PROFICIENCY TESTING SERVICE
AMERICAN ASSOCIATION OF BIOANALYSTS
205 West Levee St. ◆ Brownsville, TX 78520-5596
800-234-5315 ◆ 281-436-5357 ◆ Fax 713-781-5008

Q1-2016 Hereditary Spherocytosis (HS)

Hemolytic anemias are characterized by abnormally shortened red cell survival. The degree of anemia that results is dependent upon the bone marrow response. Normal bone marrow can increase cell production up to eight times the normal baseline level. It is only when cell destruction occurs more rapidly that the bone marrow can respond that the patient will develop anemia. Hemolytic anemias are categorized as to whether the origin of the defect that results in the hemolysis is intrinsic (intracopuscular) or extrinsic (extracorpusular).

If red cells with intrinsic defects are infused into normal recipients, theses RBCs exhibit the same shortened life span as they did in the patient. When the source of the shortened red cell survival is extrinsic, normal RBCs infused into these patients will exhibit a shortened survival. Therefore, an intrinsic defect is that of the red cell itself whereas an extrinsic defect is external to the red cell.

Intrinsic defects that cause shortened red cell survivals include genetic defects of 1) the red cell membrane, 2) production of normal red cell metabolic enzymes, 3) changes to normal hemoglobin structure (hemoglobinopathies), 4) decreased production of the global portion of hemoglobin (thalassemias) and, 5) acquired intrinsic defects (ie. PNH). Extrinsic causes of shortened red cell survival include, 1) antibody mediated red cell membrane damage, 2) both physical and immunologic cell damage resulting from infection, 3) hemolytic action of chemical or toxin exposure, 4) acquired membrane disorders that induce splenic sequestration and, 5) red cell fragmentation caused by mechanical etiologies.

Laboratory evaluation of the patient who has anemia, an elevated MCHC, spherocytes in the peripheral blood and reticulocytosis is a step wise process that begins with determining whether the patient has an autoimmune hemolytic process, a hemoglobinopathy or Hereditary Spherocytosis. To proceed down the correct diagnostic pathway, first a direct Coombs test is done to determine if the hemolysis is from an autoimmune process. In this case it was negative. Next, either a hemoglobin electrophoresis or an osmotic fragility test would need to be performed to rule out hemoglobnoipathy or Hereditary Spherocytosis. In this case the osmotic fragility was performed first and was positive therefore the electrophoresis was not deemed necessary for diagnosis. A diagnosis of spherocytosis was made for this case study patient..

Hereditary Spherocytosis (HS) is the most common cause of non-immune hemolytic anemia among people of northern European decent. Approximately 1 in 5000 people in the US have some form of this disorder. A confusing aspect of this disorder is the heterogeneity of both age of onset and severity. The most severe cases will be diagnosed when the patient is newborn but those with the mildest cases may be diagnosed only if and when an environmental stressor of some sort triggers enough of a hemolytic process to cause anemia, jaundice, splenomegaly or gallstones to arise. The explanation for this wide variety of clinical pictures probably has to do with the fact that one or more of four different protein mutations can cause HS. In most cases the disorder is transmitted as an autosomal dominant trait and diagnosing the disorders in multiple generations of the same family is common. However, nearly a quarter of newly diagnosed patients seem to have an autosomal recessive mode of inheritance. For this inheritance pattern, the heterozygous individuals will be silent carriers and only the homozygous individuals will have HS.

This case of Hereditary Spherocytosis (HS) is the prime example of a hemolytic anemia caused by a defect in the red cell membrane. The normal structural organization of the red cell membrane provides the red cell with the necessary strength and flexibility to survive the the stress of navigating the circulatory system. Red cells must move through capillaries so small as to only allow cells to travel in single file. This requires that the red cells be able to temporarily deform and, once having traversed these tight spaces, reform into their original, normal, biconcave disc shape. To successfully complete this necessary “shape-shift’, the red cells need to, 1) have an appropriate cell surface to volume ratio, 2) have a cell membrane with the necessary viscoelastic properties and, 3) have cytoplasmic viscosity of a specific nature.

HS is characterized by compromised vertical interactions between the red cell membrane skeleton and the “spectrin-ankyrin-band 3” complex as well as weak contacts between spectrin and the lipids of the inner portion of the red cell membrane bilayer. These genetic defects result in loss of cell membrane, in the form of micro vesicles, reducing the cell surface area and reducing the cell surface to cell volume ratio. These red cells then lose their viscoelastic properties (flexibility) and the ability to deform, instead assuming a round, or “spherocytic shape”. Subsequently, as these spherocytes continue to circulate, each time they slowly move through the spleen, their prolonged exposure to the acidic, oxidant-rich environment results in further damage. This cycle repeats until the cell becomes so damaged that it is finally phagocytsed in the spleen. Splenomegaly is usually associated with any disease process that involves abnormally increased workload for the spleen. In the hemolytic anemias in general and in hereditary spherocytosis specifically, abnormal red cells are being destroyed at an abnormal rate, thus increasing the splenic worked. The majority of HS patients have some degree of splenomegaly.

The main laboratory features of HS, mild to moderate anemia, reticulocytosis, increased MCHC, spherocytes on the PBS, and hyperbilirubenemia can be explained by the following sequence of events. First, the membrane defect results in the formation of spherocytes which can be seen on examination of the peripheral blood smear. Since these cells have an abnormal cell surface area to cell volume ratio, this results in them also having an increased mean corpuscular hemoglobin concentration (MCHC >36.0). As they circulate, they become increasingly damaged until they are finally hemolyzed in the spleen. The shortened red cell survival triggers the bone marrow response in order to compensate for the increased red cell destruction and we see evidence of rapid red cell production in the form of increased reticulocytosis. Once the bone marrow is no longer able to keep up with the pace of the red cell destruction we see the appearance of anemia. The rapid turnover of the red cells leads to hyperbilirubinemia.

Intrinsic defects in RBC membrane proteins results in RBC exoskeleton instability. The four proteins implicated in cases of HS are Spectrin, both alpha and beta, Ankyrin, Band 3 and Protein 4.2. The Spectrin protein is a tetramer made up of alpha and beta dimers. Normally alpha-spectrin (SPTA1) is produced at triple the amount as is Beta-spectrin (SPTB). A mutation resulting in deficient production of SPTA1 is associated with recessive forms of HS. but if the mutation results in a deficiency of SPTB, then the mutation is inherited in an autosomal dominant pattern. In addition to spectrum mutations that affect levels of both SPTA1 and SPTB, other mutations have been identified that prevents normal binding of SPTA1 to Protein 4.1. Additional mutations have been identified, one of which results in production of abnormal beta-spectrin which will not bind to Ankyrin and another results in the patient only able to produces unstable beta-Spectrin.

Ankyrin is the principal site on the RBC membrane to which Spectrin binds. If a mutation causes an Ankyrin deficiency, then deficient numbers of Ankyrin binding sites prevent adequate amounts of Spectrin to bind to the RBC membrane resulting in an unstable RBC exoskeleton. Somewhere in the range of 75-80% of patients with autosomal dominant HS have a combined Spectrin/Ankyrin deficiency. Another 10-20% of HS patients with mild to moderate autosomal dominant HS have been identified as having a Band 3 deficiency. Several mutations of Protein 4.2 have been described, many of them appearing relatively frequently in the Japanese population, and all of them have RBC morphology that includes spherocytes, ovalocytes and/or elliptocytes.

In the newborn HS patient, primary treatment involves managing the hyperbilirubiemia to prevent kerniterus. This is done primarily with phototherapy but in severe cases this treatment may prove inadequate and exchange transfusion may be necessary. Although it may be clinically indicated, performing splenectomies on patients under six years old puts these children at risk for fatal infection from encapsulated organisms Streptococcus pneumonia and Haemophilus influenza. Typically this surgical treatment is delayed as long a possible and these patients, as well as all other HS patients, are routinely immunized against these organisms prior to removal of the spleen.

Because HS patients are constantly manufacturing replacement red cells they can develop folic acid deficiency and subsequently experience what is called “a megaloblastic crisis “. Reducing the risk of this complication requires HS patients receive lifelong folic acid supplementation. Splenectomy is the standard treatment for patients with clinically severe HS and, with the exception of a rare autosomal recessive variant, normally results in full control of the disease. Historically, regardless of the severity of their HS disease, patients with splenomegaly were thought to have an increased risk of blunt splenic injury from trauma. However, medical reviewers have examined the medical records of large numbers of HS patients and have concluded any increased risk is statistically insignificant and that medical practice has changed. Currently, HS patients considered to have mild, compensated disease and with a hemoglobin >11 gm/dl, are no longer routinely offered splenectomy. There remain the group of HS patients whose hemoglobin falls within the 8-11 gm/dl range, the so-called patients with “moderate asymptomatic disease”, for whom little has improved in the way of moderating surgical prophylactic protocols. More study needs to be done for this group of intermediate HS patients.

As many as 50% of HS patients, including those with mild disease, will develop gallstones formed from bilirubin. Because even asymptomatic HS patients are at high risk for gallstone formation, it is strongly recommended that they receive regular ultrasonic evaluation. To reduce the need for future splenectomy, when gallstones are detected, the current recommendation is for prophylactic cholecystectomy.

After splenectomy, nearly all patients will see a 1-1.5 gm/dl rise in hemoglobin, the need for replacement red cell production goes down and therefore their reticulocyte counts get closer to normal range. The WBC and Platelet counts rise post splenectomy. This is believed to be a physiologic response partly due to the removal of the organ most responsible for effectively culling senescent cells from the blood. However, the fact that the WBC count rises after splenectomy does make using the WBC count as an indicator for developing post surgery sepsis a less specific indicator.

Cell Identification

Specimen 1

Code - Result	No.	Flag
Spherocyte	282	
Microcytic	28	
Erythrocyte, normal RBC	3	***
Polychromatophilic RBC	2	***
Abnormal, would refer	1	
Anisocytosis	1	
Stomatocyte	1	***
Myeloblast	1	***
Intended result:		
Total Population	319	
Intended result: Spherocyte		

Specimen 2

Code - Result	No.	Flag
Lymphocyte, normal	260	
Nucleated RBC, any stage	34	***
Lymphocyte, abnormal/atypical	10	***
Lymphocyte; atypical, Downey, var	4	***
Abnormal, would refer	3	***
Immature RBC, would refer	3	***
Myelocyte	2	***
Lymphocyte, reactive	2	***
Total Population	318	
Intended result: Lymphocyte Normal		

Specimen 3

Code - Result	No.	Flag
Monocyte, any stage	197	
Immature Neutrophil	110	
Monocyte, normal	6	
PMN with Dohle Bodies	3	
Abnormal, would refer	1	
Segmented Neutrophil (PMN, poly)	1	
Total Population	307	
Intended result: Monocyte		

consensus this sample was not evaluated. 17 of 22 Referees identified the intended result.

Specimen 4

Code - Result	No.	Flag
Segmented Neutrophil (PMN, poly)	303	
Immature Neutrophil	5	***
PMN with Toxic Granulation/Vacuoliz	5	***
Abnormal, would refer	1	***
Abnormal Granulocyte, would refer	1	***
Eosinophil, any stage	1	***
Myelocyte	1	***
PMN with Dohle Bodies	1	***
PMN with Pelger-Huet Nucleus	1	***
Total Population	319	
Intended result: Segmented Neutrophil		

Specimen 5

Code - Result	No.	Flag
Polychromatophilic RBC	180	
Macrocytic	117	
Dimorphic RBC	3	
Schistocyte (bite, blister, helmet)	3	***
Anisocytosis	2	***
Poikilocytosis	2	***
Erythrocyte, normal RBC	2	***
Abnormal, would refer	1	
Abnormal RBC, would refer	1	
Hypochromic	1	
Spherocyte	1	***
Stomatocyte	1	***
Reticulocyte (supravital stain)	1	***
Basophilic Stippling	1	***
PMN with Pelger-Huet Nucleus	1	***
Lymphocyte, normal	1	***
Total Population	**318**	
Intended result: Polychromatic RBC		

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.

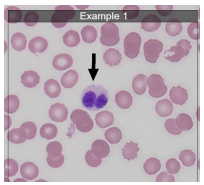
EDUCATIONAL CHALLENGES

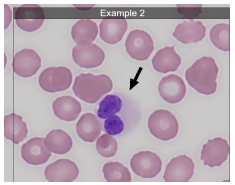
Specimen 1	No.
PMN with Pelger-Huet Nucleus	149
Abnormal, would refer	5
Segmented Neutrophil (PMN, poly)	4
(pyknotic PMN)	4
Monocyte, any stage	4
Abnormal Lymphocyte, would refer	2
Abnormal Granulocyte, would refer	2
Immature Neutrophil	2
Lymphocyte, abnormal/atypical	2
Eosinophil, any stage	1
Monocyte, normal	1
Dimorphic RBC	1
Metamyelocyte	1
Plasma Cell, any stage	1
Megakaryocyte	1
Total Population:	***180***

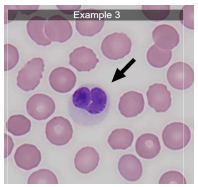
Intended result: Pseudo Pelger-Huet

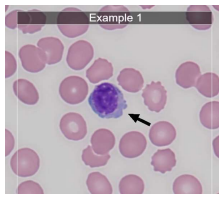
Specimen 2	No.
Nucleated RBC, any stage	118
Immature RBC, would refer	12
Lymphocyte, normal	9
Lymphocyte; atypical, Downey, variant	8
Lymphocyte, reactive	6
Plasma Cell, any stage	6
Lymphocyte, abnormal/atypical	5
Abnormal, would refer	4
Lymphocyte, normal	2
Abnormal Lymphocyte, would refer	2
Polychromatophilic RBC	1
Immature Neutrophil	1
Promyelocyte	1
PMN with Toxic Granulation/Vacuolization	1
PMN with Pelger-Huet Nucleus	1
Hairy Cell	1
Monocyte, any stage	1
Megakaryocyte	1
Total Population:	***180***

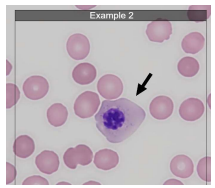
Intended result: Nucleated RBC

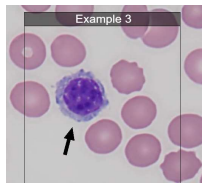












***To see the original fullsized immages, please sign on to your data entry sheet at http://www.aab-pts.org/**

Sample 16Q1-1 & 2: *History: A 74-year-old man is admitted to the ICU with acute respiratory distress. On examination he is pale with scattered petechiae on his trunk and both arms. He has an open infected wound on his right elbow that he says has failed to heal since scraping it on a doorway one week ago. He has been fatigued lately, and the day prior to admission began feeling lightheaded and unable to catch his breath. His wife brought him to the Emergency Department and he was subsequently admitted for further evaluation. CBC results: WBC 11.5, Hgb 7.2 g/dL, Hct 22.8%, Plts 29,000/µL.*

The automated CBC values are significant for anemia and thrombocytopenia with an elevation of the WBC count. The peripheral smear confirms the decrease in platelet count, which appears lower than the automated count. There is moderate aniso- and poikilocytosis of the RBCs, with spherocytes, macrocytes, elliptocytes, helmet cells, and fragmented red cells. The cells to be identified in **Specimen #1** are hypolobated PMNs with a **Pelger-Huët nucleus.** Typically, only two round nuclear lobes (bilobed) are present; the thin chromatin filament between the lobes may or may not be apparent. This gives the nucleus the appearance of spectacles ("pince-nez") or a peanut lying within the cytoplasm of the neutrophil.

The Pelger-Huët anomaly can be the result of either a congenital or acquired condition. Congenital Pelger-Huët anomaly is the result of a mutation in the lamina β-receptor (LBR) gene which helps control neutrophil differentiation and the shape of the nuclear membrane. Even though the nucleus is bilobed, these cells have a normal life span. The Pelger-Huët anomaly has an autosomal dominant inheritance pattern. Heterozygous individuals are clinically normal without any functional defects of their neutrophils; approximately half of their neutrophils will exhibit the characteristic bilobed nucleus. Individuals who are homozygous for the anomaly likewise have a clinically benign course, with almost all neutrophils demonstrating the characteristic pince-nez nuclei. This characteristic neutrophil was first reported by Dr. Karl Pelger, a Dutch hematologist, in the 1920's in two patients with disseminated tuberculosis. Since these patients subsequently died, he described the presence of this anomaly on a peripheral smear as a grim prognostic sign. A few years later, Dr. G.J. Huët, a Dutch pediatrician, described the same anomaly in a child with tuberculosis. However, his patient had a favorable outcome and he noted that these neutrophils with the bilobed nucleus continued to circulate well after his patient had recovered. He deduced that this anomaly was not a response to infection, but rather may have a genetic component. After screening various relatives of the patient, he found the same nuclear anomaly in several of them, and eventually deemed the Pelger-Huët anomaly a benign autosomal dominant inherited trait.

Acquired Pelger-Huët anomaly (also called pseudo-Pelger-Huët anomaly) can be seen in association with myelodysplastic syndrome (MDS) as well as leukemia, particularly AML and CML. Many physicians regard the Pelger-Huët anomaly as a marker for underlying myelodysplasia and associate this finding with a poor prognosis. In some individuals with MDS, the presence of Pelger-Huët cells occurs fairly late in the disease and may only be seen following extensive chemotherapy. Although most often associated with MDS, the acquired Pelger-Huët anomaly has also been .

described in vitamin B12 deficiency, folate deficiency, malaria, enteroviral infections, multiple myeloma, and following treatment with a variety of drugs (gancyclovir, sulfa drugs, tacrolimus, rituximab, and lorazepam among others).

Pelger-Huët cells should not be confused with band neutrophils, such as would occur with a left-shift. These cells are quite characteristic and close review of a peripheral smear should easily distinguish them from band neutrophils. Pelger-Huët cells have normal appearing cytoplasm without toxic granulation. Band/immature neutrophils seen with a left-shift would be expected to have increased granulation of the cytoplasm.

The cells to be identified in **Specimen #2** are **nucleated RBCs** (NRBC). These cells represent the last stage of RBC differentiation prior to extrusion of the nucleus and are normally confined to the bone marrow. Except during the neonatal period, the presence of NRBCs in the peripheral blood is typically considered abnormal and signifies bone marrow damage or stress. Most hematology analyzers are able to differentiate WBCs from NRBCs and will "flag" the results for NRBCs. Older analyzers, however, may not consistently flag NRBC levels of <5%. The number of NRBCs should be assessed based on review of the peripheral smear and are reported as the number of NRBCs per 100 WBCs.

Cells #1 and #3 in **Specimen #2** are more immature than Cell #2. Cells #1/#3 are polychromatophilic normoblasts – the nucleus is round to ovoid and composed of coarse clumped chromatin without any nucleolus. The cytoplasm is blue-gray and the nuclear:cytoplasmic (N:C) ratio is approximately 4:1. In contrast, **Cell #2** is an orthochromic normoblast. This cell again has a round nucleus with tightly condensed chromatin without nucleoli; this pyknotic nucleus is undergoing degeneration prior to being expelled with resulting formation of a reticulocyte. Note that the nucleus is smaller (N:C ratio of 1:2), and the gray-pink cytoplasm is more abundant, approaching the cytoplasmic coloration of a mature RBC.

NRBCs may be confused with small mature lymphocytes. Both have round nuclei with tightly condensed chromatin; however, the cytoplasm of a lymphocyte is typically blue to blue-gray and may contain azurophilic granules. If one is in doubt, it is often helpful to scan a peripheral smear for cells for comparison. In this peripheral smear, only a rare lymphocyte is present.

This peripheral smear is from a patient with **Myelodysplastic Syndrome** (MDS), most likely RAEB (see below). MDS is a group of hematologic disorders resulting from ineffective maturation of myeloid blood cells. In the bone marrow, the production of blood cells becomes disorderly and ineffective ("dysplastic") and cytopenias (particularly anemia and thrombocytopenia) develop. The number of myeloblasts in the bone marrow increase and may appear in the peripheral blood. MDS was previously called "pre-leukemia" and indeed approximately 30% of patients with MDS progress to acute myelogenous leukemia.

The mean age of onset of MDS is 68 years; the median age at diagnosis is between 60 -75 yrs. Males are affected slightly more often than females. The signs and symptoms exhibited by individuals with MDS are typically nonspecific and usually the result of the developing cytopenias – fatigue, chest pain, chills, shortness of breath (anemia); easy bruising, petechiae, hematomas, gum bleeding (thrombocytopenia); and increased susceptibility to infections (neutropenia). Hepatomegaly and splenomegaly can develop. Although anemia is the most common cytopenia that occurs in MDS, these patients rarely suffer side effects of severe anemia. Rather, the two most serious complications in these patients are due to thrombocytopenia or infection. It is important to keep in mind, however, that many individuals are asymptomatic and MDS is first identified based on the results of a CBC and careful review of a peripheral smear.

Although the exact cause of MDS is not known, several risk factors associated with its development have been identified. These include age (older than 60 yrs), smoking or tobacco use, exposure to excessive radiation (nuclear reactors, atomic bomb), exposure to the chemical benzene, previous radiation or chemotherapy treatment, and rare congenital disorders (Fanconi anemia, congenital neutropenia, Shwachman-Diamond syndrome).

MDS is thought to be the result of mutations that occur in the bone marrow stem cells, but the specific defect is poorly understood. Differentiation of the stem cells is impaired and a significant number of cells die within the bone marrow, never maturing to the point of being released into the circulation.

Classification of myelodysplastic syndromes has been revised in the last several years. The basic classification scheme divides the disorders into refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-T), and chronic myelomonocytic leukemia (CMML). The blast count is one of the most important prognostic indicators in MDS – individuals initially diagnosed with increased blasts have a poorer survival, and an increasing blast count in an MDS patient often results in transformation to AML. Therefore, obtaining an accurate blast count is crucial, both in the bone marrow and the peripheral blood.

Reviewing the peripheral smear of this patient, the abnormalities associated with MDS become more apparent. As previously mentioned, neutrophils with the Pelger-Huët nucleus and hypogranular cytoplasm are characteristically associated with MDS. Thrombocytopenia and anemia (low hemoglobin/hematocrit as well as the presence of NRBCs) are common findings. Several blasts are also present – using a 10-20x magnification, scan the cells around the periphery (particularly up and to the right) of Cell #1 in **Specimen #1**. A number of large cells with a high N:C ratio are present which have fine nuclear chromatin with prominent nucleoli and a thin rim of very basophilic cytoplasm. These are most likely myeloblasts, however Auer rods are not apparent and a bone marrow biopsy and aspirate, combined with flow cytometry studies would be required for a definitive characterization. The blasts seen in RAEB may be smaller in size than "normal" myeloblasts, but still have a high N:C ratio and scant cytoplasm. In poorly stained or prepared smears, these blasts can be confused with lymphocytes.

RAEB is the most clinically aggressive subtype of MDS; patients have a high risk of progression to AML, which develops in up to one-third of patients. The median survival is 9-12 months. Patients with RAEB have 5-20% blasts in the bone marrow. Blasts (<20%) may be present in the peripheral blood. Irrespective of the blast count, the presence of Auer rods is associated with a poorer survival. Treatment is largely supportive. Blood transfusions are used for anemia and low-dose chemotherapy may reduce the blast burden. Allogeneic bone marrow transplantation has been used successfully in some younger patients.

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