California Association for Medical Laboratory Technology

Distance Learning Program

Hematology Case Studies: Platelets

by

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Course Number: DL-985
1 .0 CE/Contact Hour
Level of Difficulty: Intermediate

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HEMATOLOGY CASE STUDIES: PLATELETS

OBJECTIVES:
After completing this course the participant will be able to:
1. Differentiate among the causes of thrombocythemia.
2. Explain how to determine the platelet count when the count is above the upper reportable range of the analyzer.
3. Estimate the platelet count from the blood smear.
4. List the signs and symptoms of Essential Thrombocythemia.
5. Enumerate the causes of thrombocytopenia.
6. Discuss the causes of pseudothrombocytopenia.
7. Explain the methods of determining the causes of pseudothrombocytopenia.

Case #1

A 44-year-old woman comes in for a complete blood count (CBC) as part of a routine physical exam. The results from the hematology analyzer, Cell-Dyn 1700 ® (Abbott Diagnostics), are:

WBC	7.5 K/µL		RBC	4.22 M/µL
Lym	28.7 %		HGB	12.4 g/dL
MID	10.4 %		HCT	38.6 %
Gran	60.9 %		MCV	91.4 fL
			MCH	29.3 pg
PLT	>>>> K/µL		MCHC	32.0 g/dL
			RDW	13.5 %

MID cells may include less frequently occurring and rare cells correlating to monocytes, eosinophils, basophils, blasts, and other precursor white cells.

Questions:

1. What is abnormal about her CBC?
2. Which parts can be reported?
3. What procedures can be done regarding the abnormal result?
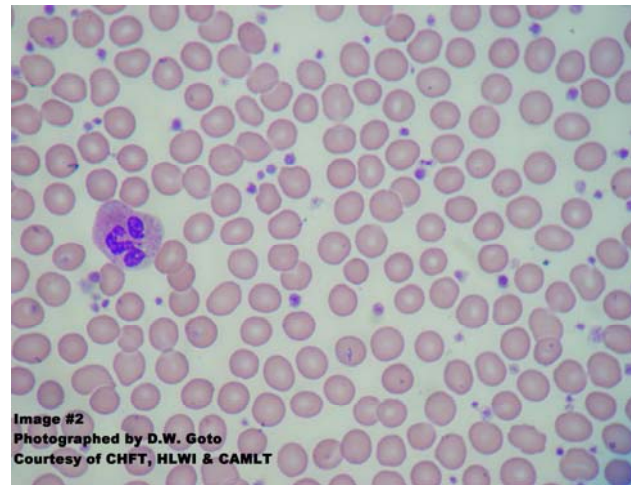
Answers:
1. The platelet count is above the upper reportable range.
2. The WBC histogram and 3-part differential are normal and can be reported. The RBC histogram is normal and can be reported.
3. To determine the platelet count:
   a. Make a 1:1 dilution of the whole blood and re-run the platelet count. Correct the platelet count for the dilution.
   b. Make a smear of the whole blood and examine for platelet morphology and numbers.

Discussion:

The platelet count on 1:1 diluted blood was 534, so the platelet count is 2 x 534 = 1,068 K/µL (normal is 150-400 K/µL).

On blood smears made from EDTA-blood and stained with a Romanowsky stain, platelets are round or oval, 2-4 µm in diameter, and separated from one another. The platelet count can be estimated from the smear. At 1000x magnification (oil immersion), this is equivalent to about 7-30 platelets per oil immersion field (OIF). Count the number of platelets in 10 oil immersion fields. Divide the total by 10 to get the average number of platelets per field. Each platelet seen on the smear equates to approximately 15,000/µL. Multiply the average number per OIF to get the platelet estimate[1]. See Image #1. In this case the average number of platelets per field was 70. The estimate equals 70 x 15,000 = 1,050 K/µL. Thus the platelet estimate derived from the smear in Images #1 and #2 correlates with the corrected platelet count of 1,068 K/µL.

Image #1
Photographed by D.W. Goto
Courtesy of CHFT, HLWI & CAMLT

Image #2
Photographed by D.W. Goto
Courtesy of CHFT, HLWI & CAMLT

The causes of increased platelet counts include:

Inflammatory disorders	Iron deficiency anemia
Splenectomy	Chronic granulocytic leukemia
Polycythemia vera	Undetected cancer
Essential (primary) thrombocythemia	

Since the patient had no symptoms, no history of splenectomy, and normal WBC and RBC hemograms, all except essential (primary) thrombocythemia can be eliminated or are unlikely.

Essential (Primary) Thrombocythemia[2]

Essential thrombocythemia (ET) is a myeloproliferative disease. These diseases are a group of disorders that share features that include the clonal overproduction of one or more blood cell lines. Clonal diseases begin with a mutation in one or more bone marrow cell lines. Myeloproliferative diseases include polycythemia vera, myelofibrosis, chronic granulocytic leukemia, and essential thrombocythemia.

In ET there is overproduction of megakaryocytes, the precursor to platelets (thrombocytes). Abnormalities in platelet aggregation and adhesiveness tests suggest defective platelet function[3]. In about half the patients with ET there is a mutation of the JAK2 (Janus kinase 2) gene in their blood cells. In the others the cause is unknown.

ET occurs mostly in adults. There are about 0.1 to 2.4 new cases per 100,000 in the U.S. each year. The disease does not ordinarily shorten life expectancy, but serious complications can occur, so the patient needs to be followed by a physician.

Many patients have no symptoms. In others signs, symptoms and complications of ET result from the increased numbers of platelets in the peripheral blood. Since platelets are involved in the process of clot formation in response to blood vessel injury, the most common complication of ET is blockage of blood vessels by excess platelets (thrombosis). Less often the increased platelets cause bleeding.

Signs, symptoms, and complications include:
- Burning or throbbing in the feet
- Headache, dizziness, and weakness or numbness on one side to the body or other signs of inadequate blood flow to the brain
- Thrombosis (abnormal clotting)
- Unexpected or exaggerated bleeding (infrequent, associated with very high platelet count)
- Enlarged spleen
- Complications of pregnancy

Diagnosis of ET may occur when a higher than normal platelet count occurs on a routine blood count (as with this patient), or on a blood count that is ordered on a patient who has a blood clot, unexpected bleeding, or an enlarged spleen and there is no other cause for the increased numbers of platelets. In ET the platelet count is over 600 K/µL blood and remains high in subsequent counts. Although the diagnosis cannot be made by laboratory tests alone, the following may be useful: JAK2 mutation in blood cells, slightly lower than normal blood hemoglobin and slightly higher WBC count, no evidence of other myeloproliferative diseases, and an examination of the bone marrow. The bone marrow will show a significant increase in megakaryocytes and masses of platelets.

Treatment of patients with ET is based on the risk of clotting or bleeding complications. If there are no signs or symptoms, the patient is seen for regular checkups. If the patient has high risk as determined by previous clotting or bleeding episodes, a history of a clot, cardiovascular risk factors--diabetes, high cholesterol, smoking, hypertension, obesity--therapy may be considered.

Drug therapy may include aspirin, hydroxyurea, anagrelide, or interferon alfa. Aspirin, although decreasing clotting, may increase the risk of bleeding. When the platelet count is very high and the patient suffers acute clotting, plateletpheresis may be done on an emergency basis.

This patient had no symptoms and was given follow-up appointments.

Case #2

A 38-year-old female inpatient has the following results on her initial complete blood count on Coulter Gen-S ® (Beckman-Coulter):

WBC	8.9 K/µL	RBC	4.86	M/µL
NE	57.9 %	HGB	14.4	g/dL
LY	33.4 %	HCT	42.5	%
MO	6.3 %	MCV	87.4	fL
EO	1.9 %	MCH	29.8	pg
BA	0.5 %	MCHC	34.0	g/dL
		RDW	12.5	%
		PLT	64	K/fL

Suspect/Definitive Messages/Flags: MPV 6.9 fL
Micro/Fragmented Red Cells
Giant Platelets   R flag on Platelet Count & MPV
Platelet clumps   Comments:
            Do not verify platelets; review first
            and redraw if necessary

Questions
1. What is abnormal about the blood count?
2. Which parts of the CBC can be reported?
3. What would you do to investigate the abnormal result?

Answers:
1. The platelet count is abnormally low and there are flags for microcytic or fragmented RBC, giant platelets, or platelet clumps.
2. The WBC histogram and differential are normal and can be reported.
3. The platelet and RBC histogram patterns are consistent with platelet clumps, fragmented red cells, or microcytic red cells. Make and review the smear (See Image #3) for platelet clumps, fragmented red cells, or small red cells before verifying the platelet count.
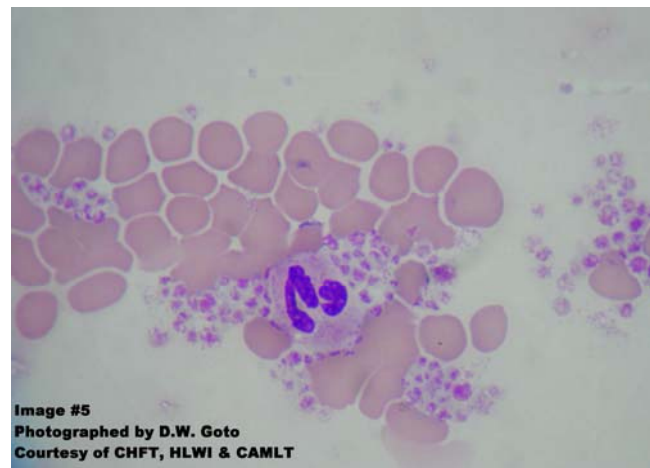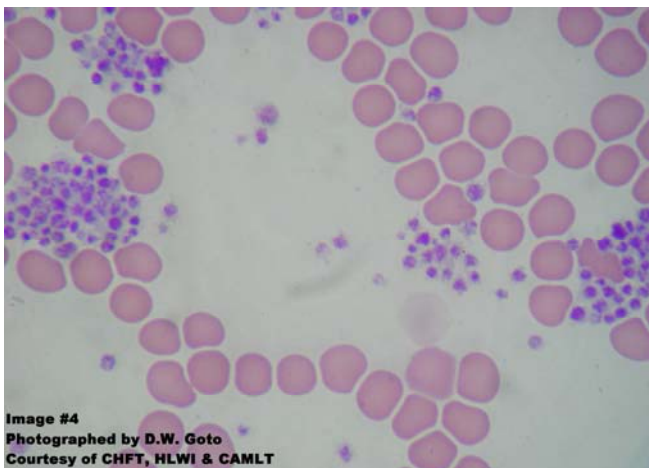

Image #3
Photographed by D.W. Goto
Courtesy of CHFT, HLWI & CAMLT
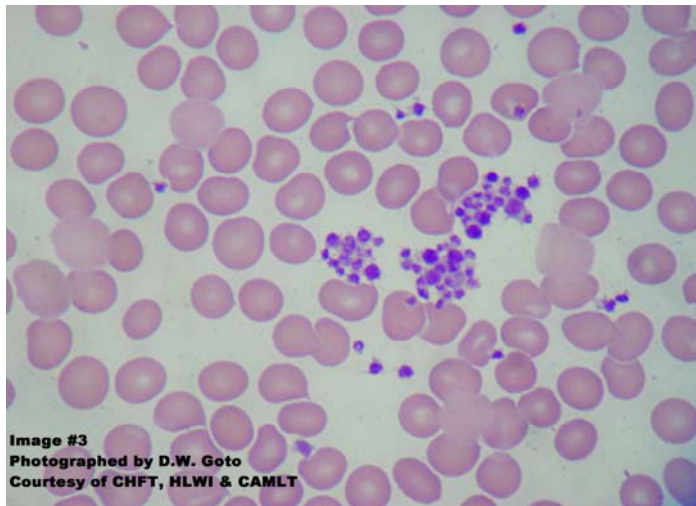
Discussion:
    The platelet count was below normal, a condition known as thrombocytopenia.
The causes of decreased platelet counts are[4]:

- Decreased Production
    Leukemia or lymphoma
    Cancer treatments such as radiation or chemotherapy
    Various anemias
    Toxic chemicals
    Medications: diuretics, chloramphenicol
    Viruses: chickenpox, mumps, Epstein-Barr, parvovirus, AIDS
    Alcohol in excess
    Genetic conditions: Wiskott-Aldrich, May-Hegglin, Bernard-Soulier syndromes
- Abnormal distribution
    Splenomegaly with sequestration in the spleen
- Increased destruction
    Autoimmune diseases: Idiopathic (immune) thrombocytopenic purpura
    Medications: quinine, antibiotics containing sulfa, Dilantin®, vancomycin,
            rifampin, heparin-induced thrombocytopenia
    Surgery: man-made heart valves, blood vessel grafts, bypass machines
    Infection: septicemia
    Pregnancy: about 5% of pregnant women develop mild decrease
    Thrombotic thrombocytopenic purpura
    Disseminated intravascular coagulation
- Pseudothrombocytopenia
    Partial clotting of specimen
    EDTA-platelet clumping
    Platelet satellitism around WBCs
    Cold agglutinins
    Giant platelets

Results of the blood smear evaluation (Case #2, Image #3):
        The smear showed numerous platelet clumps (make sure to examine the edges of the
smear since the clumps may migrate there; Images #4 and #5). There were no giant platelets,
fragmented RBC, or small RBC. To obtain an automated platelet count, obtain a blood specimen
drawn into Sodium Citrate (NaCitrate).

Image #4
Photographed by D.W. Goto
Courtesy of CHFT, HLWI & CAMLT

Image #5
Photographed by D.W. Goto
Courtesy of CHFT, HLWI & CAMLT

Results of the platelet count on the NaCitrate specimen (Case #2, Image #6):

There were no flags or error messages. The platelet count of 289 K/µL needs to be corrected for the dilution of the blood by liquid NaCitrate as follows:

289 x 1.1 (dilution factor) = 318 K/µL

The diagnosis is EDTA-platelet clumping. This condition may persist for decades without any evidence of abnormal hemostasis. EDTA-platelet clumping needs to be recognized and documented in the patient's chart to prevent unnecessary treatment for thrombocytopenia, and to guide future laboratory tests.

Image #6
Photographed by D.W. Goto
Courtesy of CHFT, HLWI & CAMLT
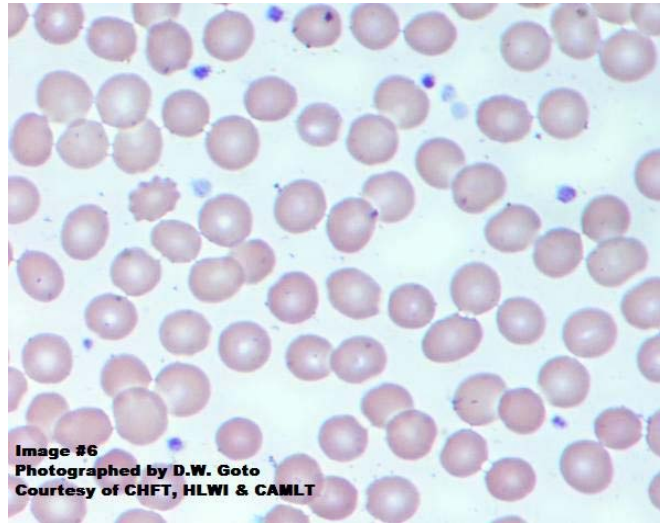
Causes of pseudothrombocytopenia are as follows:

Partial clotting of the specimen:

With a low platelet count the first procedure is to examine the specimen for evidence of clotting as well as to make a smear and look for evidence of platelet clumping. When blood clots, platelets adhere to the clot and are removed from the fluid blood. If evidence of micro-clots or clumping is seen, obtain a new specimen.

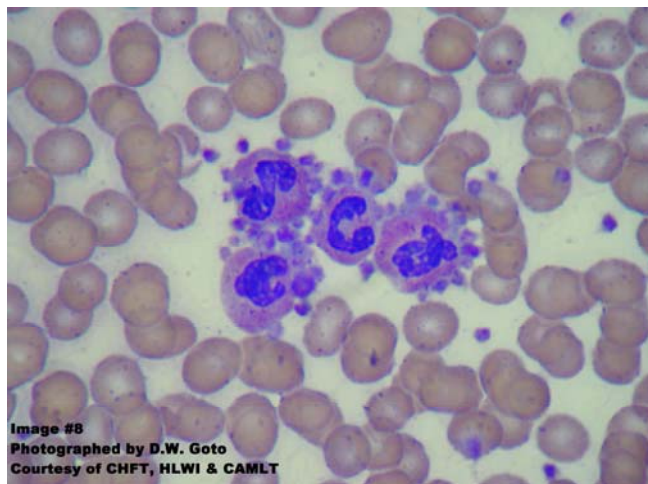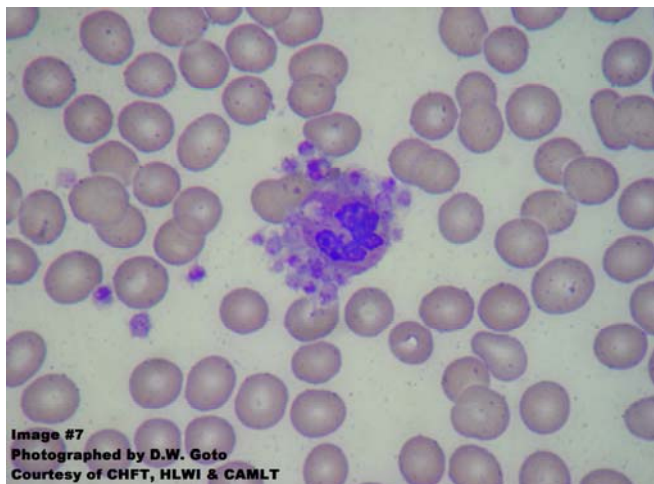EDTA-Induced Platelet Agglutination (EIPA) (EDTA-platelet clumping):

EIPA is an *in-vitro* phenomenon due to the presence of naturally occurring autoantibody against a cryptantigen on the GPIIb/IIIa platelet receptor. Under normal *in vivo* conditions this antigen is not accessible for antibody binding (crypt or hidden antigen). When calcium is chelated by EDTA, the GPIIb protein undergoes a structural change that exposes the cryptantigen. The antibody can then bind to the exposed site and crosslink to other platelets causing agglutination. The condition occurs in 0.1 to 2% of hospitalized patients[5].

Platelet satellitism

In this phenomenon platelets rosette around neutrophils or rarely around other cells. The satellite platelets are not counted by automated cell counters, resulting in spurious thrombocytopenia. Platelet satellitism is caused by EDTA-dependent antiplatelet and antineutrophil IgG antibodies in the patient's plasma (5).

The phenomenon has not been associated with any disease state or drug and is thought to be benign.

The diagnosis is made by making a blood smear and looking for platelet rosettes: Images #7 and #8. This needs to be documented in the patient's chart.

Image #7
Photographed by D.W. Goto
Courtesy of CHFT, HLWI & CAMLT

Image #8
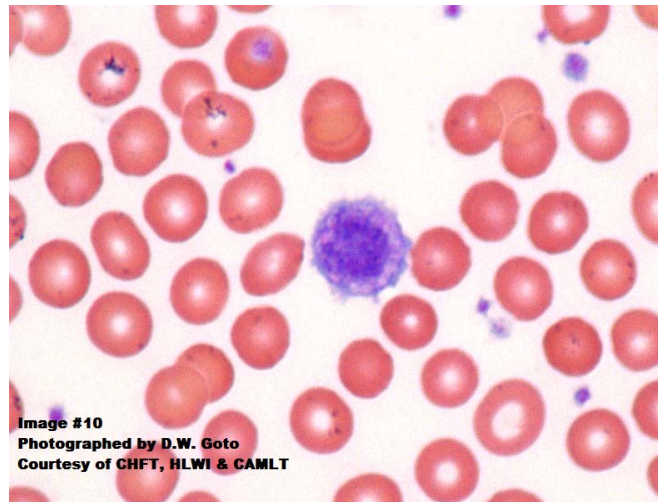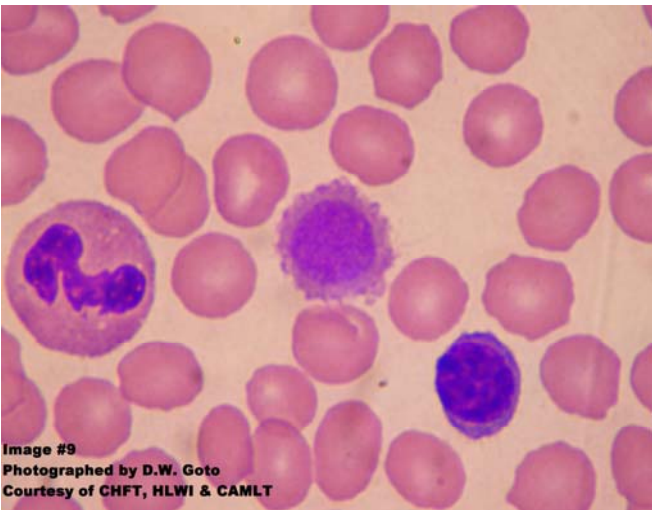Photographed by D.W. Goto
Courtesy of CHFT, HLWI & CAMLT

Cold agglutinins

Spontaneous EDTA-independent agglutination associated with cold antibodies is rare. The condition should be considered when agglutination occurs in citrate and heparin as well as EDTA anticoagulants. This phenomenon is temperature dependent. The specimen should be maintained at 37° C or warmed to 37° C to obtain an accurate platelet count[6].

Giant platelets

   Giant platelets that are 36 fL or larger will be counted as red cells (See Images #9 and #10) in most automated electronic platelet counters, resulting in spuriously low platelet counts. Low platelet counts along with instrument flagging of giant platelets should prompt the operator to confirm the abnormal platelet count by blood smear review/platelet estimate or perform a manual platelet count. The confirmatory method of choice employs a manual platelet count using phase-contrast microscopy. Manual platelet counts include three steps: dilution of the blood with simultaneous lysis of RBCs with ammonium oxalate; sampling the diluted suspension into a measured volume using a hemocytometer; and counting the platelets in that volume[1]. When significant numbers of giant platelets are counted as red cells, spuriously low platelet counts cannot be reported. The platelet estimate or manual platelet count must be reported in the place of automated platelet count.

Image #9
Photographed by D.W. Goto
Courtesy of CHFT, HLWI & CAMLT

Image #10
Photographed by D.W. Goto
Courtesy of CHFT, HLWI & CAMLT

ACKNOWLEDGMENTS

REFERENCES
1. McPherson RA, Rincus, MR. *Henry's clinical diagnosis and management by laboratory methods*. 21st ed. Philadelphia, PA: W.B. Saunders Company, 2006.
2. www.lls.org
3. McKenzie S. *Clinical Laboratory Hematology*. Upper Saddle River, NJ: Pearson Prentice-Hall; 2004:525
4. http://home.columbus.rr.com/allen/thrombocytopenia.htm
5. http://www.pathoindia.com/newspath107.html
6. Schimmer A, Mody M, Sager M, et al. Platelet Cold Agglutinin: a flow cytometric analysis, *Transfusion Science*, Vol 19:3, Sept 1998

Review Questions
Course #DL-985

Chose the **one** best answer.

1. 360 platelets are counted in 10 oil immersion fields on a conventionally made blood smear. The platelet estimate is
   a. 36,000/μL
   b. 54,000/μL
   c. 360,000/μL
   d. 540,000/μL

2. If the number of platelets is above the reportable range on an automated instrument, the first recommended procedure is to
   a. prepare a smear and count the number of platelets/10 OIF
   b. do a manual platelet count
   c. report the number of platelets beyond the reportable range without further analysis
   d. dilute the blood and run the diluted blood through the automated instrument

3. Causes of increased platelet counts include all but which of the following:
   a. splenectomy
   b. platelet satellitism
   c. Chronic granulocytic leukemia
   d. Essential Thrombocythemia

4. The most common symptom of Essential Thrombocythemia is
   a. thrombosis
   b. bleeding
   c. burning of the feet
   d. enlarged spleen

5. Of the following causes of thrombocytopenia which is classified as increased destruction?
   a. chickenpox
   b. disseminated intravascular coagulation
   c. chloramphenicol
   d. May-Hegglin Anomaly

6. EDTA induced platelet aggregation is caused by
   a. fibrin strands in the blood specimen
   b. EDTA bridges between platelets
   c. a cryptantigen-antibody reaction
   d. reaction between platelets and the glass slide

7. A blood specimen is taken in NaCitrate. The platelet count on an automated instrument is 305,000/µL. What is the corrected platelet count?
    a. 33,550/µL
    b. 277,300/µL
    c. 335,500/µL
    d. 305,000/µL

8. A blood smear is made on a patient with a low platelet count. Platelets are seen attached to the periphery of neutrophils. Which of the following applies to this finding?
    a. Neutrophils are attempting to phagocytose platelets.
    b. The patient may exhibit bleeding problems.
    c. It is found in patients who are taking sulfonamides.
    d. It is caused by an EDTA dependent antiplatelet-antineutrophil antibody.

9. The best part of the smear to see agglutinated platelets is
    a. the edge
    b. the central part
    c. the thick part
    d. agglutinated platelets are not seen on smears

10. Cold agglutinin-caused platelet agglutination can be diagnosed by
    a. drawing blood into NaCitrate.
    b. maintaining blood at 37° C.
    c. drawing blood into heparin.
    d. cooling the blood to 15° C.

Course #DL-985 - HEMATOLOGY CASE STUDIES: PLATELETS
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1.	a	b	c	d		6	a	b	c	d
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3.	a	b	c	d		8	a	b	c	d
4	a	b	c	d		9	a	b	c	d
5	a	b	c	d		10	a	b	c	d

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