Blood Cell Identification – Graded

Case History

This peripheral blood smear is from a 20-year-old woman who recently emigrated from Africa. She presents with persistent cyclical fevers. Laboratory data include: WBC = 4.8 x 10E9/L; RBC = 2.67 x 10E12/L; HGB = 8.3 g/dL; HCT = 30.2%; MCV = 70 fL; RDW = 18; PLT = 148 x 10E9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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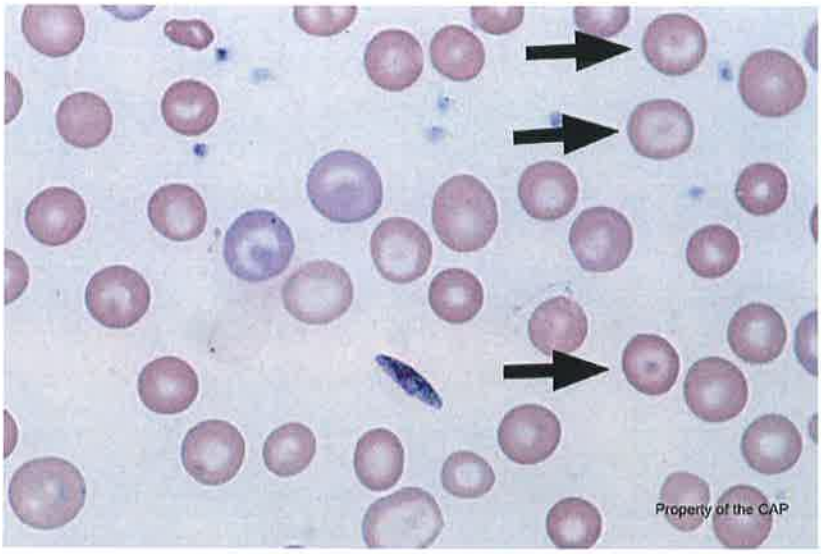
BCP-21

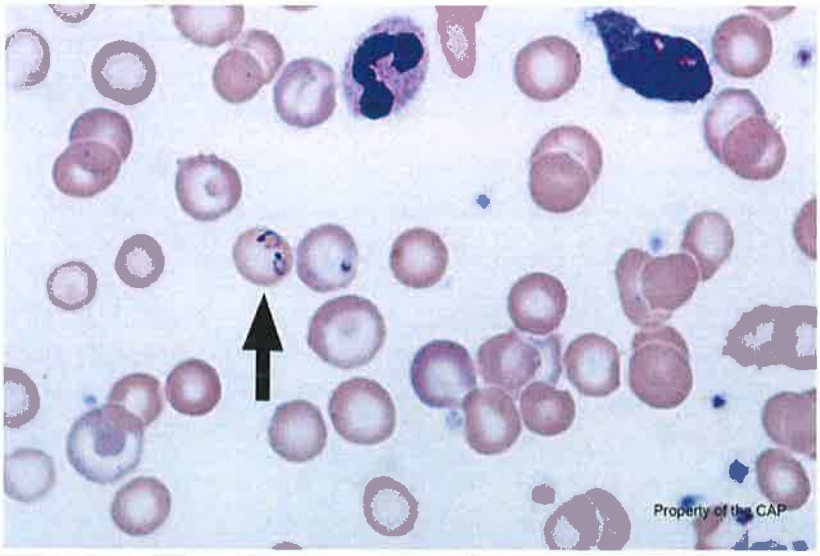


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Target cell (codocyte)	76	100.0	4758	97.8	Good

The arrowed cells are target cells (codocytes), as correctly identified by 100.0% of referees and 97.8% of participants. Target cells are thin red cells with an increased surface membrane-to-volume ratio. They often appear flattened-out on blood smears, revealing sometimes a greater-than-normal diameter. Target cells are believed to arise from disturbances in red cell membrane cholesterol and lecithin content or decreased cytoplasmic hemoglobin content. Target cells are characterized by a central hemoglobinized area within the surrounding area of pallor, which in turn is surrounded by a peripheral hemoglobinized zone giving target cells the appearance of a bull's-eye. Target cells associated with hemoglobin C may have a slightly reduced or normal MCV, whereas those associated with hemoglobin E disorders or hemoglobin H disease exhibit microcytosis of varying degree. Target cells are usually seen in thalassemias, iron deficiency anemia, following splenectomy, or in patients who are jaundiced or who have chronic liver disease. In the latter two conditions, the MCV may be normal or increased. Target cells may also appear as artifacts from slow drying the slides in a humid environment or made from specimens anticoagulated with excessive EDTA. The drying artifact results in the presence of numerous target cells in some fields, but none or few in others.

Blood Cell Identification – Graded

BCP-22

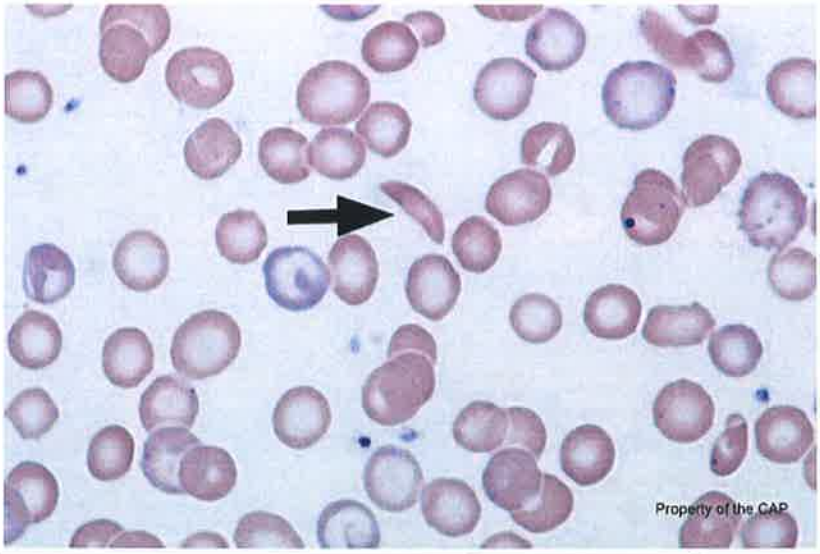


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Plasmodium sp. (malaria)	62	81.6	4142	85.2	Good
Babesia sp.	1	1.3	52	1.1	Unacceptable

The arrowed organism(s) are *Plasmodium* sp. (Malaria), as correctly identified by 81.6% of referees and 85.2% of participants. There are four species of *Plasmodium* that cause the clinical disease known as malaria: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. The different shapes and appearance of the various stages of development and their variations between species are distinctive. The ring forms of all four types of malaria are usually less than 2 µm in diameter. Trophozoites range from 3 to 8 µm, depending on the species. Schizonts and gametocytes range from approximately 5 to 11 µm. Two species have enlarged infected erythrocytes (*P. ovale* and *P. vivax*). Schüffner stippling (a golden brown to black pigment in the cytoplasm of the infected erythrocyte) is most conspicuous in infections with *P. ovale* and *P. vivax*. Multiple stages of organism development are seen in the peripheral blood with all species except *P. falciparum*, where the peripheral blood usually contains only ring forms and gametocytes (unless infection is very severe). Multiple ring forms within one erythrocyte are also most common with *P. falciparum* and are not seen with *P. malariae*. Mixed infections occur in 5% to 7% of patients. Potential look-alikes include platelets overlying red blood cells, clumps of bacteria or platelets that may be confused with schizonts, masses of fused platelets that may be confused with a gametocyte, precipitated stain, Babesia infection, and contaminating microorganisms (bacteria, fungi, etc.). Often infected cells are present in low numbers and difficult to identify in thin blood films. Use of a thick smear or other concentration methods will increase the sensitivity of microscopic examination.

Blood Cell Identification – Graded

BCP-23

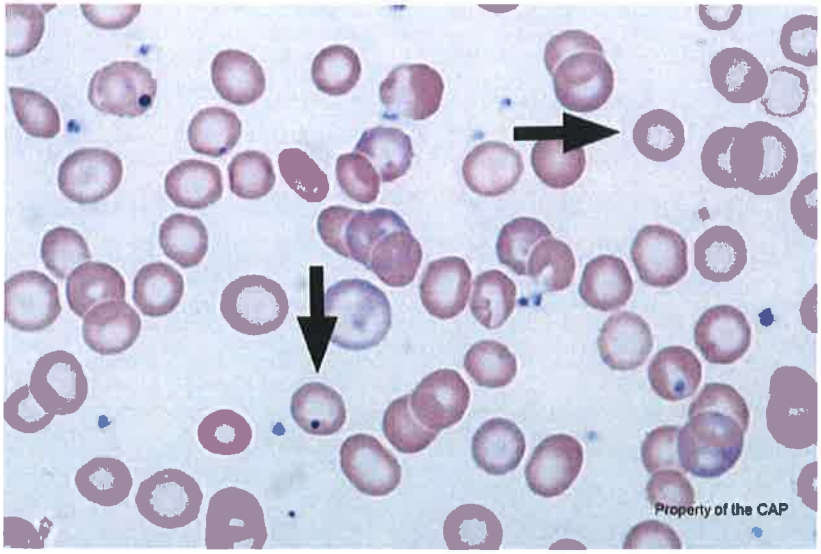


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Sickle cell (drepanocyte)	70	92.1	4451	91.5	Good
Fragmented red cell	3	4.0	202	4.2	Unacceptable
Ovalcyte (elliptocyte)	1	1.3	138	2.8	Unacceptable

The arrowed cell is a sickle cell (drepanocyte), as correctly identified by 92.1% of referees and 91.5% of participants. Red cells appearing in the shape of a thin crescent with two pointed ends are called sickle cells. The polymerization/gelation of deoxygenated hemoglobin S may cause red cells to appear in one or more of the following forms: crescent-shaped, boat-shaped, filament-shaped, holly-leaf form, or envelope cells. These cells usually lack central pallor. Sickle cells may be seen particularly in the absence of splenic function or after splenectomy in patients with the various forms of sickling disorders including hemoglobin SS disease, hemoglobin SC disease, SD disease, and S-beta-thalassemia.

Blood Cell Identification – Graded

BCP-24

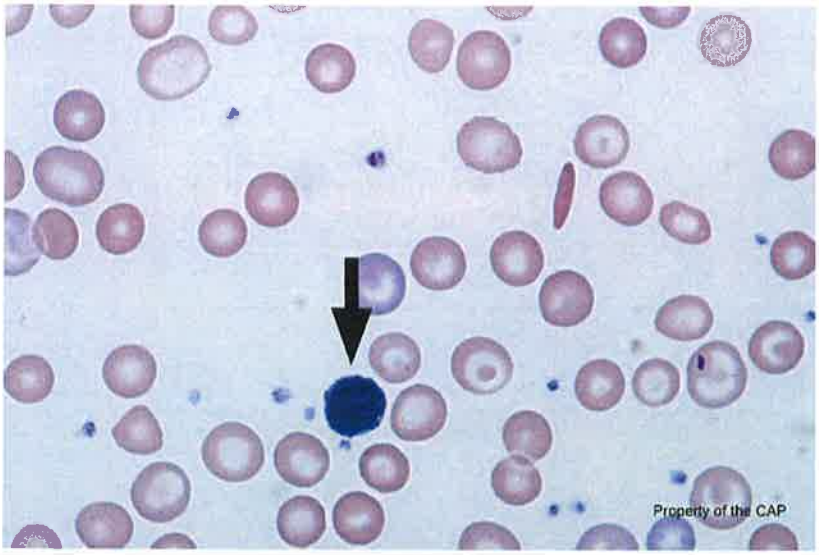


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Howell-Jolly body (Wright stain)	76	100.0	4823	99.2	Good

The arrowed inclusion(s) are Howell-Jolly bodies, as correctly identified by 100.0% of referees and 99.2% of participants. Howell-Jolly bodies are small round dark purple homogeneous intracellular inclusions that measure about 1 µm in diameter. They are larger, more rounded, and darker staining than Pappenheimer bodies and are composed of DNA. They are formed in the process of red cell nuclear karyorrhexis or when an aberrant chromosome becomes separated from the mitotic spindle and remains behind when the rest of the nucleus is extruded. Normally, the spleen is very efficient in removing Howell-Jolly bodies from red cells, but if the spleen is missing or hypofunctional, they may be readily found in the peripheral blood. Howell-Jolly bodies are usually present singly in a given red cell. Multiple Howell-Jolly bodies within a single red cell are less common and typically seen in megaloblastic anemia.

Blood Cell Identification – Graded

BCP-25



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte	67	88.2	4293	88.3	Good
Nucleated red cell, normal or abnormal morphology	5	6.6	220	4.5	Unacceptable
Lymphocyte, reactive	2	2.6	92	1.9	Unacceptable
Basophil, any stage	1	1.3	103	2.1	Unacceptable
Megakaryocytic cell	1	1.3	35	0.7	Unacceptable

The arrowed cell is a lymphocyte, as identified by 88.2% of referees and 88.3% of participants. While most lymphocytes encountered as a normal constituent of peripheral blood leukocytes are fairly homogeneous, they may exhibit a range of morphologic features. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with a nuclear:cytoplasmic ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei that may be slightly indented or notched. The chromatin is diffusely dense or coarse and clumped. Nucleoli are not visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. Although difficult to visualize in this image, most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Infrequent lymphocytes may demonstrate a small clear zone, or "hof", adjacent to one side of the nucleus.

Case Presentation:

This peripheral blood smear is from a 20-year-old woman who recently emigrated from Africa. She presents with persistent cyclical fevers. Laboratory data includes: WBC = 4.8 × 10E9/L; RBC = 2.67 × 10E12/L; HGB = 8.3 g/dL; HCT = 30.2%; MCV = 70 fL; RDW = 18; PLT = 148 × 10E9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Sickle cell and *Plasmodium falciparum*

Hemoglobin A is the major normal adult hemoglobin subtype, accounting for ~97% of hemoglobin after two years of age, and consisting of two identical alpha chains and two identical beta chains. Hemoglobin functions by reversibly binding with oxygen, allowing for effective delivery throughout the body via the circulatory system. Hundreds of unique hemoglobin variants have been identified with the vast majority being due to single amino acid substitutions. Sickle cell trait is due to a substitution of glutamic acid for valine in the 6th position of the beta chain in one beta globin gene (*HBB*) resulting in formation of Hb S. This heterozygous state represents the most common hemoglobinopathy in the United States. Sickle cell trait is also common in sub-Saharan Africa and to a lesser extent the Mediterranean, Middle East, and India. Sickle cells are not typically seen in blood smears from individuals with isolated sickle cell trait. Although usually associated with a benign clinical course, rarely the heterozygous state can be associated with clinical symptoms including severe vaso-occlusive crisis.

Sickle cell disease, on the other hand, causes moderate to severe chronic hemolytic anemia due to homozygous HBB mutation and first manifests in childhood. Sickle cells are observed in these patient's blood smears because of polymerization of Hb S, which occurs in hypoxemic conditions commonly encountered in the microcirculation. The severity of disease varies in individuals, but repeated vaso-occlusive episodes are the norm. These vaso-occlusive events may result over time in auto-splenectomy, painful crises, acute chest syndrome, priapism, and stroke. Despite therapy, the mainstay of which is red blood cell transfusion to reduce the percentage of Hb S, daily folic acid, and hydroxyurea to increase fetal hemoglobin, the average patient with sickle cell disease in the United States succumbs to disease related morbidity/mortality within their forties to fifties. Currently only allogenic hematopoietic stem cell transplantation is curative, but newer treatment modalities are being investigated including gene therapy in hopes to improve and prolong life.

Malaria is frequently cited as the most significant parasitic infection in humans globally, claiming more lives of children than any other infectious disease. Worldwide, malaria accounts for 1-2 million deaths annually. Malaria infection requires female anopheline mosquitos, which bite and infect susceptible human hosts. *Plasmodium falciparum* is the most fatal form of malaria and its incidence is highest in sub-Saharan Africa. Most patients with *P. falciparum* become symptomatic within a month of exposure and commonly present with cyclic chills, fever, and splenomegaly. Currently prophylactic and therapeutic antimicrobials are available; however, significant concern is raised regarding developing drug resistant-strains.

For decades, the apparent association of malaria and sickle cell anemia has been noted. Subsequent research has confirmed a protective advantage in patients with sickle cell anemia resulting in decreased infection and death by *P. falciparum*. This protective advantage is thought to be responsible for the increased incidence of sickle cell trait in malaria endemic areas, most notably sub-Saharan Africa. The precise mechanisms involved in protection from malarial infection are the subject of ongoing investigation.

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Hematology and Clinical Microscopy Committee

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5. Beet EA. Sickle cell disease in Northern Rhodesia. *East Afr Med J.*1947;24(6):212–222.
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7. Lell B, May J, Schmidt-Ott RJ, Lehman LG, et al. The role of red blood cell polymorphisms in resistance and susceptibility to malaria. *Clin Infect Dis.* 1999;28(4):794-799.

Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from a 45-year-old man with a previous history of axillary lymphadenopathy who now presents with lymphocytosis. Laboratory data include: WBC = 19.4 x 10E9/L; RBC = 2.75 x 10E12/L; HGB = 8.5 g/dL; HCT = 34.8%; MCV = 97 fL; RDW = 20; and PLT = 138 x 10E9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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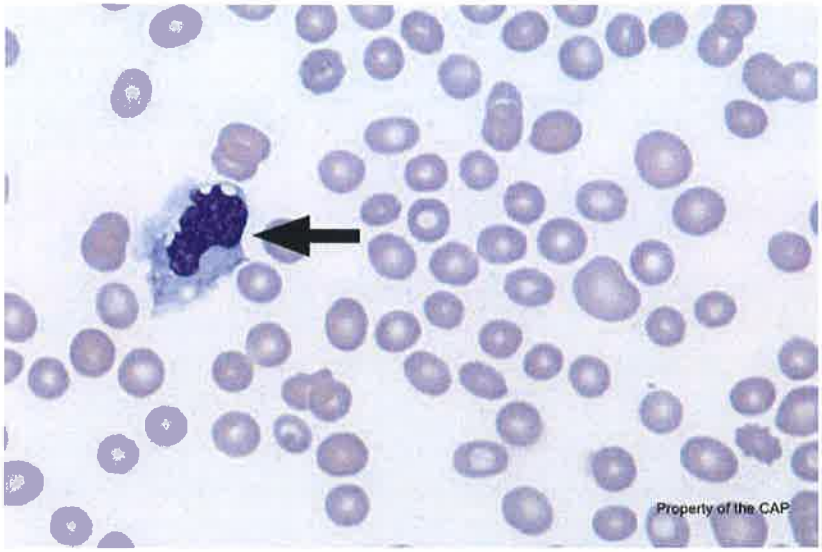
BCP-26

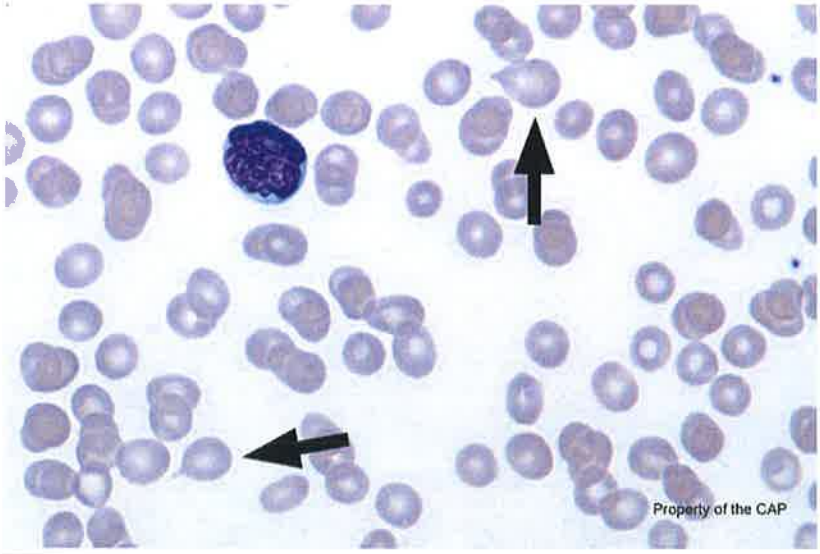


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Monocyte	72	94.7	4468	93.3	Educational
Lymphocyte, reactive	2	2.6	121	2.5	Educational
Monocyte, immature (promonocyte, monoblast)	1	1.3	33	0.7	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	1.3	5	0.1	Educational

The arrowed cell is a mature monocyte, as correctly identified by 94.7% of referees and 93.3% of participants. It has an indented nucleus, abundant pale blue-gray cytoplasm, moderately condensed chromatin, cytoplasmic vacuoles, and fine azurophilic cytoplasmic granules. Monocytes are 12-20 µm in diameter with a nuclear to cytoplasmic ratio of 4:1 to 2:1.

Blood Cell Identification – Ungraded

BCP-27

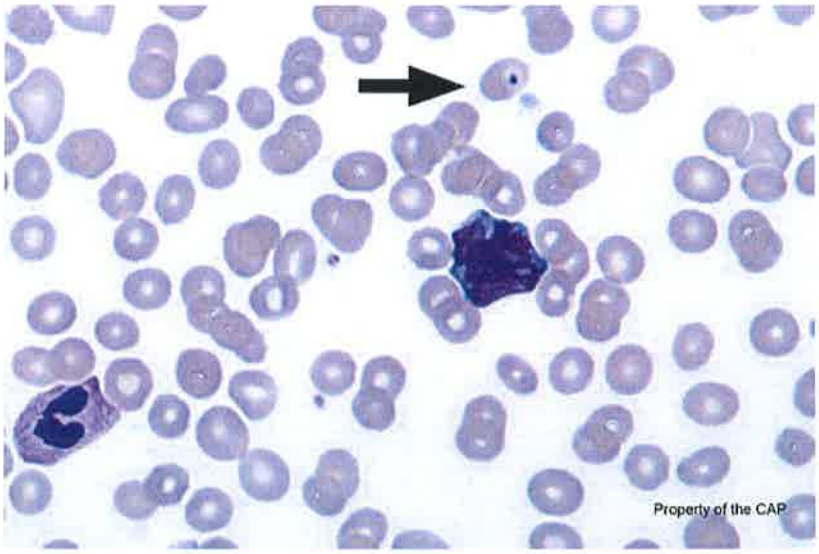


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Teardrop cell (dacrocyte)	76	100.0	4687	99.7	Educational

The arrowed cells are teardrop cells/dacrocytes, as correctly identified by 100.0% of referees and 99.7% of participants. These cells are pear-shaped erythrocytes with an elongated cytoplasmic extension or tail that has a blunted or rounded end. They are commonly seen in patients with bone marrow fibrosis or infiltration by a neoplastic process such as bone marrow involvement by lymphoma. Dacrocytes may also be seen in patients with severe anemia of other types including those related to hemolysis, renal disease, and pernicious anemia.

Blood Cell Identification – Ungraded

BCP-28

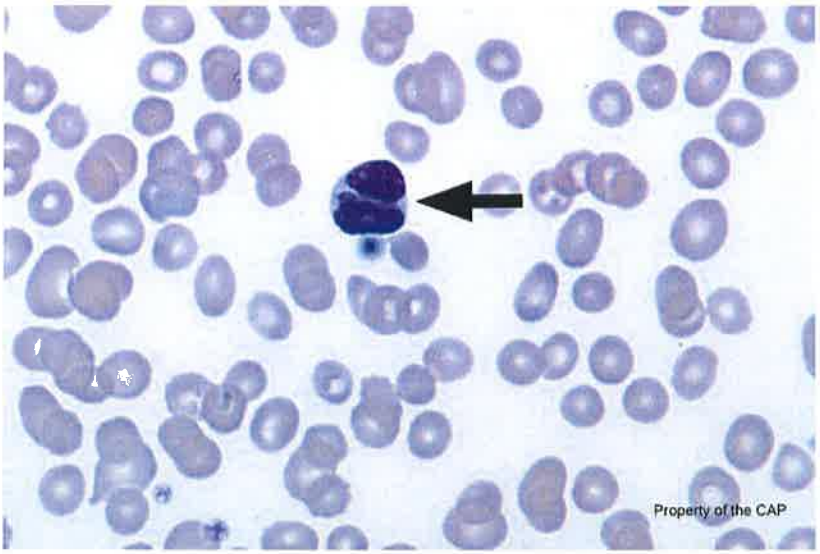


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Erythrocyte with overlying platelet	74	97.4	4527	96.3	Educational
Erythrocyte, normal	1	1.3	10	0.2	Educational
Howell-Jolly body (Wright stain)	1	1.3	32	0.7	Educational

The arrowed cell is an erythrocyte with overlying platelet, as correctly identified by 97.4% of referees and 96.3% of participants. Erythrocytes are mature red blood cells that are biconcave disc-shaped (diameter 6.7-7.8 µm) with central pallor accounting for approximately one-third of their diameter. A normal red blood cell should have the diameter of a resting lymphocyte. The overlying cell is a platelet which can be distinguished from a Howell-Jolly body based on size (platelets are larger) and granularity.

Blood Cell Identification – Ungraded

BCP-29

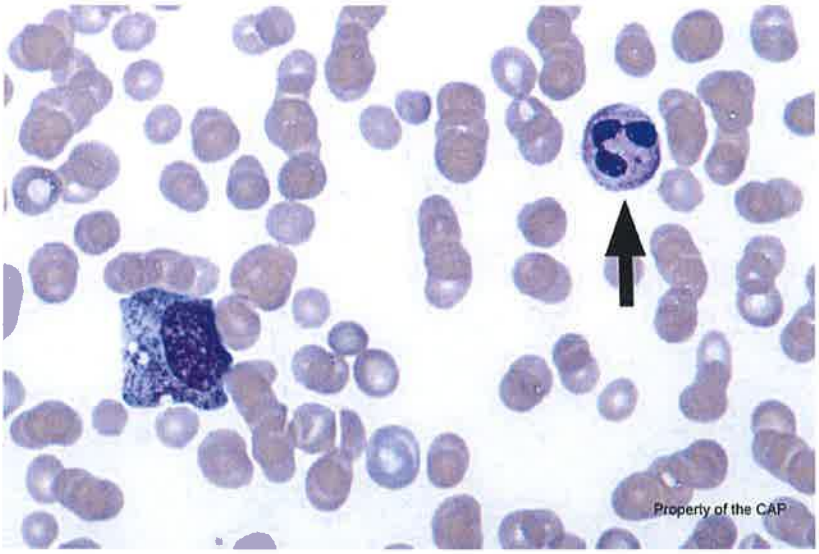


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Malignant lymphoid cell (other than blast)	42	55.3	2448	52.2	Educational
Lymphocyte, reactive	15	19.7	654	13.9	Educational
Lymphocyte	5	6.6	447	9.5	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	3	4.0	199	4.2	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	2	2.6	186	4.0	Educational
Lymphocyte, large granular	1	1.3	13	0.3	Educational
Monocyte, immature (promonocyte, monoblast)	1	1.3	94	2.0	Educational
Neutrophil, segmented or band	1	1.3	85	1.8	Educational
Neutrophil, metamyelocyte	1	1.3	20	0.4	Educational

The arrowed cell is a malignant lymphoid cell (other than blast), specifically a lymphoma cell, as correctly identified by 55.3% of referees and 52.2% of participants. These cells have prominent nuclear clefts or grooves, which is classically a feature of follicular lymphoma. The nuclei of these cells may appear binucleated, folded, indented, or convoluted. The cells are small to intermediate in size with coarse chromatin, indicating that they represent a mature neoplastic lymphocyte. The cytoplasm is agranular and varies from scant to moderate. A reactive lymphocyte or resting lymphocyte should not have a prominent cleft. Reactive lymphocytes also typically have more abundant cytoplasm than the cell pictured.

Blood Cell Identification – Ungraded

BCP-30



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, segmented or band	70	92.1	4172	88.8	Educational
Neutrophil, toxic	5	6.6	484	10.3	Educational
Leukocyte with *Anaplasma/Ehrlichia*	1	1.3	3	0.1	Educational

The arrowed cell is a segmented neutrophil, as correctly identified by 92.1% of referees and 88.8% of participants. It is the most frequent cell found in the blood. Neutrophils have a diameter of 10-15 µm and the nucleus is segmented or lobated with 2-5 lobes being normal. A filament should connect all of the lobes. Neutrophil segments have condensed chromatin. The cytoplasm contains specific granules and pale cytoplasm. If the granules are very numerous, dark, and prominent, designation as "toxic granulation" may be appropriate. In this case, the appearance is normal.

Case Presentation:

This peripheral blood smear is from a 45-year-old man with a previous history of axillary lymphadenopathy who now presents with lymphocytosis. Laboratory data include: WBC 19.4 x 10E9/L; RBC = 2.75 x 10E12/L; HGB 8.5 g/dL; HCT = 34.8%; MCV 97 fL; RDW = 20; PLT 138 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Follicular lymphoma

Follicular lymphoma is a B-cell derived malignancy of germinal center cell origin. Although the most common clinical presentation is peripheral lymphadenopathy, it infrequently presents with peripheral blood lymphocytosis. Up to 10% of cases may show variable degrees of peripheral blood involvement in the form of circulating lymphoma cells. At least 40% of cases have bone marrow involvement with paratrabecular lymphoid aggregates/infiltrates.

Follicular lymphoma is a common low-grade B-cell non-Hodgkin lymphoma that accounts for approximately 20% of the lymphomas diagnosed in the United States. It tends to affect middle-aged to older patients with a median onset in the 6th decade. Patients often present with widespread lymphadenopathy and splenomegaly. Extranodal disease can also occur, including involvement of GI tract. As noted above, marrow involvement is frequently seen. Cases with widespread involvement may also have peripheral blood involvement. Follicular lymphoma is seen in both men and women, with a male to female ratio approximately 1:1.7. Cases can occur in children (pediatric-type follicular lymphoma), although these cases often have a different clinical course with near normal overall survival and localized disease.

Review of the peripheral blood when peripheralized follicular lymphoma is present may show occasional atypical lymphocytes or, less commonly, frank lymphoctyosis. Cells in the peripheral blood often have nuclear clefts or irregular nuclear contours and are called centrocytes. Occasional larger cells with prominent nucleoli may be admixed consistent with centroblasts, although the majority of the cells should be small. Morphologically these larger cells may be concerning for possible blasts. Although morphologic features are suggestive, definitive diagnosis of follicular lymphoma usually required additional studies, such as immunophenotyping for definitive diagnosis. Bone marrow examination and/or lymph node biopsy may be helpful to determine the extent of disease and to exclude transformation to diffuse large B-cell lymphoma if numerous large cells are present. Lymphoid aggregates in the bone marrow are often characteristically paratrabecular, although nodular and diffuse infiltrates can also occur.

Flow cytometric immunophenotyping can be performed to distinguish follicular lymphoma from other types of B-cell lymphoproliferative disorders involving the peripheral blood. Follicular lymphoma is characterized by expression of CD10, CD19, CD20, CD22, CD38 (dim), and monoclonal surface light chain. This immunophenotype is consistent with lymphomas of follicle center cell origin including follicular lymphoma, Burkitt lymphoma, and diffuse large B-cell lymphoma. Morphologic and cytogenetic correlation is required to exclude the other lymphomas with a similar immunophenotype. In rare cases, CD10 may be negative, leading to a non-specific immunophenotype that could be confused with marginal zone lymphoma or lymphoplasmacytic lymphoma.

Follicular lymphomas demonstrate a characteristic genetic translocation t(14;18)(q32q21); *BCL2/IGH* which is seen in up to 90% of cases, and this translocation is most common in lower grade follicular lymphomas (those with fewer large cells). A minority of cases (10-15%), have *BCL6* rearrangements. Both of these cytogenetic abnormalities can also be seen in diffuse large B-cell lymphoma, so correlation with histologic findings is essential for diagnosis. Patients with follicular lymphoma may have concomitant diffuse large B-cell lymphoma (DLBCL) at

the time of diagnosis or may transform to DLBCL after treatment. Approximately half of patients will transform to DLBCL, with a transformation risk of 2-3% per year. Grading is required in tissue biopsies of follicular lymphoma. The grade can be assigned as 1-2, 3A or 3B and correlates with prognosis and the numbers of large cells that are present. Low-grade (1-2) cases tend to behave indolently with long survival and patients may initially be managed with a 'watch and wait' approach if asymptomatic. High-grade disease is more aggressive and requires chemotherapy.

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