

**California Association for  
Medical Laboratory Technology  
Distance Learning Program**

**NEUTROPHILIA**

by

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**Course DL-989  
1.0 CE/Contact Hour  
Level: Basic**

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## NEUTROPHILIA

### ABSTRACT:

The production and distribution of normally functioning neutrophils is vital to host defenses. In order to understand the normal condition compared to changes that occur in disease states, the development, structure, function, and kinetics of neutrophils will be discussed.

There are a number of causes for increase in neutrophils. In this course we present two cases of neutrophilia and compare the etiology, laboratory findings, and microscopic morphology for each. Other causes of neutrophilia will be discussed.

### OBJECTIVES:

After completing this course the participant will be able to:

1. Outline the maturation stages of neutrophils.
2. Discuss how neutrophils protect the body against foreign invaders.
3. Explain the difference between shift neutrophilia and absolute neutrophilia.
4. Compare the microscopic morphology of neutrophils in infections to those in chronic myelocytic leukemia.
5. Discuss the other findings and causes of neutrophilia in infections compared to chronic myelocytic leukemia.
6. List other causes of neutrophilia.

**Case #1:** A 59-year-old patient with high fever and chills

WBC	27.2 x 10 <sup>3</sup> /μL	Reference interval 4.1 – 11.0 x 10 <sup>3</sup> /μL	RBC	5.03 x 10 <sup>6</sup> /μL	Reference interval 4.20 – 6.30 x 10 <sup>6</sup> /μL
Lym	9.3 %	13.0 – 48.5 %	HGB	15.2 g/dL	12.0 – 18.0 g/dL
MID	2.7 %	0.1 – 11.0 %	HCT	45.5 %	37.0 – 51.0 %
Gran	88.0 %	46.5 – 82.0 %	MCV	90.5 fL	80.0 – 97 fL
			MCH	30.2 pg	26.0 – 32.0 pg
PLT	222 x 10 <sup>3</sup> /μL	140 – 440 x 10 <sup>3</sup> /μL	MCHC	33.4 g/dL	31.0 – 36.0 g/dL
			RDW	11.6 %	11.5 – 14.5 %

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

Performed on Abbott Cell-Dyn 1800

1. What is abnormal about the CBC?
2. Which parameters can be reported?
3. What procedures can be done regarding abnormal result(s)?

**Case #2:** A 35-year-old male complaining of fatigue

		Reference interval			Reference interval
WBC	>>> 10 <sup>3</sup> /μL	4.1 – 11.0 x 10 <sup>3</sup> /μL	RBC	4.33 x 10 <sup>6</sup> /μL	4.20 – 6.30 x 10 <sup>6</sup> /μL
Lym	5.8 %	13.0 – 48.5 %	HGB	13.5 g/dL	12.0 – 18.0 g/dL
MID	7.1 %	0.1 – 11.0 %	HCT	38.9 %	37.0 – 51.0 %
Gran	87.1 %	46.5 – 82.0 %	MCV	89.9 fL	80.0 – 97 fL
			MCH	31.2 pg	26.0 – 32.0 pg
PLT	189 x 10 <sup>3</sup> /μL	140 – 440 x 10 <sup>3</sup> /μL	MCHC	34.7 g/dL	31.0 – 36.0 g/dL
			RDW	13.6 %	11.5 – 14.5 %

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

Performed on Abbott Cell-Dyn 1700

1. What is abnormal about the CBC?
2. Which parameters can be reported?
3. What procedures can be done regarding abnormal result(s)?

**DISCUSSION:**

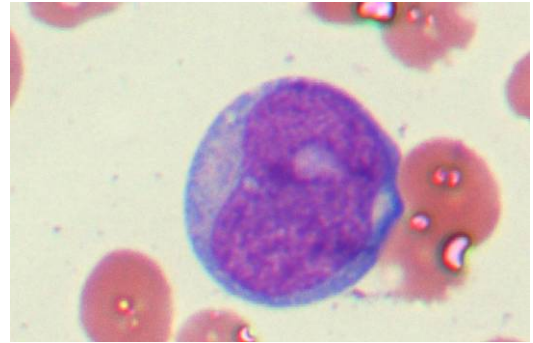
Neutrophils are one of the three granulocyte types (eosinophils, basophils, and neutrophils) found in peripheral blood. Neutrophils are the most numerous leukocyte, (white blood cell [WBC]), normally 55% to 75% of the leukocytes in the adult. The neutrophil is so named because the granules in mature neutrophils stain with the neutral dyes in Wright's stain, appearing faint pink-lavender.

**Development of Neutrophils:**

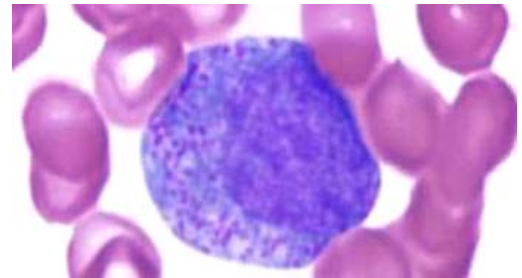
Neutrophils are produced in the bone marrow from pluripotential hematopoietic stem cells. These hematopoietic stem cells can differentiate into all the blood cell types. One line of differentiation begins with a stem cell (CFU-GEMM) capable of developing into granulocytes, erythrocytes, monocytes, and megakaryocytes. The next differentiation is the CFU-GM (colony forming unit granulocyte, monocyte). This in turn differentiates into CFU-G, the neutrophil cell line. This neutrophil stem cell is no longer capable of producing other blood cell types. Myeloblasts are the first morphologically recognizable cells in the neutrophil cell line. Neutrophils go through six morphologic stages before release into the peripheral blood: myeloblast, promyelocyte, myelocyte, metamyelocyte, band, and polymorphonuclear (PMN) neutrophil. The first three of these stages are capable of replication as well as differentiation (maturation). The last three stages are only able to mature. The maturation stages and morphology of immature neutrophils are shown in the following sequence:

## Maturation and Morphology of Immature Granulocytes

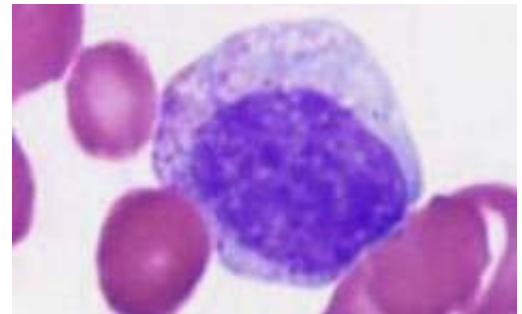
- **Myeloblast:** the first and earliest granulocyte
  - Is a large cell (15  $\mu\text{m}$ )
  - High nucleus to cytoplasm (N:C) ratio (5:1)
  - Round or oval nucleus with loose light staining euchromatin
  - 1-2 nucleoli
  - Has minimal light blue cytoplasm
  - Contains **no cytoplasmic granules**
  - Begins to produce myeloperoxidase granules (MPO)
  - Comprises 1% of the nucleated cells in the bone marrow
  - Takes 18 hours to mature



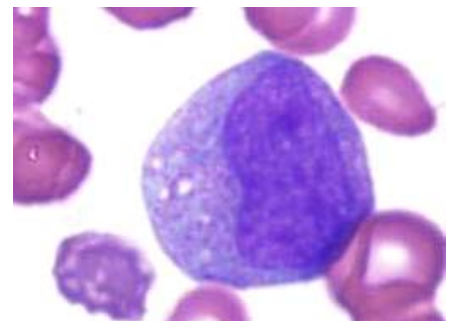
- **Promyelocyte:** larger than a myeloblast (20  $\mu\text{m}$ )
  - High N:C ratio (3:1)
  - Loose chromatin with nucleoli
  - Dark blue cytoplasm
  - Contains **large nonspecific cytoplasmic granules** containing myeloperoxidase (MPO)
  - Comprises 3-4% of nucleated bone marrow cells
  - Takes 24 hours to mature



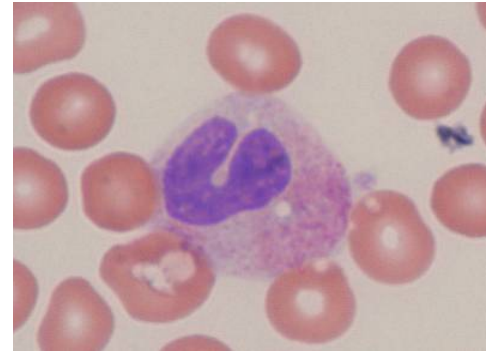
- **Neutrophilic Myelocyte:** medium cell size (12  $\mu\text{m}$ )
  - High N:C ratio (3:1)
  - Round, oval, or slightly indented nucleus with darker blue heterochromatin
  - Last stage of cell division
  - Has active RNA, therefore, the cytoplasm is blue
  - Contains MPO and secondary granules containing leukocyte alkaline phosphatase
  - Comprises 12% of bone marrow nucleated cells
  - Takes 100 hours to mature



- **Neutrophilic Metamyelocyte:** size (11  $\mu\text{m}$ )
  - N:C ratio (2:1)
  - last mononuclear stage, no mitosis
  - Nucleus is kidney or horseshoe shaped, and has condensed heterochromatin
  - Has a prominent Golgi apparatus – clear area located at the indentation site of the nucleus
  - Cytoplasm is similar to the mature cell
  - Comprises 18% of bone marrow cells
  - Takes 72 hours to mature

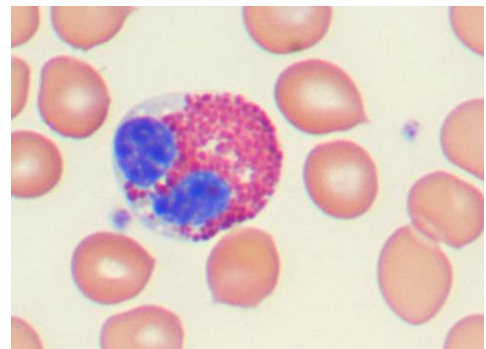


- **Band**
  - Same size as a mature neutrophil (10-12  $\mu\text{m}$ )
  - N:C ratio has reversed (1:2)
  - Nucleus is **band-** or **sausage-shaped** without segmentation
  - Cytoplasm is filled with small neutrophilic granules
  - Last immature stage
  - Comprises 11% of bone marrow cells and 0-3% of peripheral WBCs
  - Stored in the bone marrow and released when there is an increased demand for neutrophils
  - **Shift to the left** is an increase in immature cells indicating increased demand for WBCs in peripheral blood
  - Takes 48 hours to mature



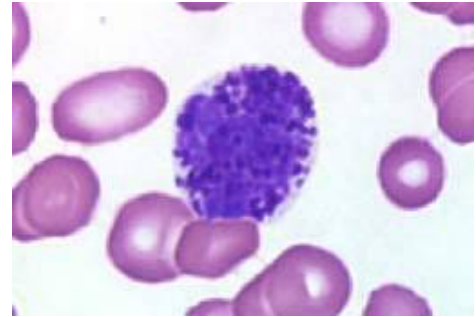
### Morphology of Mature Granulocytes

- **Neutrophils**
  - Also known as segmented neutrophils, segs, polymorphonuclear cells, polys, and PMNs
  - N:C ratio is 1:3, and the size is 10-12  $\mu\text{m}$
  - Average nucleus contains 3-5 segments connected by narrow filaments
  - **Hyposegmented** is less than 3 segments, and may indicate a shift to the left or an anomaly
  - **Hypersegmented** is more than 5 segments and may indicate infection or megaloblastic anemia
  - Cytoplasm contains very small neutral granules
  - Granules can become larger upon bacterial infection producing **toxic granulation**, which are numerous, large, basophilic granules
  - Makes up 55-75% of all peripheral WBCs
  - Average time spent in the blood is 10 hours
- **Eosinophils**
  - Average size is 13  $\mu\text{m}$
  - Nucleus is generally bilobed
  - Cytoplasm is **bright red** or **orange** which is due to large specific, secretory granules containing peroxidase, acid phosphatase, aryl sulfatase, beta-glucuronidase, etc. that stain red with the eosin component of Wright's stain
  - Makes up 3% of WBCs in the peripheral blood



- **Basophils**

- Is the smallest granulocyte at 10  $\mu\text{m}$
- The nucleus is difficult to see due to heavy granulation
- Cytoplasm contains large specific, secondary granules that contain **heparin** and **histamines**, which stain purple with Wright's stain. These granules are water-soluble and sometimes appear as holes in the cell if the cells are not fixed well during staining.
- Makes up to 0.5% of peripheral WBCs
- *Note:* **Tissue mast cells** are similar to basophils but are larger and have no developmental relationship with basophils. Mast cells have a **mesenchymal** (connective tissue) origin and have granules containing **serotonin** (basophilic granules contain no serotonin).



### **Function of Neutrophils:**

The major role of neutrophils is to protect the body against infectious agents. Their granules contain substances that are bactericidal, hydrolytic, and activate the complement cascade. Neutrophils have three granule types. The azurophilic or primary granules contain myeloperoxidase, defensins, cathepsin, lysozyme, and several other proteins and enzymes. The specific or secondary granules contain lactoferrin, collagenase, lysozyme, and other factors. The gelatinase or tertiary granules contain gelatinase, acetyltransferase, and lysozyme. In addition, secretory vesicles are present. They contain stores of surface membrane bound receptors. When the neutrophil is activated, the secretory vesicles fuse with the plasma membrane, releasing receptors help prime the neutrophil for antimicrobial action.

Neutrophils protect by migrating to the source of infection or irritation and destroying the foreign substance. They are attracted by chemo-attractants released at the site of infection. The neutrophils follow the concentration gradient of the chemotactic agents, which also prime the neutrophil for subsequent activation. After arriving at the area of infection, the neutrophils adhere to the blood vessel wall and migrate through the wall to the site of infection. The chemo-attractant agents bind to receptors on the neutrophils, setting in motion morphologic changes and metabolic activation.

At the site the neutrophils project pseudopodia that surround the foreign particles, initiating phagocytosis. This process is enhanced if the foreign organism has antibodies or complement factors attached. The organism is engulfed into a phagosome formed by invagination of the neutrophil's cell membrane.

The contents of the neutrophil's azurophilic (primary) granules are discharged into the phagosome. The myeloperoxidase catalyzes the production of hypochlorite, a potent anti-microbial. Other oxidative killing mechanisms involve the production of hydrogen peroxide, superoxide anion and singlet oxygen. These substances kill the phagocytized organism. The dead and dying neutrophils, with their contents, form the exudate (pus) seen at the site of the infection.

Specific (secondary) granules are released into the extracellular space. Some of their products activate the complement cascade. Collagenase helps hydrolyze the extracellular matrix, aiding locomotion of the neutrophil through the tissues. Tertiary granules contain gelatinase that plays a similar role in locomotion.

**Neutrophil kinetics:**

Neutrophils take seven to eleven days to go through the maturation stages in the bone marrow. The last three maturation stages form a resting pool of PMN neutrophils, bands, and metamyelocytes in the bone marrow. The PMNs are released into the peripheral blood to replace those that go into the tissues. When there is increased demand the bone marrow is stimulated to release cells from the neutrophil storage pool: the PMNs, bands and then the metamyelocytes enter the peripheral blood. If the demand is great enough there is stimulus for increased production of neutrophils.

PMNs normally spend ten hours in peripheral blood before migrating into the tissues. Once in the peripheral blood the neutrophils enter the circulating granulocytic pool (CGP) or the marginal granulocytic pool (MGP). The MGP is still in the vascular space but these cells are adherent to the blood vessel walls, especially in small vessels of post-capillary venules. These pools are about equal in size. The cells in these pools can go from one pool to another. For instance, exercise or epinephrine injection can cause demargination and a transient (about 30 min.) increase in the CGP. However the total blood granulocytic pool (TBGP) remains unchanged. The temporary increase in the WBC count at this time should not be confused with an absolute increase in the TBGP. In these demargination cases there is no increase in immature forms in peripheral blood.

**NEUTROPHILIA:**

Neutrophilia refers to higher than normal numbers of neutrophils (over 11,000/ $\mu$ L) in peripheral blood. This can be a temporary shift of marginal to circulating neutrophils without an increase in TBGP or an absolute increase in the size of the TBGP. Absolute neutrophilia involves an increase in cells from the bone marrow.

Most cases of absolute neutrophilia are associated with infections caused by cocci (e.g. staphylococci, pneumococci, streptococci, meningococci, gonococci), bacilli (e.g. *E. coli*, *Pseudomonas aeruginosa*, *Actinomyces* species), certain fungi, spirochetes, rickettsia, and parasites. Viruses are less likely to cause significant neutrophilia, but rather an increase in lymphocytes. During early infection the neutrophil count can decrease due to increased margination of cells near the site of infection. This is followed by neutrophils entering the circulation from the bone marrow and an increase in the TBGP.

The total leukocyte count in infections is usually below 50,000/ $\mu$ L. A shift to the left (release of younger neutrophils) is due to increased demand and release of band cells and metamyelocytes. If the infection continues there is increased rate of production of neutrophils. As the infection subsides the inflow from the bone marrow decreases and the WBC count falls.

In severe infections there may be such an increase in the leukocyte count, over 30,000 to 50,000/ $\mu$ L, that the blood picture may resemble leukemia. This is called a leukemoid reaction. The differences between a leukemoid reaction and leukemia are:

<b>Finding</b>	<b>Leukemoid reaction</b>	<b>Chronic granulocytic leukemia</b>
Total leukocyte count	30,000 to >50,000/ $\mu$ L	May be well over 100,000/ $\mu$ L
Immaturity of neutrophils	No blasts or promyelocytes	Blasts and promyelocytes may occur
Basophils	Normal %	May be increased
Toxic granulation	Frequently present	Not usually present
Döhle bodies	Frequently present	Not usually present
RBC morphology	Usually normal	May be abnormal
Leukocyte alkaline phosphatase	Increased	Decreased
Philadelphia chromosome	Absent	Usually present

Other causes of neutrophilia are<sup>2</sup>:

Hematologic disorders: chronic myelocytic leukemia, polycythemia vera, myelofibrosis, myeloid metaplasia.

In these disorders there are neoplastic transformations in clonal hematopoietic stem cells resulting in excessive production of granulocytes and their precursors. The granulocyte production is not under physiologic control of increased demand but is uncontrolled. In some of these disorders other hematologic cell lines may also be involved.

Non-infectious inflammation: burns, postoperative state, acute myocardial infarction, gout, acute glomerulonephritis, rheumatic fever, hypersensitivity reactions.

Neutrophilia in severe burns shows a shift to the left and the presence of degenerative forms, including toxic granulation and Döhle bodies.

Neutrophilia post-operatively is caused by release of adrenocortical hormones as a result of tissue injury.

Metabolic: diabetic ketoacidosis, preeclampsia, uremia.

Poisoning: lead, mercury, digitalis, camphor, antipyrine, phenacetin, quinidine, pyrogallol, turpentine, arsphenamine, insect venoms.

Acute hemorrhage, especially into peritoneal, pleural, joint or intracranial cavities.

Neutrophilia is probably due to pain and release of epinephrine and corticosteroids. During the first one to three hours after an acute hemorrhage, neutrophilia occurs due to a shift from the marginal pool to the circulating pool.

This is followed by release of neutrophils from the marrow.

Malignant neoplasms: probably due to tumor necrosis factor or production of neutrophilic growth factors in rapidly growing neoplasms.

Acute or long-term administration of corticosteroids.

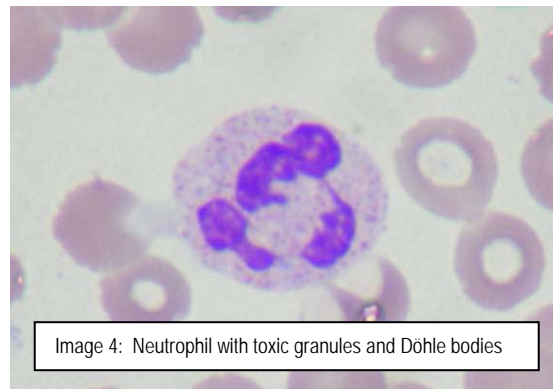
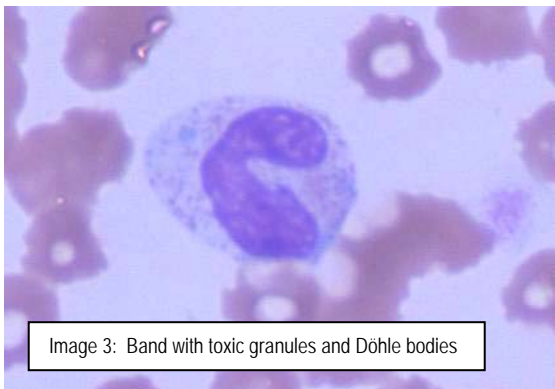
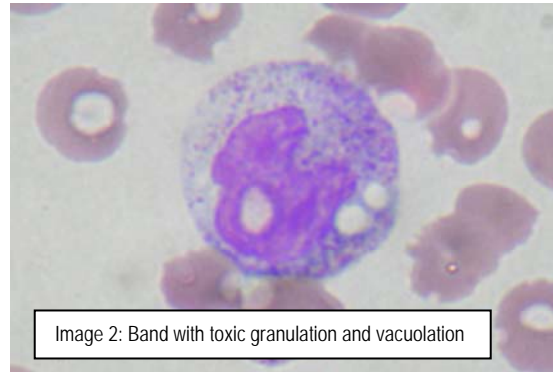
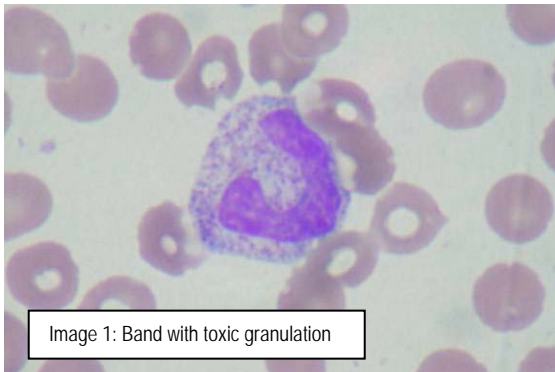
Physiologic: strenuous exercise and epinephrine cause transient neutrophilia. Can also be seen in pregnancy, labor, and in newborns.

### Case #1: 59-year-old patient with high fever and chills

Answers:

1. The WBC count is above normal and the differential is abnormal.
2. The RBC parameters and platelet numbers are normal and can be reported.
3. Perform a manual WBC differential.

Manual differential:		Reference interval
PMN Neutrophils	26 %	40 – 75 %
Bands	60 %	0 – 9 %
Lymphocytes	12 %	16 – 46 %
Monocytes	2 %	0 – 12 %
PMN toxic granulation	2+	none
PMN vacuolation	1+	none
PMN Döhle bodies	1+	none



The blood picture shows increased neutrophils, 86% total, with a shift to the left but no immaturity beyond bands.

The neutrophils also show changes that are characteristic of infection: toxic granulation, vacuolation, and Döhle bodies.

Toxic granulation: accumulation of dense azurophilic, peroxide positive granules occurring with rapid production of neutrophils; associated with infection. They may also occur in toxemia of pregnancy, vasculitis, or in patients receiving chemotherapy. They may also be an artifact of staining if the stain is too basic.

**Döhle bodies:** seen as oval, single, or multiple cytoplasmic inclusions composed of aggregated strands of rough endoplasmic reticulum (RNA). They are associated with rapid production of neutrophils; found in patients with severe infection, burns, aplastic anemia and in patients receiving chemotherapy. In these conditions Döhle bodies represent toxic changes. They are also observed in hereditary disorders: May-Hegglin anomaly and in Chédiak-Higashi syndrome.

**Vacuolization:** round clear unstained areas randomly dispersed in the cytoplasm. When seen in neutrophils, their presence suggests very severe infection.

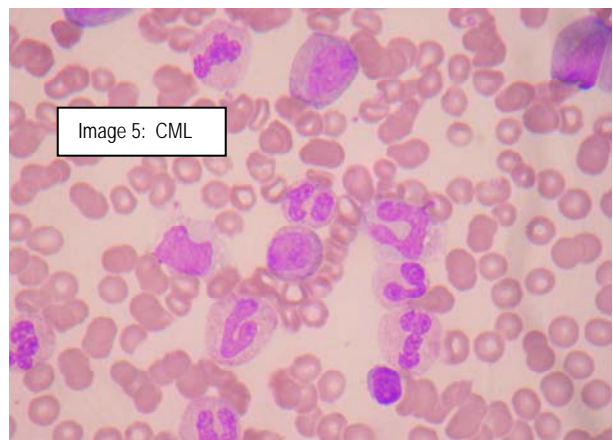
**Diagnosis:** Neutrophilia due to bacterial infection. Neutrophils show a ‘shift to the left’— increased bands, toxic granulation, vacuolation, and Döhle bodies, all associated with increased demand due to the infection.

**Case #2: 35-year-old male complaining of fatigue**

Answers:

1. The WBC count is above normal and the differential is abnormal.
2. Although the Hgb and Hct are slightly low for a male, the RBC parameters and platelet numbers are normal and can be reported.
3. a. The WBC count is too high to be reported by the automated hematology instrument: do a 1:1 dilution of whole blood and re-run WBC count.  
The WBC count on the diluted blood was  $68.9 \times 10^3/\mu\text{L}$ .  
Correct for the dilution:  $68.9 \times 2 = 137.8 \times 10^3/\mu\text{L}$ .
- b. Perform a manual WBC differential because of the abnormal automated differential and to corroborate the high WBC count.

Manual differential:		Reference interval
Neutrophils	15 %	40 – 75 %
Bands	36 %	0 – 9 %
Metamyelocytes	17 %	0 – 1 %
Myelocytes	3 %	0 – 1 %
Promyelocytes	2 %	0 %
Blasts	8 %	0 %
Lymphocytes	10 %	16 - 46 %
Monocytes	8 %	0 – 12 %
Basophils	1 %	0 – 2%



**Diagnosis:** The very high white count and the immaturity of the neutrophils that goes back to blast cells, but with predominance of more mature forms, are characteristic of chronic myelocytic leukemia (CML). The near normal RBC numbers and normal morphology as well as normal platelet numbers also are indicative of CML rather than acute myelocytic leukemia (AML), which has anemia, sometimes severe, and decreased platelets. CML may have anemia but it is mild. Platelet numbers in CML are normal to increased.

In chronic myelocytic leukemia there are other characteristics that can sometimes be observed on the blood smear: increased numbers of basophils, eosinophils and/or platelets,

including giant platelets or megakaryocytic fragments. Occasionally nucleated RBC may be found along with RBC abnormalities—aniso-poikilocytosis, basophilic stippling, and polychromasia.

Other laboratory findings are

1. The presence of the Philadelphia (Ph) chromosome. This is a cytogenetic abnormality involving a translocation between the long arms of chromosomes 22 and 9. The translocation results in a shortened chromosome 22, called the Philadelphia chromosome, named after the city in which the discovery was made. The Ph chromosome is found in 90 to 95% of the cases of typical CML. Its presence is diagnostic of the disease.
2. Low levels of leukocyte alkaline phosphatase. This is in contrast to neutrophilia caused by infection in which there are high levels of leukocyte alkaline phosphatase.

### **CONCLUSION:**

Neutrophil function, development, and kinetics were discussed as a background for understanding neutrophilia. Photomicrographs illustrated the maturation morphology in the neutrophil line. The two cases illustrate the differences associated with neutrophilia due to infection compared to neutrophilia due to chronic myelocytic leukemia. Other causes of neutrophilia were also discussed.

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- California Association for Medical Laboratory Technology (CAMLT)

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## Review Questions

Course #DL-989

Choose the **one** best answer.

1. The primary function of neutrophils is to:
  - a. produce antibodies against foreign substances
  - b. protect against parasitic infections
  - c. protect against bacterial infections
  - d. produce chemo-attractants
  
2. In the maturation stages of neutrophils, which stage first contains granules?
  - a. promyelocyte
  - b. metamyelocyte
  - c. myeloblast
  - d. myelocyte
  
3. The neutrophil is named for
  - a. the neutralizing substances it produces
  - b. the reaction of its granules to components of Wright's stain
  - c. not taking sides in the body's war against infection
  - d. the presence of neutros in the cytoplasm
  
4. A patient has a 25,000/ $\mu$ L WBC count. The differential shows 18 % band cells and the presence of toxic granules in some of the neutrophils. The most likely cause of the neutrophilia is
  - a. a bacterial infection
  - b. chronic myelocytic leukemia
  - c. metabolic ketoacidosis
  - d. acute hemorrhage
  
5. Which of the following is not true of a leukemoid reaction?
  - a. neutrophil granules may stain darker than normal
  - b. neutrophils show immaturity
  - c. decreased reaction to leukocyte alkaline phosphatase
  - d. increase in the WBC count above normal
  
6. Döhle bodies are composed of
  - a. rough endoplasmic reticulum
  - b. DNA
  - c. tertiary granules
  - d. coalesced secondary granules

7. Myelocytes are capable of
  - a. replication but not differentiation
  - b. replication and maturation
  - c. differentiation but not replication
  - d. maturation but not replication
  
8. Which of the following substances is found in primary granules of neutrophils?
  - a. gelatinase
  - b. plasminogen activator
  - c. collagenase
  - d. myeloperoxidase
  
9. The circulating granulocytic pool is
  - a. increased by cortisone injection
  - b. decreased by epinephrine injection
  - c. increased with exercise
  - d. half the number of cells in the marginal granulocytic pool
  
10. Causes of neutrophilia include all but which of the following?
  - a. hemorrhage
  - b. severe burns
  - c. viral infection
  - d. polycythemia vera

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| 1. | a | b | c | d | 6  | a | b | c | d |
| 2. | a | b | c | d | 7  | a | b | c | d |
| 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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(strongly agree) 5 4 3 2 1 (strongly disagree)
- I will use what I learned from this Distance Learning course.  
(strongly agree) 5 4 3 2 1 (strongly disagree)
- The time to complete this Distance Learning course was: \_\_\_\_\_ hours
- What did you like or dislike about this Distance Learning course ?