on the Flagging line of each specimen.

Cell Identification - Educational Challenge

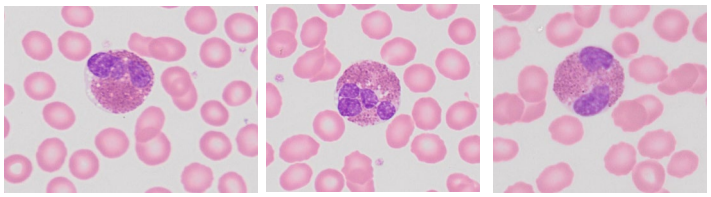
Specimen 6	No.
Plasma Cell, any stage	105
Abnormal Lymphocyte, would refer	7
Abnormal, would refer	6
Lymphocyte, abnormal/atypical	6
Lymphocyte; atypical, Downey,	5
Myelocyte	3
Blast, undifferentiated	3
Lymphocyte, normal	3
Lymphocyte, reactive	3
Nucleated RBC, any stage	2
Polychromatophilic RBC	1

Specimen 7	No.
Eosinophil, any stage	139
Segmented Neutrophil (PMN, poly)	2
PMN with Toxic Granulation/Vacuolizatic	2
Metamyelocyte	1

Total Population: **0**
Intended result: Plasma Cell



Total Population: **0**
Intended result: Eosinophil, any stage



*To see the original fullsized immages, please sign on to your data entry sheet at http://www.aab-pts.org/

Sample 19Q2 - Clinical Discussion

Plasmacytoid Lymphocytes:
History: A 21-year-old college student is brought by her mother to the Emergency Department with a 3-day history of a high fever (up to 102.2F), severe headache, nausea, and myalgias. She recently returned from a mission trip to Columbia to she and her classmates lived in huts in a rural river region in Santander. They were vaccinated for yellow fever and took malaria prophylaxis. She has otherwise been healthy and no one else in the family is ill. Her mother does not know if any of her daughter's classmates also became ill.

On physical examination, the patient is in obvious distress, mildly hypotensive, febrile, has enlarged cervical and axillary lymph nodes and a petechial rash on her lower legs and thorax. CBC results are as follows: WBC 4.1 x 10^3/µL, Hgb 10.2 g/dL, Hct 29.4%, Plts 64,000/µL. Identify the indicated cells.

Clinical Discussion

In this educational challenge, a 21-year-old female college student is seen in the Emergency Department with complaints of fever (up to 102.2ºF), headache, nausea, and myalgias. The symptoms began approximately 3 days ago. She recently returned from Columbia, where she and her classmates performed mission work in the Santander region of the Andes Mountains. The mission group lived in huts in one of the river valleys and was all vaccinated for yellow fever. Upon further questioning, she does recall some mosquito bites, however states she and her classmates all used insect repellent and took malaria prophylaxis. None of her classmates have been ill since their return to the United States, and everyone else in her family is well. She has no underlying medical conditions, does not take any medication on a regular basis, does not drink alcohol, and denies use of illicit drugs.

On physical examination, she appears ill and in a moderate degree of distress. She is febrile, hypotensive for her age, and tachycardic. On physical examination, lymph nodes in her cervical area and both axillary regions are moderately enlarged and slightly tender. She has a diffuse petechial rash on both lower extremities and over her thorax. Blood samples are collected for a CBC and various coagulation, chemistry and microbiology tests. Initial CBC results are as follows: WBC 4.1 x 10^3/µL, Hgb 10.2 g/dL, Hct 29.4%, Plts 64,000/µL. Review of the peripheral smear shows mild to moderate aniso- and poikilocytosis. Target cells, and red cell fragments are present. The platelet count is decreased, with an occasional large platelet seen. Granulocytes show normal lobation with typical primary and secondary cytoplasmic granules. Although normal-sized lymphocytes are present, many of the cells are enlarged and atypical with abundant cytoplasm. These cells can be characterized as **plasmacytoid lymphocytes and plasma cells**.

A plasmacytoid lymphocyte is a lymphocyte in the process of differentiation to a plasma cell. Such cells are slightly larger than a normal lymphocyte and have abundant, deep blue cytoplasm. The nuclear chromatin is not as condensed as the mature lymphocyte and a nucleolus may be evident. Plasma cells are typically larger than the mature lymphocyte with very basophilic cytoplasm and a characteristic eccentrically placed nucleus. A prominent area of clearing next to one side of the nucleus ("nuclear hof") is common and corresponds to the location of the Golgi apparatus. The presence of an occasional plasmacytoid lymphocyte or plasma cell in a peripheral smear is not unusual; however, the large number seen in this patient merits further investigation. The differential diagnosis would include reactive processes such as viral and bacterial infections, autoimmune disorders, hypersensitivity reactions, and lymphoproliferative disorders.

A variety of serologic tests were performed and the patient was eventually diagnosed with **dengue fever**. The dengue viruses are transmitted by the bite of an infected *Aedes* (*Ae. aegypti* or *Ae. albopictus*) mosquito. As with other mosquito-borne diseases (Zika, chikungunya), the mosquitoes become infected when they bite an individual already infected with the virus. After virus incubation/replication for 5-10 days, the mosquito is "infected" and can transmit the virus for the rest of its life. The virus is thus spread to other people through a bite. Instances of vertical transmission from a dengue-infected mother to her fetus or infant during delivery have also been reported. The dengue-infected mosquitoes are common in tropical and subtropical parts of the world, with the highest levels of disease transmission observed in Southeast Asia and the western Pacific islands (Samoa, Solomon Islands, Cook Islands, Tonga). In recent years the incidence of the disease has increased dramatically in regions of Latin America and the Caribbean. Although dengue is seen in the United States, almost all of the cases have been in individuals who were travelers from other countries. In 2019, the highest numbers of travel-associated cases seen in the U.S. were in California, Florida, and New York.

There are four serotypes of dengue virus (DENV-1, -2, -3, and -4). These are RNA viruses belonging to the same family that includes West Nile virus, Yellow Fever virus, and St. Louis Encephalitis virus. Recovery from infection with one infers immunity to that serotype only. An individual can still contract dengue from any of the other three serotypes. Subsequent infection by a different serotype has been shown to increase the risk of developing severe dengue. The World Health

Organization (WHO) now estimates that about half of the world's population is at risk for contracting dengue.

Infection with the dengue virus can be associated with no signs or symptoms; this is the case in approximately 50%-75% of individuals. In the mild form of dengue fever, symptoms appear within 4 to 10 days following the mosquito bite. A marked elevation in temperature (up to 104ºF) is characteristic, accompanied by eye pain, muscle/joint/bone pain, a diffuse erythematous rash (beginning on the lower extremities and progressing to the chest), swollen lymph nodes, and nausea or vomiting. Easy bruising and petechiae may occur. Leukopenia is frequent, as is thrombocytopenia. Even though these symptoms can be uncomfortable, lasting up to 7 days, most individuals recover within one to two weeks.

A small number of individuals develop severe dengue, also called dengue shock syndrome and dengue hemorrhagic fever, with severe abdominal pain, protracted vomiting, mucosal (gums, nose) bleeding, hematuria/hematochezia/hematemesis, and profound fatigue. This is a medical emergency and such individuals require immediate hospitalization. Potentially deadly complications can occur due to plasma leakage syndrome with fluid accumulation, respiratory distress, and severe bleeding, ultimately progressing to profound shock and organ damage. With access to appropriate medical care, the fatality rate is <1%.

Treatment for dengue fever is supportive care. A vaccine to prevent dengue (Dengavaxia®) has been developed and is approved for use in individuals ages 9-45 years old in countries with a high incidence of dengue fever. In May 2019, Dengavaxia® was approved by the FDA in the U.S. for use in children 9-16 years old in the U.S. territories of American Samoa, Guam, Puerto Rico and the U.S. Virgin Islands. The vaccine is not approved for use in children younger than 9 years of age since these children appear to be at increased risk of developing severe dengue fever within two years of receiving the vaccine.

Diagnosing dengue fever can be difficult since the presenting signs and symptoms can resemble those of diseases such as malaria, typhoid fever, influenza, and leptospirosis. Travel history is important, particularly international travel. Although tests exist for detection of both an antibody response to the virus as well as the virus itself, results are typically received either after the patient has already recovered or too late to aid in specific treatment. Review of a peripheral blood smear can be helpful in suspecting dengue as a cause of infection. There are reports that plasmacytoid lymphocytes have been observed in 64% to 73% of dengue infections.