Blood Cell Identification – Graded

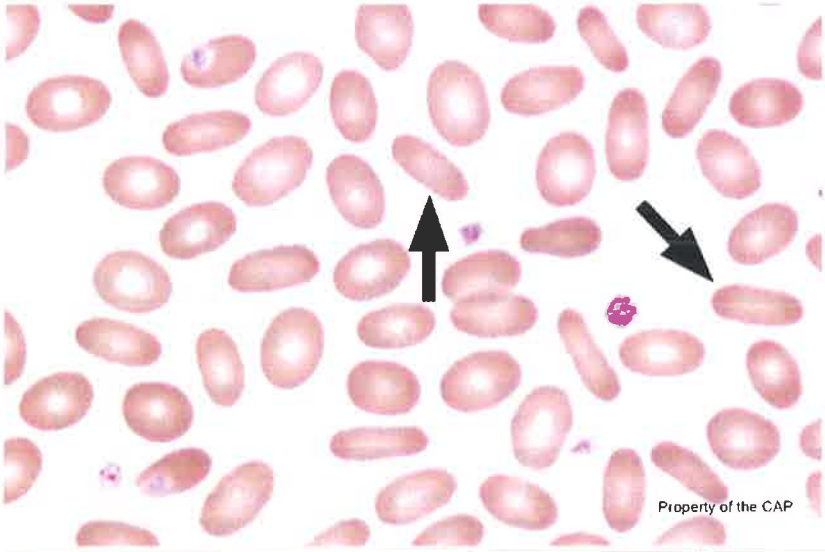
Case History

This peripheral blood smear is from a 15-month-old boy with no previous medical history. Laboratory data include: WBC = 10.4 x 10E9/L; RBC = 4.37 x 10E12/L; HGB = 13.1g/dL; HCT = 37.4%; MCV = 77 fL; MCHC = 34.0 g/dL; RDW = 16%; and PLT = 363 x 10E9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

To access the online Hematology Glossary, please click the hyperlink below:
http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution%20Folders/WebContent/pdf/hematology-glossary.pdf

BCP-11

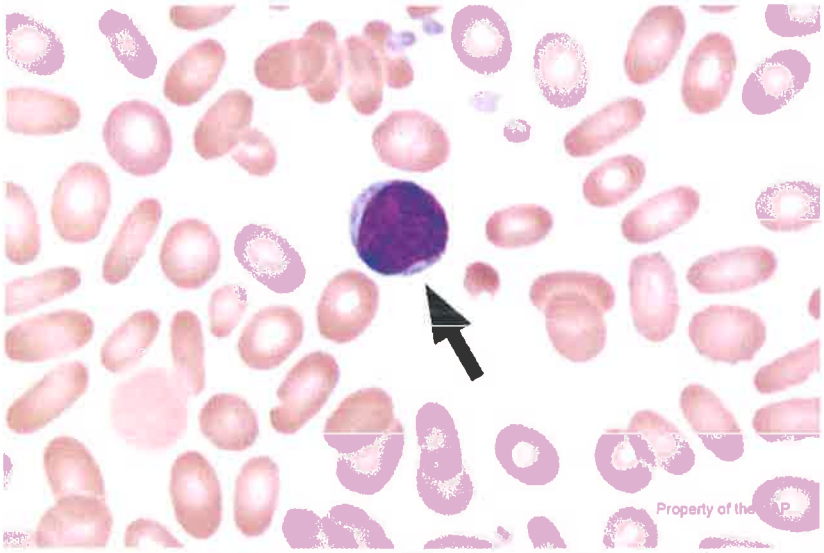


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Ovalocytes (elliptocytes)	96	100.0	5863	99.8	Good

The arrowed objects are ovalocytes (elliptocytes), as correctly identified by 99.8% of the participants and 100.0% of the referees. The terms elliptocytes and ovalocytes are used to describe the red blood cells appearing in the shape of a pencil or thin cigar, with blunt ends and parallel sides. A small number of elliptocytes/ovalocytes may be present on the smears of normal individuals (< 1%), whereas a moderate to marked elliptocytosis/ovalocytosis (> 25%) is observed in patients with hereditary elliptocytosis. Elliptocytes are also commonly increased in iron deficiency. Some ovalocytes may superficially resemble oval macrocytes but they are not as large and tend to be less oval. The ends of ovalocytes are always blunt and never sharp, unlike those of sickle cells.

Blood Cell Identification – Graded

BCP-12

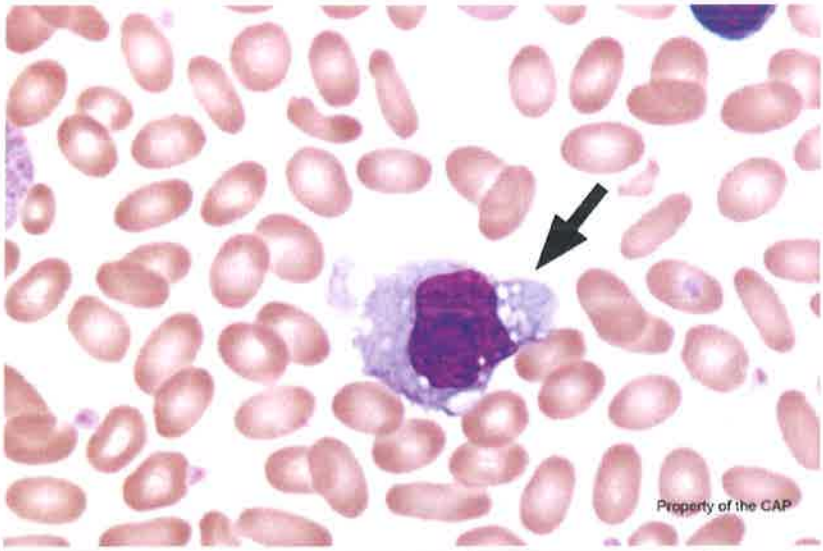


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte	96	100.0	5656	99.4	Good

The arrowed object is a lymphocyte, as correctly identified by 99.4% of the participants and 100.0% of the referees. While most normal lymphocytes are fairly homogeneous, they can exhibit a range of normal morphology. Lymphocytes are usually easily recognized by their round-to-oval nuclei, occasionally 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. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm.

Blood Cell Identification – Graded

BCP-13

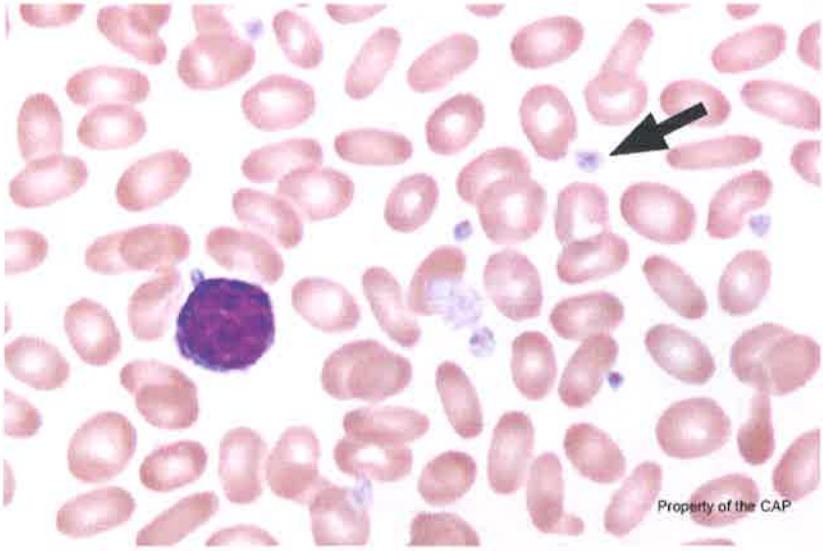


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Monocyte	94	97.9	5529	97.2	Good
Monocyte, immature (promonocyte, monoblast)	2	2.1	30	0.5	Unacceptable

The arrowed object is a monocyte, as correctly identified by 97.2% of the participants and 97.9% of the referees.. Monocytes are typically larger than neutrophils and lymphocytes. The majority of monocytes are round with smooth edges, but some may have pseudopod-like cytoplasmic extensions. The cytoplasm is abundant, with a gray or gray-blue ground-glass appearance, and may contain vacuoles or fine, evenly distributed azurophilic granules. The nucleus is usually indented, often resembling a three-pointed hat, but it can also be folded or band-like. The chromatin is condensed, but is usually less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus. The monocyte with granulation can usually be distinguished from large granular lymphocytes on the basis of its distinct nuclear features.

Blood Cell Identification – Graded

BCP-14

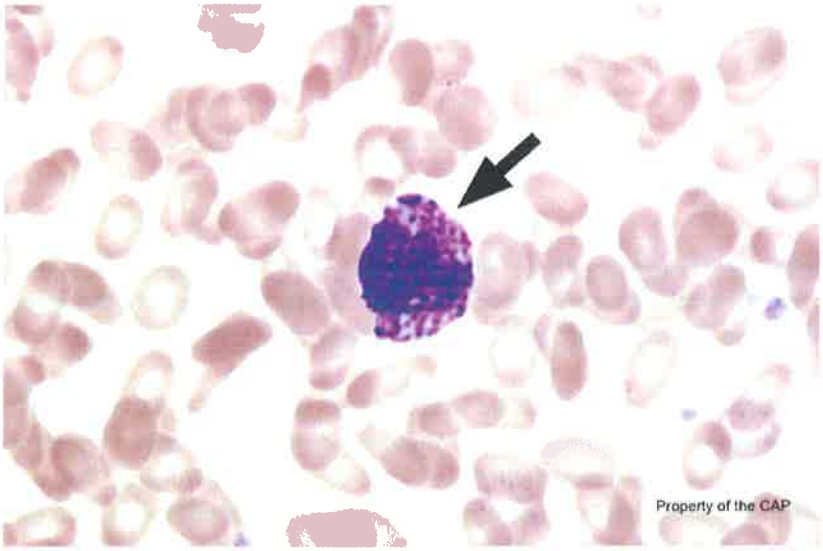


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Platelet	96	100.0	5641	99.1	Good

The arrowed object is a platelet, as correctly identified by 99.1% of the participants and 100.0% of the referees. Normal platelets, in fact fragments of megakaryocytic cytoplasm, are typically smaller than normal red cells. In contrast, giant platelets are larger than the typical red cell. Fine, purple-red granules are typical dispersed throughout the normal platelet cytoplasm or are sometimes aggregated at the center. These granules are platelet alpha granules and are absent or reduced in hypogranular forms. Platelets may be variable in shape, but most normal platelets are round or very slightly elliptical. Some have short cytoplasmic projections or ruffled margins.

Blood Cell Identification – Graded

BCP-15



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Basophil, any stage	96	100.0	5655	99.4	Good

The arrowed object is a basophil, as correctly identified by 99.4% of the participants and 100.0% of the referees. Basophils are characterized by the presence of numerous coarse and densely stained granules of varying sizes and shapes. The granules are larger than the granules of neutrophils, and most a roughly spherical. The granules are typically blue-black, but some may be purple-red, when stained using Wright-Giemsa preparations. The granules are unevenly distributed and frequently overlay and obscure the nucleus. Basophilia may be seen in several contexts, including in association with myeloproliferative neoplasms, in hypersensitivity reactions, with hypothyroidism, iron deficiency, and renal disease.

Case Presentation:

This peripheral smear is from a 15-month-old boy with no previous medical history. Laboratory data include: WBC = 10.4 x 10E9/L; RBC = 4.37 x 10E12/L; HGB = 13.1 g/dL; HCT = 37.4%; MCV = 77 fL; MCHC = 34.0 g/dL; RDW = 16%; and PLT = 363 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Hereditary Elliptocytosis

The features in this case are compatible with hereditary elliptocytosis (HE). The CBC parameters are within normal range, but the peripheral smear is notable for a prominent number of elliptocytes. Notably, the smear is negative for spherocytes, without red blood cell fragments, and an erythroblastic response is not seen.

HE is part of a family of autosomal dominantly inherited disorders resulting from mutations in genes encoding components of the skeleton of the erythrocyte membrane. Inherited mutations commonly ascribed to HE include those in genes encoding the α-spectrin (*SPTA1*), β-spectrin (*SPTB*) or band 4.1 (*EPB41*) proteins. These proteins make up the horizontally oriented cytoskeletal elements present along the inner cell membrane surface of erythrocytes. Owing to defective production or defective function of these proteins, the erythrocytes of affected experience a decrease in mechanical stability and a loss of the normal (and stable) erythrocyte bi-concave shape. These affected cells are often more easily fragmented as they pass through the microcirculation and are more readily destroyed by the spleen. Thus, the erythrocytes of patients with HE experience a shorter average life-span. The notable HE peripheral smear elliptocytosis has been attributed to persistence of the ellipsoidal shape that affected red blood cells must undertake to pass through small-caliber vascular structures.

Question 1. Which statement is CORRECT?

- **A. Hereditary elliptocytosis typically shows an autosomal recessive pattern or inheritance**
- **B. Protein abnormalities in hereditary elliptocytosis affect erythrocyte nuclear membrane structure**
- **C. The red blood cells of patients with hereditary elliptocytosis are often more easily fragmented**
- **D. Premature splenic destruction of erythrocytes in patients with hereditary elliptocytosis is never seen.**

The prevalence of HE is estimated at 3-5 cases per 10,000 in the US population. Akin to other red blood cell disorders, HE is encountered much more frequently in areas of endemic Malaria, owing to resistance to infection by elliptocytic red blood cells.

Despite the autosomal dominant inheritance in HE, there is variability patient-to-patient in the severity of disease (ie. incomplete penetrance). Patients with HE may be asymptomatic, with diagnosis made incidentally on examination of a blood smear; some patients may experience a clinically significant hemolysis; rare cases of hydrops fetalis have been attributed to HE. Indeed, the HE clinical spectrum includes several related entities: "common" hereditary elliptocytosis, typically identified incidentally and most commonly seen in patients of African descent; hemolytic HE, in which anemia (often associated with an unrelated intercurrent illness) motivates laboratory investigation; hereditary pyropoikilocytosis, in which a severe hemolytic anemia is manifest, in combination with a peripheral blood picture remarkable for red blood cell fragments; spherocytic elliptocytosis, seen more commonly in patients of European descent, in which an admixture of elliptocytes and spherocytes is noted; and Southeast Asian ovalocytosis, in which erythrocytes are relatively more rigid and do not have a shortened lifespan.

Question 2. Which statement is CORRECT?

A. **Rare cases of hydrops fetalis have been attributed to HE**
B. **HE patients typically experience a clinically significant hemolysis**
C. **HE is more commonly encountered in Malaria-endemic areas given that HE erythrocytes are more susceptible to infection by Malarial parasites**
D. **The Southeast Asian form of HE is characterized by erythrocytes with a shortened lifespan relative to other HE subtypes**

Diagnosis of HE is typically straightforward, based on morphologic evaluation of a peripheral smear in concert with evaluation of CBC parameters. Cases of HE are typified by elliptocytes making up the majority of erythrocytes. Diagnosis requires elliptocytes numbering greater than 15%, in order to reduce the likelihood of over diagnosis in cases of iron deficiency or other medical conditions in which a minor population of ovalocytes/elliptocytes may be observed. Although less frequently performed in the present day, the osmotic fragility test has been classically employed to assist in the subtyping of a putative HE case: non-hemolytic HE often shows a normal range osmotic fragility; osmotic fragility is increased in cases of hemolytic HE, hereditary pyropoikilocytosis or spherocytic HE; osmotic fragility is typically decreased in cases of Southeast Asian ovalocytosis.

It should be noted that some HE variants will also show abnormal results when tested for eosin-5-maleimide (EMA) binding (typically by flow cytometry). This test, typically used to assist in the diagnosis of hereditary spherocytosis (HS), may be positive in a variety of non-HS cases including hereditary pyropoikilocytosis. In contrast, EMA testing may be negative in cases of Southeast Asian ovalocytosis.

Many HE patients do not require medical intervention or specific treatment. Those patients with clinically significant hemolysis require supportive care as indicated. Long term surveillance for cholecystitis, splenomegaly and other sequelae of chronic hemolysis are indicated.

Question 3. Which statement is CORRECT?

A. **EMA testing is highly specific for HE**
B. **The osmotic fragility assay is diagnostic of HE**
C. **Elliptocytes number greater than 15% in HE**
D. **Splenomegaly is not of clinical concern in patients with HE**

Etienne R. Mahé, MD, MSc, FRCPC, FCAP
Hematology and Clinical Microscopy Committee

References:

1. Gallagher PG, Glader B. Hereditary Spherocytosis, Hereditary Elliptocytosis, and Other Disorders Associated with Abnormalities of the Erythrocyte Membrane (Chapter 27), In: Greer JP, et al. *Wintrobe's Clinical Hematology*. 13th Edition. 2014: Wolters Kluwer, UK.

2. Da Costa L, et al. Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. *Blood*. Rev (2013)

3. Barcellini W, et al. Hereditary red cell membrane defects: diagnostic and clinical aspects, *Blood Transfusion*. (2011)

Answers to Questions:

Question 1 answer: C: The red blood cells of patients with hereditary elliptocytosis are often more easily fragmented

With the exception of patients with Southeast Asian ovalocytosis, the erythrocytes of patients with HE are often more easily fragmented. In contrast, hereditary elliptocytosis typically shows an autosomal dominant pattern of inheritance; protein abnormalities in hereditary elliptocytosis affect the erythrocyte cell membrane (erythrocytes do not possess nuclei); and premature erythrocyte destruction by the spleen may be seen in patients with HE.

Question 2 answer: A: Rare cases of hydrops fetalis have been attributed to HE

Rare cases of hydrops fetalis have indeed been attributed to HE. In contrast, HE patients may not experience clinically significant hemolysis; HE is more commonly encountered in Malaria-endemic areas given that HE erythrocytes are more resistant to infection by Malarial parasites; and the Southeast Asian form of HE is characterized by erythrocytes with a relatively longer lifespan relative to other HE subtypes.

Question 3 answer: C: Elliptocytes number greater than 15% in HE

Diagnostic criteria require elliptocytosis of at least 15%. In contrast, EMA testing is not specific for HE; the osmotic fragility assay is not diagnostic of HE, but rather can assist in HE subtyping; and monitoring for splenomegaly is highly recommended in patients with HE.

Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from an 18-month-old girl presenting with anemia. Laboratory data includes: WBC = 9.9 × 10E9/L; RBC = 2.34 × 10E12/L; HGB = 7.1 g/dL; HCT = 21.3%; MCV = 62 fL; MCHC = 38 g/dL RDW = 37%; and PLT = 382 × 10E9/L. Absolute retic = 543 × 10E9/L (normal range 30 – 130 × 10E9/L). Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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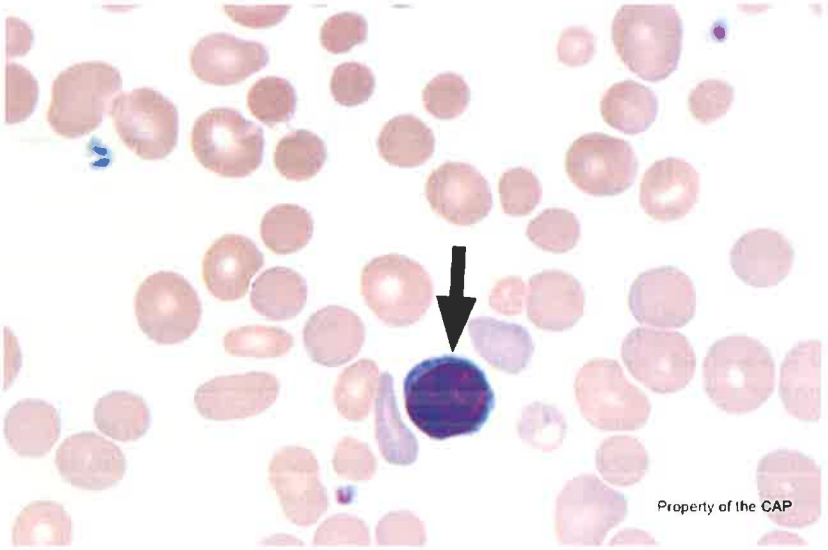
BCP-16

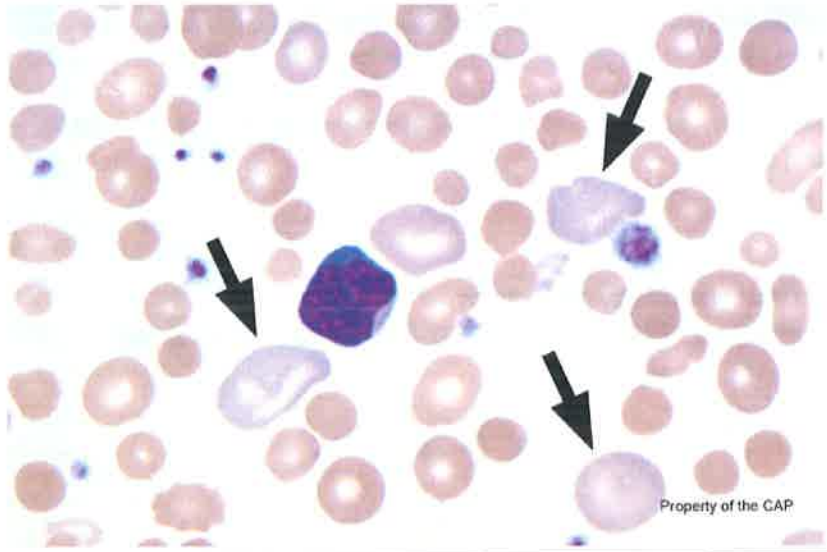


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte	95	100.0	5560	98.9	Educational

The arrowed object is a lymphocyte, as correctly identified by 98.9% of the participants and 100% of the referees. Small lymphocytes are usually only slightly larger than erythrocytes and contain a round to oval nucleus with clumped chromatin and relatively sparse lightly basophilic cytoplasm. Small lymphocytes usually have an inconspicuous nucleolus. Nucleated red blood cells are smaller than lymphocytes and have more condensed chromatin and blue-grey cytoplasm.

Blood Cell Identification – Ungraded

BCP-17

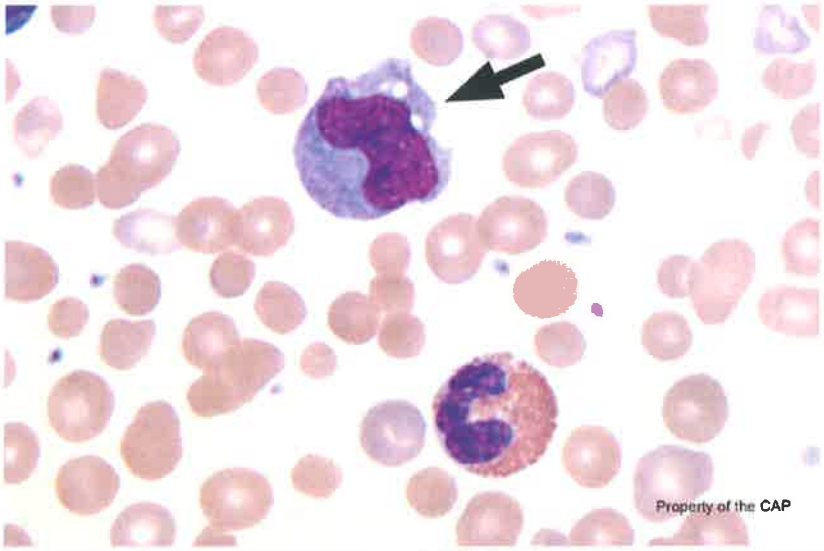


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
polychromatophilic (non-nucleated) red blood cells	95	100.0	5448	98.4	Educational

The arrowed objects are polychromatophilic (non-nucleated) red blood cells, as correctly identified by 98.4% of the participants and 100% of the referees. Mature erythrocytes are round to oval disc shaped cells measuring ~7 µm in diameter, and contain central pallor occupying less than one of the cell diameter. In contrast, polychromatophilic red blood cells (RBCs) are reticulocytes with blue-grey cytoplasm and occasionally contain fine basophilic granules. Polychromatophilic RBCs are larger than mature erythrocytes and typically lack the consistent central pallor of the more mature counterparts.

Blood Cell Identification – Ungraded

BCP-18

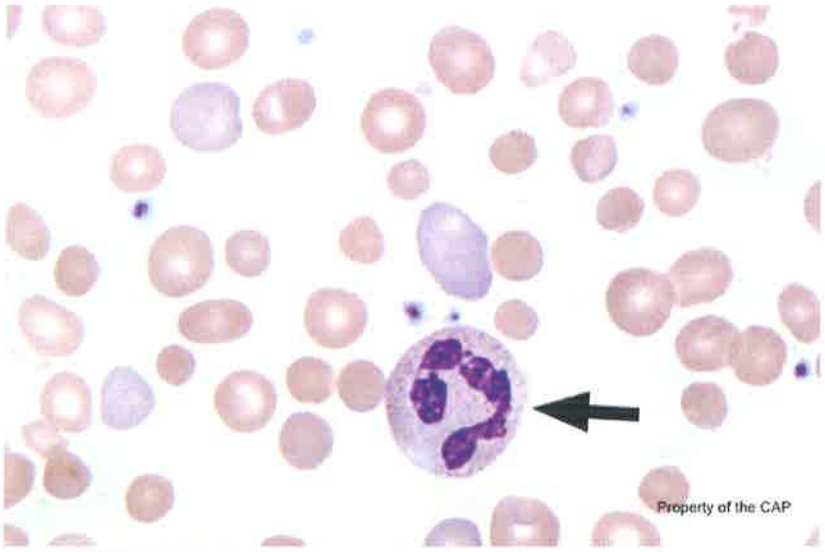


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Monocyte	94	99.0	5334	96.3	Educational
Monocyte, immature (promonocyte, monoblast)	1	1.0	78	1.4	Educational

The arrowed object is a monocyte, as correctly identified by 96.3% of the participants and 99.0% of the referees. In a normal peripheral blood smear, the monocyte is relatively infrequent, representing up to 10% of peripheral white blood cells. Monocytes are approximately 12-20 µm in size with convoluted or folded nucleus and abundant blue-grey cytoplasm. Cytoplasmic vacuolization is common (as seen in the image), and coarse eosinophilic vacuoles can occasionally be appreciated. In contrast to a lymphocyte, the monocyte has a fine and lacy chromatin pattern.

Blood Cell Identification – Ungraded

BCP-19

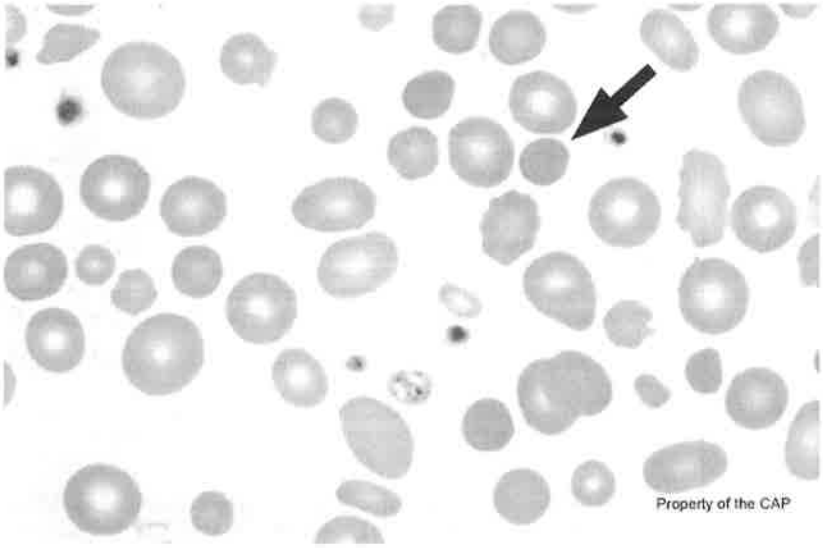


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, segmented or band	92	96.8	5291	95.6	Educational
Neutrophil, toxic	3	3.2	127	2.3	Educational

The arrowed object is a neutrophil, segmented or band, as correctly identified by 95.6% of the participants and 96.8% of the referees. Neutrophils are 10-15 µm in size and contain moderate pale pink cytoplasm containing fine, eosinophilic granules. The mature neutrophil has a segmented nucleus usually comprised of two to five lobes with condensed nuclear chromatin. Segmented neutrophils are mature granulocytes and usually represent the most predominant while cells in adult blood. A band neutrophil is nearly mature, with a curved, band-like nucleus that has not yet segmented.

Blood Cell Identification – Ungraded

BCP-20



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Spherocyte	93	97.9	5326	96.2	Educational
Microcyte (containing increased central pallor)	2	2.1	172	3.1	Educational

The arrowed object is a spherocyte, as correctly identified by 96.2% of the participants and 97.9% of the referees. Spherocytes are densely staining, spherically shaped red blood cells that lack central pallor and have a decreased diameter compared to normal red blood cells. Spherocytes may be found in patients with the red blood cell membrane disorder, hereditary spherocytosis, or in patients with immune hemolytic anemia. Increased spherocytes may also be seen as an artifact in very thin areas of a blood film.

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The College uses Exception Reason Codes that signify the proficiency testing (PT) for an analyte has not been graded. The Exception Reason Code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all of the analytes with an Exception Reason Code and investigate the acceptability of performance with the same rigor as if it were an unacceptable performance. The actions accredited laboratories should take include but are not limited to:

Code	*Exception Reason Code Description*	*Action Required*
11	Unable to analyze.	Document why the specimens were not analyzed (eg, instrument not functioning or reagents not available). Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
20	No appropriate target/response; cannot be graded.	Document that the laboratory performed a self-evaluation using the data presented in the Participant Summary and compared its results to a similar method, all method, or all participant statistics if provided. If comparison is not available, perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
21	Specimen problem.	Document that the laboratory has reviewed the proper statistics supplied in the Participant Summary. Perform and document alternative assessment for the period that commercial PT was not tested to the same level and extent that would have been tested. Credit is not awarded in these cases.
22	Result is outside the method/ instrument reportable range.	Document the comparison of results to the proper statistics supplied in the Participant Summary. Verify detection limits.
24	Incorrect response due to failure to provide a valid response code.	Document the laboratory's self-evaluation against the proper statistics and evaluation criteria supplied in the Participant Summary. Perform and document the corrective action of any unacceptable results. Document corrective action to prevent future failures.
25	Inappropriate use of antimicrobial.	Document the investigation of the result as if they were unacceptable and review the proper reference documents to gain knowledge of the reason your response is not appropriate.
26	Educational challenge.	Response to the CAP is not required. Laboratory should document its review.
27,31	Lack of participant or referee consensus.	Document that the laboratory performed a self-evaluation and compared its results to the intended response when provided in the Participant Summary. If comparison is not available, perform and document alternative assessment (ie, split samples) for the period that commercial PT reached non-consensus to the same level and extent that would have been tested.
28	Response qualified with a greater than or less than sign; unable to quantitate.	Document that the laboratory performed a self-evaluation and compared its results to the proper statistics supplied in the Participant Summary. Verify detection limits.
30	Scientific Committee decision.	Document that the laboratory has reviewed the proper statistics supplied in the Participant Summary.
33	Specimen determined to be unsatisfactory after contacting the CAP.	Document that the laboratory has contacted the CAP and no replacements specimens were available. Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
40	Results for this kit were not received.	Document why results were not received, corrective action to prevent recurrence and the laboratory's self-evaluation of the results by comparing results to the proper statistics and evaluation criteria supplied in the Participant Summary. If PT specimens were not analyzed, perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
41	Results for this kit were received past the evaluation cut-off date.	
42	No credit assigned due to absence of response.	The Participant Summary indicates which tests are graded (see evaluation criteria) and which tests are Not Evaluated/Educational. Updates to grading will also be noted. If a test is educational, the laboratory is not penalized for leaving a result(s) blank. The code 42 that appears on the evaluation is **not** a penalty. However, if a test is graded (regulated and non-regulated analytes) and your laboratory performs that test, results cannot be left blank. The laboratory is required to submit results for **all** challenges within that test or use an appropriate exception code or indicate test not performed/not applicable/not indicated. Exceptions may be noted in the Kit Instructions and/or the Result Form. Document corrective actions to prevent future failures.
44	This drug is not included in our test menu. Use of this code counts as a correct response.	Verify that the drug is not tested on patient samples and document to ensure proper future reporting.
45	Antimicrobial agent is likely ineffective for this organism or site of infection.	Document that the laboratory performed a self-evaluation of written protocols and practices for routine reporting of antimicrobial susceptibility reports to patient medical records. Document that routine reporting of this result to clinicians for patient care is compliant with specific recommendations of relevant Medical Staff and Committees (eg, infectious Diseases, Pharmacy and Therapeutics, Infection Control). Response to the CAP is not required.
77	Improper use of the exception code for this mailing.	Document the identification of the correct code to use for future mailings.
91	There was an insufficient number of contributing challenges to establish a composite grade.	Document the investigation of the result as if it were an unacceptable result. Perform and document the corrective action if required.
35, 43, 88, 92	Various codes.	No action required.

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FH(1-4, 6, 9-10, 13)-B 2017: HEREDITARY SPHEROCYTOSIS

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This activity is approved for continuing education credit in the states of California and Florida.

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Author	Commercial Interest	Your Role	What was received
Yuri D. Fedoriw, MD	None	Author	
Stephanie A. Salansky, MEd, MS, MT(ASCP)	None	Planner	

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Learning Objectives

Upon completing the reading and answering the learning assessment questions, you should be able to:

1. Describe the clinical presentation of hereditary spherocytosis (HS).
2. Identify the morphologic and common laboratory features of HS.
3. Explain the underlying erythrocyte defects of HS.
4. Understand the importance and findings of ancillary studies in the diagnosis of patients with HS.

FH(1-4, 6, 9-10, 13)-B 2017: HEREDITARY SPHEROCYTOSIS

Case Presentation:

This peripheral blood smear is from an 18-month-old baby girl presenting with anemia. Laboratory data include: WBC = 9.9 × 10E9/L; RBC = 2.34 × 10E12/L; HGB = 7.1 g/dL; HCT = 21.3%; MCV = 62 fL; MCHC = 38 g/dL; RDW = 37%; and PLT = 382 × 10E9/L. Absolute reticulocyte count = 543 × 10E9/L (normal range = 30 - 130 × 10E9/L).

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

INTRODUCTION

Hereditary spherocytosis (HS) is one of several inherited disorders of the red blood cell cytoskeletal proteins associated with hemolytic anemia. While prevalent worldwide, the highest incidence of HS is among those of Northern European ancestry, with the most severe forms of disease presenting in infancy and early childhood. The severity of disease is partially predicted by which of the structural proteins is defective, but that alone does not explain the variable clinical presentation and symptomatology. Ultimately, disruption of the erythrocyte cytoskeletal scaffold results in loss of the expected biconcave shape, membrane instability, and hemolysis. In the spleen, the defective and ridged erythrocytes are further damaged, and they enter systemic circulation as classic spherocytes lacking central pallor and visibly smaller than normal red blood cells (RBCs).

GENETICS AND CLINICAL PRESENTATION

Erythrocyte morphology and function are maintained by a complex framework of interacting cytoskeletal proteins. These include Ankyrin, Spectrin, Band 3, Protein 4.1, and Protein 4.2, among others. HS is caused by mutations or deficiencies of these structural proteins, with changes to the surface area to volume ratio, and associated hemolysis. Most cases of HS are inherited in an autosomal dominant fashion, with mutations of Ankyrin being the common. Severe forms of disease are rare, and have an autosomal recessive inheritance pattern.

As the underlying genetics are variable, so is the clinical presentation of HS. In classic cases, patients present in early childhood with mild-to-moderate hemolytic anemia, intermittent jaundice, and splenomegaly. In these, and mild forms of HS, the bone marrow can typically compensate for hemolysis, and the patients can be followed safely. A family history of hemolysis or consequences of hyperbilirubinemia, including gallstones and jaundice are common. Depending on clinical features, the patients may undergo splenectomy for definitive treatment. Removing the spleen limits further damage to the erythrocyte, although the molecular defect remains. Severe cases are resistant to splenectomy.

DIAGNOSIS

Although none of the clinical or conventional laboratory tests are specific, the constellation of findings is often diagnostic.

PERIPHERAL BLOOD EVALUATION

The laboratory plays an important role in establishing the diagnosis of HS. Ongoing extravascular hemolysis is reflected in a mild-to-moderate anemia, and an increased red cell distribution width (RDW) reflecting the combination of reticulocytosis and circulating spherocytes. The defective cellular structure of HS is associated with an increased mean corpuscular hemoglobin concentration (MCHC), typically greater than 36 g/dL.

A well-prepared peripheral blood smear from a freshly collected sample confirms the findings from the complete blood count and red cell indices. Classic spherocytes lack the typical central pallor, and appear smaller and more dense than normal erythrocytes (Figure 1). Importantly, spherocytes are not specific for HS, and can be seen as an artifactual change, and in the setting of immune mediated hemolytic anemia or thermal injury. As the hemolysis in HS is not immune mediated, the direct antiglobulin (Coombs) test is negative. Schistocytes and red cell fragments suggestive of intravascular hemolysis are similarly not present and would suggest alternative etiologies.

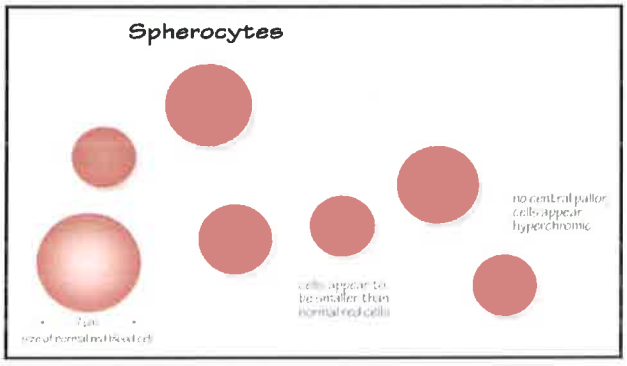

Figure 1. Spherocytes. Spherocytes depicted in this figure are smaller than the other red blood cells in the image and appear round with dense, homogenous cytoplasm that lacks central pallor (Wright-Giemsa). Used with permission from the Color Atlas of Hematology. EF Glassy (Ed), pp 100.

ANCILLARY STUDIES

Additional studies are used to support the clinical diagnosis. For technical and practical reasons, the ancillary tests are not uniformly available or well standardized. A number of newer assays are being developed, but not included in the discussion below. Bone marrow evaluation is not necessary, but would demonstrate erythroid hyperplasia as the hematopoietic system responds to ongoing hemolysis.

OSMOTIC FRAGILITY TESTING

The membrane defects of HS decrease the surface area-to-volume ratio of erythrocytes. As such, spherocytes are more susceptible to lysis in hypotonic solution than normal RBCs. In other words, normal RBCs can accommodate progressively hypotonic solutions without lysis, while spherocytes of HS lyse under even modestly hypotonic conditions. Typically, a range of buffered salt solutions ranging from 0.2% to 0.9% NaCl is used, and percent hemolysis at each concentration is measured spectrophotometrically. Samples can be incubated at 37°C for 24 hours to increase the sensitivity of detection. Curves are generated reflecting the percent hemolysis at a given saline concentration, and plotted against a normal or expected pattern. Osmotic fragility assays are useful screening tests but cannot discern HS from immune mediated hemolytic disease with spherocytes, although other laboratory studies can help in this context.

FLOW CYTOMETRIC EVALUATION OF BAND 3

Flow cytometric assays for HS have been developed, with the most conventional based on the detection of the Band 3 protein by fluorescently labeled eosin-5-maleimide (EMA). Although not entirely sensitive, defects in the majority of cytoskeletal proteins seen in HS will result in decreased binding of EMA to Band 3, and decreased fluorescence intensity as identified by flow cytometric analysis.

The combination of clinical and laboratory features described above is typically sufficient for effective diagnosis. Rare cases with equivocal test results or atypical presentations may require molecular testing or electrophoretic methods for definitive diagnosis.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis includes other causes of non-immune hemolytic anemia, both hereditary and acquired. These include other hereditary erythrocyte membrane defects such as hereditary elliptocytosis (HE) and pyropoikilocytosis (HP). The underlying defects of HE and HP are biologically similar, but red cell morphology is distinctly different. Other causes of extravascular hemolysis, including autoimmune hemolytic anemia, can be associated with spherocytosis, although, as discussed above, other clinical factor and laboratory studies can help definitively establish these diagnoses.

PROGNOSIS AND THERAPY

The prognosis and clinical course of HS is highly variable, and closely related to the degree of ongoing hemolysis. Many patients can be treated symptomatically, with only occasional hemolytic episodes under physiologic stress in adulthood. Splenectomy may be necessary in some cases, prolonging the red cell lifespan. Consequences of chronic hemolysis and hyperbilirubinemia, including pigmented gallstones (cholelithiasis) and gall bladder inflammation (cholecystitis) can be managed medically or surgically.

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