PROFICIENCY TESTING SERVICE
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Q3-2016 - Platelet Satellitosis

Case History

This 38 year old landscaper is seeking medical care after falling from a ladder and injuring his shoulder. Laboratory values are as follows: WBC - 8.7, RBC - 5.18, Hgb 14.6, Hct - 45.2, normal RBC indices, Platelets -96,000.

Modern automated hematology analyzers can generate a remarkable array of information regarding patients WBCs, RBCs and platelets. These analyzers use updated versions of traditional hematology laboratory automated technologies, such as electronic impedance and flow-cytometry. Constantly improving software is employed to analyze and characterize tremendous amounts of raw data. This evolution in laboratory analyzer capabilities, when combined with state of the art LIS and middle-ware resources, enables the most advanced hematology laboratories to generate huge volumes of highly, accurate and precise information. Much of this impressive improvement has come about because complex algorithms have been developed that allow "non-normal" specimen conditions to be identified or "flagged". The "flags" alert the operator, or in some instances, the LIS, that a suspect condition may exists and usually will trigger further evaluation.

However, as impressive as the "flagging" abilities of these analyzers are , they still are only as accurate as the initial raw data the analyzer "sees". We know that certain specimen characteristics may adversely affect the accuracy of these analytical methods and these results from these specimens should be double-checked, using methods that can circumvent these known "analyzer blind spots".

As required by Quality Assurance standards, our laboratory procedures and the manufacturer provided instrument manuals have specifics sections devoted to identifying the kinds of specimens characteristics that may affect results accuracy. It is these "non-normal" conditions that generate the majority of what, in the hematology laboratory, are sometimes referred to as a "smear review" or an "eyes- on" review.

For these reasons, laboratories frequently develop additional review criteria, especially for unique patient populations (e.g. oncology or renal dialysis facilities). Whether identified by the analyzer flagging software settings, critical value and/or delta check settings programmed into the LIS or by the professional reviewing the data before final release, the goal of all of these methods is to reach an appropriate balance between ensuring timely reporting of results while at the same time reporting only accurate test results. In other words, reaching the fine line between reviewing too few (i.e. false normal) and too many (i.e. false "non-normal") .

The methodologies used in modern automated hematology analyzers separate platelets from RBCs and WBCs by using the size differential between these cells lines. Gating is used to set the low and high size limits for what the analyzer will identify as a platelet. Very small RBCs and/or RBC fragments may overlap the top end of the size range assigned to platelets. This can results in the analyzer incorrectly identifying them as platelets, thereby generating an incorrect platelet count than is higher than it actually is. The newest advanced hematology analyzers have made efforts to correct this problem by adding additional analytics to flag specimens suspect for "RBC fragments". Presumably these flag would results in both the RBC and platelet count results being further evaluated prior to results being released.

In contrast, to falsely elevated platelet counts, falsely low counts , "pseudo thrombocytopenia" , occur if the sample exhibits either platelet clumping or platelet satelliting. Again, the newest analyzers have modified the software used to analyze platelet data and attempt to address this issue with a "platelet clumping" flag. The flag would, as above, presumably generate further evaluation prior to results being reported. However, as of yet, there is not a "platelet satelliting" flag.

The importance of recognizing the patients whose samples demonstrate platelet satellitism isn't so much the abnormality itself but rather the affect on determining accurate platelet counts. This is the dilemma posed by this particular sample. In this particular laboratory the platelet satelliting seen on this patient sample did not generate a graphic fingerprint that could trigger any analyzer generated flag alert. However, this laboratory did have in-house criteria established that required a manual smear review when the sample was the initial CBC being ordered and the platelet results were outside of the normal range of 150-400 K. The purpose of the manual smear review was to determine if the numeric count matched the smear estimate. In this case study, the two did not match. The platelet count of 96K was not reported but was instead reported as " normal platelet numbers by blood smear estimation" and the comment " platelet satelliting present, redraw requested" was added to the report.

Platelet satellitism is a real phenomenon and not an artifact. The involved cell, platelets, neutrophils/bands and occasionally monocytes, exhibit normal morphology and have normal function. Although there have been recent articles suggesting there may be an association between vasculitis, lupus or mantle cell lymphoma with platelet satellitosis, an additional eighty plus cases of healthy individuals have been reported. Therefore, at present, the predominant thinking remains that there is no definitive relationship between the satelliting and any medication or medical condition.

What is known is that platelet satelliting is transient and seen primarily in EDTA anti-coagulated blood that has been cooled to room temperature. The amount of satelliting observed is time dependent, the longer the sample is incubated at RT, the more satelliting is observed, peaking somewhere in the 60 minute range. Usually if the sample is recollected using a different anticoagulant, like citrate, heparin or ACD, the satelliting will no longer be observed. However, there have been a few reports where the phenomenon persists despite changing the type of anticoagulant that the blood is collected in.

Research has determined that the platelet to neutrophil (band, neutrophil) attachment is mediated by the presence, in the blood of these patients, of an IgG immunoglobulin that attaches to the glycoprotein IIb/IIa complex on the platelet membranes and also the FC receptor III on the surface of the affected WBCs. Incubation at 37°C, may decrease, but not eliminate, the attachment.

The presence of this protein can be demonstrated with mixing studies. When the plasma of the implicated blood is mixed with ABO compatible blood, transfer of the phenomenon to the originally unaffected blood sample will occur. Even more convincing, is the experiment where the suspect plasma is preincubated with anti-IgG and then mixed with an ABO compatible sample. In this case, the satelliting is not seen to appear in the originally unaffected sample.

Cell Identification

Specimen 1

Code - Result	No.	Flag
Monocyte, any stage	291	
Monocyte, normal	9	
PMN with Toxic Granulation/Vacuolization	3	***
Lymphocyte; atypical, Downey, variant	2	***
Lymphocyte, reactive	2	***
Eosinophil, any stage	1	***
Abnormal, would refer	1	***
Total Population	309	

Intended result: Monocyte, any stage

Specimen 2

Code - Result	No.	Flag
Platelet, giant	279	
Platelet Satellitosis around PMN	19	
Abnormal Platelet, would refer	2	
Megakaryocyte	2	***
Basophil, any stage	1	***
Segmented Neutrophil (PMN, poly)	1	***
Abnormal, would refer	1	
Stain artifact	1	***
Stomatocyte	1	***
Lymphocyte, normal	1	***
Platelet, normal	1	***
Platelet Clumping	1	***
Total Population	303	

Intended result: Platelet, giant

Specimen 3

Code - Result	No.	Flag
Immature Neutrophil	191	
PMN with Toxic Granulation/Vacuolization	75	
Segmented Neutrophil (PMN, poly)	40	
Lymphocyte, normal	1	***
Abnormal Granulocyte, would refer	1	
Metamyelocyte	1	***
PMN with Dohle Bodies	1	***
Total Population	310	

Intended result: Immature Neutrophil

Specimen 4

Code - Result	No.	Flag
Erythrocyte, normal RBC	288	
Spherocyte	11	***
Abnormal, would refer	3	***
Microcytic	3	***
Polychromatophilic RBC	3	***
Lymphocyte, normal	1	***
Anisocytosis	1	***
Total Population	308	

Intended result: Erythrocyte, normal RBC

Specimen 5

Code - Result	No.	Flag
Segmented Neutrophil (PMN, poly)	282	
Hypersegmentated Neutrophil	10	***
Segmented Neutrophil (PMN, poly)	9	
Granulation/Vacuolization	2	***
Platelet Satellitosis around PMN	2	
Monocyte, normal	1	***
Erythrocyte, normal RBC	1	***
PMN with Dohle Bodies	1	***
PMN)	1	***
PMN with Pelger-Huet Nucleus	1	***
Total Population	**310**	

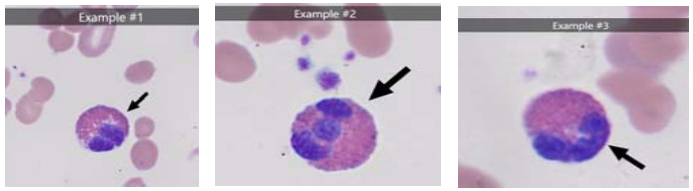
Intended result: Segmented Neutrophil (PMN, poly)

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.
Nucleated RBC, any stage	190
Immature RBC, would refer	3
Plasma Cell, any stage	3
Lymphocyte, normal	2
Reticulocyte (supravital stain)	1
Hypersegmentated Neutrophil	1
Total Population:	***200***
Intended result: Nucleated RBC	

Specimen 2	No.
Eosinophil, any stage	198
Hypersegmentated Neutrophil	1
Plasma Cell, any stage	1
Total Population:	***200***
Intended result: Eosinophil	



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

Sample 16Q3-1: ***History:*** *This 67-year-old man with a history of hemolytic anemia, atrial fibrillation, COPD, and hypertension was admitted to the hospital by his cardiologist after presenting with increasing shortness of breath and edema in both lower extremities. His past surgical history is significant for gastric bypass surgery, splenectomy, and cholescystectomy. Physical examination showed sclera icterus, 1+ edema bilaterally in lower extremities, tachycardia, atrial fibrillation, and a soft cardiac murmur. He was dizzy when standing and was afebrile. He was admitted for further evaluation of congestive heart failure. Subsequent laboratory tests showed the following: LDH 5056 U/L (RR: 125-220 U/L), total bilirubin 3.2 mg.dL (RR: 0.2-1.2 mg/dL), plasma hemoglobin 480 mg/dL (RR: 0.0-30.0 mg/dL), and haptoglobin <6 mg/dL. His CBC results were as follows: WBC 17.5, Hgb 8.3 g/dL, Hct 26.2%. Plts 341,000/µL. Identify the indicated cells in Specimen 1 and Specimen 2.*

The automated CBC values are significant for leukocytosis and anemia. The majority of participants correctly identified Cell ID #1 as **nucleated RBCs** (**NRBCs)**. Since red cell maturation occurs in the bone marrow, immature RBCs are not often seen in the peripheral blood. If the NRBC enters the circulation prematurely from the bone marrow, one or more of the maturation stages may be evident. In such instances, the stages typically seen are the late polychromatophilic normoblast or the orthochromic normoblast. The cytoplasm is blue-gray, changing to gray-pink as hemoglobinization occurs. In the polychromatophilic normoblast, the nucleus occupies much of the cell, while in the later orthochromatic stage, the nucleus becomes more pyknotic and eccentrically placed. Eventually the nucleus is observed at the periphery of the RBC membrane and is extruded. Although NRBCs bear some resemblance to lymphocytes, there are important features that can be used to distinguish between these cells. A lymphocyte is typically round or ovoid with a nucleus that occupies the majority of the cell (N:C ratio ranges from 5:1 to 2:1); the cytoplasm is blue. In contrast, the NRBC has an N:C ratio of approximately 1:2, with an often eccentric nucleus surrounded by abundant cytoplasm. Although a small number of NRBCs are normally present in neonates, their presence in the blood of adults is consistent with bone marrow replacement or infiltration of the bone marrow, compensatory erythropoiesis (such as due to severe anemia or hemorrhage), extramedullary hematopoiesis (chronic hemolytic anemia, myelofibrosis, leukemia), and disorders associated with hypoxia or hyposplenism. The number of NRBCs in the circulation depends on the severity of the anemia and the ability of the bone marrow to respond.

The cells to be identified in Cell ID #2 are **eosinophils**. The eosinophils present are mature and normal in appearance, with a segmented or bi-lobed nucleus (which has been described as resembling a pair of Ray-Ban® or aviator-style sunglasses) composed of clumped chromatin. There is abundant cytoplasm containing many coarse, round, uniform, refractile orange-red granules. Occasionally, a few granules overlie the nucleus.
Further history was obtained and the patient stated he had been given a diagnosis of warm autoimmune hemolytic anemia for which he underwent a splenectomy in 2005 and had no “flares” of hemolytic anemia which required treatment since that time. He had numerous RBC transfusions prior to his splenectomy.

Review of the peripheral smear shows anisocytosis with hypochromia, spherocytes, polychromasia, basophilic stippling of RBCs, rare Howell-Jolly bodies, and giant platelets. The findings on the peripheral blood smear, in conjunction with an elevated LDH and plasma hemoglobin, and decreased haptoglobin are consistent with a hemolytic process. Additional laboratory tests were significant for a reticulocyte count of 9.5%, BNP 1072 pg/mL (RR: 0-100 pg/mL), and a troponin I level within reference range. The admitting diagnosis was congestive heart failure and hemolytic anemia. Following admission, his hemoglobin dropped from 8.3 g/dL to 5.4 g/dL, necessitating RBC transfusion. His antibody screen was positive with all cells tested and an antibody panel showed a panagglutinin reactive at the antihuman globulin (AHG) phase only; no underlying alloantibodies were detected. The direct antiglobulin test (DAT) was 3+ with IgG; negative with complement; an eluate showed reactivity against all panel cells tested without specificity. The findings were consistent with the presence of a warm-reactive IgG autoantibody with resultant **autoimmune hemolytic anemia (AIHA)**.

The clinical expression of AIHA is extremely variable, ranging from mild anemia that occurs over a period of months to sudden onset of hemolysis with severe anemia. The clinical signs and symptoms include pallor, fatigue, weakness, tachycardia, dyspnea, jaundice, and splenomegaly or hepatosplenomegaly. Laboratory findings include normochromic, normocytic anemia, polychromasia with reticulocytosis, spherocytes, decreased haptoglobin, increased total and unconjugated (indirect) bilirubin, increased LDH, increased urine bilinogen (urobilinogen), and positive DAT.

The detection of RBC autoantibodies is not uncommon in the transfusion service. These antibodies are typically classified as either cold autoantibodies, if optimum serologic reactivity is at room temperature (25C) or lower, or warm autoantibodies, if reactivity occurs at 37C or the antihuman globulin (AHG) phase of testing. Although cold- or warm-reactive RBC autoantibodies may be detected in a patient, only a small number have an associated hemolytic anemia. One published retrospective study found that only 29% of patients with warm-reactive autoantibodies experienced a hemolytic anemia. More often, these antibodies create havoc in obtaining compatible (or serologically incompatible) blood for the patient.
prevented by other therapy.

Splenectomy is successful in approximately two-thirds of patients who are refractory to steroids and can result in a complete remission. However, splenectomy brings the life-long risk of predisposure to infections, particularly by encapsulated organisms. More recently, rituximab has been used for treatment of refractory WAIHA. This is a monoclonal antibody directed against the CD20 antigen which is found on early B cells and plasma cells (the cells which produce the autoantibody). Good results have also been seen with use of alemtuzumab (another monoclonal antibody that targets CD52, found on both B and T cells). These agents are now used prior to consideration of splenectomy, and may be considered as first-line therapy in some patients.