CM-B 2018: Neutrophilia

INTRODUCTION

A complete blood count (**CBC**) is a blood test used to broadly evaluate the health status and can detect a wide range of disorders, including anemia, infection, and leukemia. A complete blood count measures red blood cells, which carry oxygen; white blood cells (WBCs)/leukocytes, which fight infection; and platelets, which help with blood clotting. Additional measures include hemoglobin, the oxygen-carrying protein in red blood cells and hematocrit, the proportion of red blood cells to the fluid component/plasma in blood.

Leukocytosis refers to an increase in the total number of WBCs due to any cause. It can be classified according to the component of white cells that contribute to an increase in the total number of WBCs. Therefore, leukocytosis may be caused by an increase in the granulocytes (neutrophils, monocytes, eosinophils, basophils), or lymphocytes or immature cells (eg, blasts). A combination of any of the above may be involved. Leukocytosis is a reflection of an underlying condition rather than a disease itself and is a nonspecific finding that can result from a variety of reactive and neoplastic causes. It may be present as a result of infection, such as bacterial, viral, or parasitic infections; inflammatory conditions such as chronic inflammation or autoimmune disorders; or medication (eg, steroids). It may also be seen with bone marrow involvement by a metastatic malignancy as well as primary marrow neoplasms such as leukemia. This continuing education activity will focus on neutrophilia, a specific type of leukocytosis, with the following objectives.

Case History:

A 40-year-old female presents with fever and cough. No hepatosplenomegaly is identified on physical exam. A CBC is performed and shows WBC = 24.06 x10E9/L, Hg = 6.5 g/dL, Platelets = 407x10E9/L. The instrument flags the case for manual review due to leukocytosis and immature granulocytes (IGs). A peripheral smear is made. The red blood cells show slight anisopoikilocytosis, echinocytes, and basophilic stippling. The platelets show normal morphology. No blood parasites are seen. The granulocytes show toxic granulation with Döhle bodies. A 200 WBC manual differential count is performed due to the increased WBC. Neutrophils and bands = 72%; metamyelocytes = 6%; myelocytes = 14%; promyelocytes = 2%; blasts = 1%; lymphocytes = 2%; monocytes = 1%; eosinophils = 2%; and basophils = 0%.

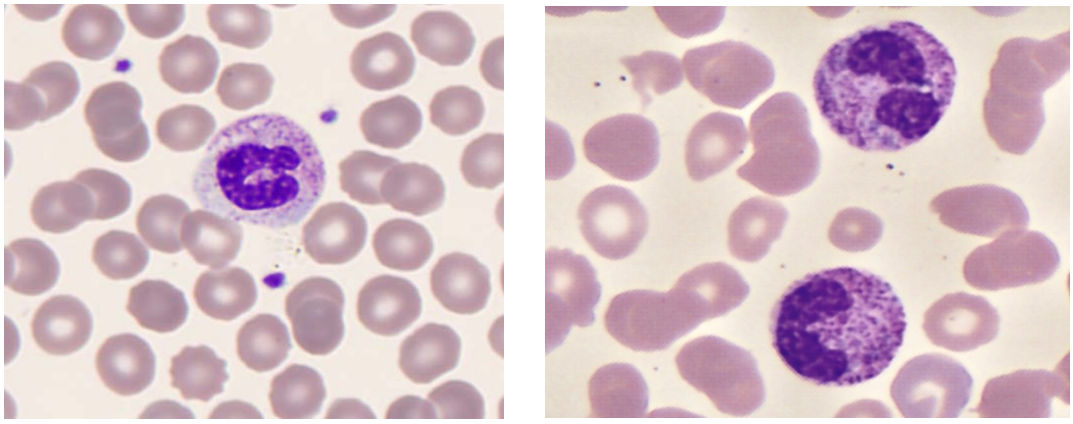


Figure 1: Peripheral smear showing neutrophils with toxic granulation (oil 1000x)

The differential diagnosis for this presentation includes a leukemoid reaction and also a myeloproliferative neoplasm such as chronic myeloid leukemia (CML). The clinical presentation and morphology of the granulocytes are compatible with a leukemoid reaction. Note that the manual differential performed included both bands and neutrophils under the same denomination. Should they have been counted separately? The automated instrument flagged “Immature granulocytes (IGs)”. What are IGs?

DISCUSSION

Neutrophils

Neutrophils are the most abundant human immune cells. They are rapidly recruited to sites of infection, where they perform lifesaving antimicrobial functions. Although reference ranges differ between laboratories, an absolute neutrophil count reference range somewhere near 2.0–7.7×10E9/L (40% – 80% of WBC)[1, 30]. People of African and Middle Eastern descent may have lower counts, which are still normal. The normal absolute neutrophil count is highest at birth (5.7-20.7x10E9/L at 0-3 days) and slowly declines; infants and children (<5 years) have slightly higher normal levels (1.5-8.5x10E9/L) than adults ranges.[31] Reference ranges are even more difficult to establish for preterm babies, term babies, or the ranges at the first 48 -72 hours. Particularly given these normal variations, age specific reference ranges must be established/verified and appropriate for the population served by the testing laboratory.

Neutrophils develop in the bone marrow from hematopoietic stem cells in a process called “granulopoiesis,” which normally takes 10-14 days. Bone marrow neutrophil also considered the “storage pool” can be divided into 3 compartments: (i) the stem cell compartment composed of hematopoietic stem cells and pluripotent progenitors; (ii) the mitotic compartment composed of proliferating, lineage-committed myeloblasts, promyelocytes, and myelocytes; and (iii) the post-mitotic compartment composed of metamyelocytes, band cells, and mature neutrophils. The post-mitotic cells (metamyelocytes, band cells, and neutrophils) constitute 95% of the circulating neutrophils in the body. Mature neutrophils are short-lived (with a life span up to 5 days) and highly motile, as they can enter parts of tissue where other cells/molecules cannot.[1,2] A neutrophil is usually 10-15 µm in diameter with N:C ratio of 1:3. Normally, neutrophils contain a nucleus divided into 2–5 segments connected by a filament. The neutrophil’s nuclear chromatin is clumped with no discernible nucleoli. The cytoplasm contains at least four different types of granules:

(1) primary granules, also known as azurophilic granules; (2) secondary granules, also known as specific granules; (3) tertiary granules; and (4) secretory vesicles. The granules are formed through a continuous process of vesicles budding from the Golgi apparatus. The content of these structures change during the development of the granulocyte resulting in a continuum of granule development with overlapping contents.[3,26] The Wright-Giemsa and other modifications of the Romanowsky cytochemical stains used in the laboratory are designed to stain the primary and secondary granules. The primary granules are the main storage site of the most toxic mediators, including elastase, myeloperoxidase, cathepsins, and defensins. The secondary and tertiary granules contain lactoferrin and lysozyme among other substances. The secretory vesicles in human neutrophils contain human serum albumin, suggesting that they contain extracellular fluid that was derived from endocytosis of the plasma membrane. Granules are released only when receptors in the plasma membrane or phagosomal membrane are activated, triggering movement of the granules to the cell membrane for secretion of their contents by degranulation. This control mechanism is important to minimize tissue damage, as the neutrophil is highly enriched in tissue-destructive proteases. Upon stimulation, neutrophils quickly migrate out of circulation into targeted tissue and congregate at a focus of infection. This extravasation and tissue localization is mediated by cytokines expressed by activated endothelium, mast cells, and macrophages including tumor necrosis factor (TNF) alpha, TNF beta, G-CSF, GM-CSF, IL-8, and C5a. Neutrophils express and release cytokines, which in turn amplify inflammatory reactions by recruiting several other cell types.

In addition to activating other cells of the immune system, neutrophils play a key role in the front-line defense against invading pathogens. Neutrophils have 3 methods for directly attacking micro-organisms: phagocytosis (ingestion), degranulation (release of soluble anti-microbials), and generation of neutrophil extracellular traps (NETs). In degranulation, the neutrophil release a diverse array of antimicrobial proteins and enzymes intracellularly into membrane-bound organelles, called phagosomes, which contain engulfed small microorganisms. At the same time, neutrophils release reactive oxygen species and cytokines outside the cells to kill extracellular bacteria and recruit additional leukocytes to the region of infection or inflammation. A study by Brinkmann[4] and colleagues described a novel mechanism by which neutrophils eliminate bacteria. Neutrophils, on activation by a range of mediators such as interleukin-8 (IL-8), lipopolysaccharide, and interferon-α generate a web of extracellular fibers known as NETs, composed of deoxyribonucleic acid (DNA), histones, and antimicrobial granule proteins. These are highly effective at trapping and killing invasive bacteria. Neutrophils from this “storage pool” in the bone marrow are released into the circulation—“circulating pool” by the actions of chemokine receptor signaling involving CXCR4 and CXCR2. In the periphery neutrophils are stored in the “marginated pools,” or vascular pools located in the lungs, spleen, and liver.[3-5] Demargination or mobilization of neutrophils into the circulation or tissues from the marginated pools is promoted by inflammatory reactions by cytokines and other cell signaling proteins plus environmental factors like hypoxia. During infections, the mitotic/proliferative compartment increases the cell divisions that can double the number of mature neutrophils produced. A small fraction of these neutrophil precursors escape the marrow and are found in the blood, and can be measured by the IG%. Another mechanism involves the release of postmitotic cells (neutrophils, bands, metamyelocytes) from the marrow into the circulation. This leads to an increase in the I/T ratio.[11]

Bands

The morphologic definition of band is a granulocyte with condensed chromatin and a nucleus that is indented to more than half of the distance to the farthest nuclear margin, but in no area is the chromatin condensed to a single filament. Bands are round to oval and 10-18 µm in diameter, with a nuclear to cytoplasmic ratio of 1:1.5 to 1: 2. The shape of the nucleus can be a curved or coiled band with no limits on the level of indentation as long as it does not completely segment the nucleus into lobes connected by a filament. Bands normally constitute 5%-10% of the nucleated cells in the blood. Other names for bands include schilling, band cell, stab cell, stab neutrophil, and staff cell. Experimental studies show that the some of the granulocytic lineage functionalities such as the chemotaxis, and oxygen-dependent bacterial killing develop in the later stage of granulocyte differentiation and are seen at both the band and neutrophil stage, suggesting similar functional capabilities for these 2 cell types. In current literature, band and segmented neutrophil are both considered within the category of "neutrophil."[1,23]

Flagging

Flagging is an indication from the instrument that a reported result is abnormal and the cells in question are quantitatively or qualitatively outside the established normal ranges programmed into the instrument or the instrument detects a technical problem with the sample. Therefore, all immature cells are flagged as they are not an expected finding in the peripheral blood. In the current context, immature cells were perhaps previously classified under "left shift" or monocytes. A manual review is required for further delineation. The criteria for manual review can be chosen judiciouly based on scientific literature, striking the right balance for manual smear reviews, that can optimize use of resources—the most scarce being medical technologists. The consensus guidelines from *International Society for Laboratory Hematology* can be used to generate criteria for manual review.[28]

Performing differentials: Band vs. Segmented Neutrophils

The impetus for delineating bands came from its utility in diagnosis of sepsis, for which band count was considered an important parameter especially in the neonate population.[9] The band count is used widely in various established diagnostic algorithms to diagnose bacterial infection in newborns and infants less than 3 months of age. The neonatologists use the I/T Neutrophil Ratio, calculated as the total number of immature cells (promyelocytes + myelocytes + metamyelocytes + bands) divided by the total number of cells in the neutrophils cell line (immature + segmented neutrophils).[10] One can see that to determine the I/T ratio a manual differential must be performed. There are several major technical limitations of the manual band count. Lack of consistent interpretation of the band cell identification criteria leads to considerable variation in reference ranges.[6,7,8,21] Distinction between these cell types can be particularly difficult when the nucleus is twisted or folded. Precision of the band count is poor due to both limited counting sample (100-200 cells) and individual technologist variation. The 95% confidence limits for a standard 100-cell differential count are unacceptably wide—for 5% bands the 95% confidence limits are 1%-12%, for 10% bands the limits are 4%-18%, and for 15% bands the limits are 8%-24%.[22] In the study by Manroe et al[9] which established the reference ranges in infants and is considered a classic reference in the field, the investigators relied on a single experienced technologist to perform more than 90% of the manual differential counts. This practice is not practical in a laboratory that depends on several medical technologists to perform differentials. Although a 500 cell differential can decrease this variability, routine counting of 500 cells is also not practical in a busy hematology laboratory. Further, we now know that elevation of the band count is not specific for infection, and may be secondary to inflammatory processes, tissue damage or necrosis,

neoplasia, intoxication, metabolic abnormalities, hemorrhage, hemolysis, or drugs. Conditions in infants that can result in increased bands without infection include meconium aspiration, hypoglycemia, prolonged labor, oxytocin induction, pneumothorax, and/or asphyxia. The College of American Pathologists Hematology & Clinical Microscopy Committee and the Clinical and Laboratory Standards Institute recommend reporting bands with segmented neutrophils to represent the total absolute neutrophil count (ANC).[19] In the case study presented above, while performing a differential the bands and neutrophils were counted together.

The total WBC and ANC have repeatedly shown to be much better predictors of bacterial infection, to which the band count adds no additional useful information. The current recommendations include the use of WBC, total ANC, and IGs (described in the next paragraph). Critical values for leukocytes are generally determined from the total WBC with both a low and high value along with the ANC.

(Neutrophil% +Band%) x WBC = ANC

The ANC Critical low value, when a patient cannot adequately fight infection, for most laboratories is less than 0.500 x 10E9/L. Current guidelines for the management of severe sepsis and septic shock[27] include the following inflammatory variables for diagnosis of sepsis:

1. WBC count > 12,000/ µL (12 x 10E9/L)
2. Leukopenia < 4,000/ µL (4 x 10E9/L)
3. Normal WBC count with >10% immature forms

Severe neutropenia can be seen in overwhelming sepsis due to absolute depletion of cells in the bone marrow storage pool in association with increased loss from blood into tissues. It can also be seen in significant viral infections as well as post chemotherapy treatment.

IGs

IGs are generally used to denote cells more immature than a band neutrophil and include metamyelocytes, myelocytes, and promyelocytes. Although myeloblasts are immature granulocytes, they are not included in this definition. IG counts can be performed both manually and by automated instruments. Modern automated hematology analyzers are capable of performing CBCs and differential leukocyte counts (DIFFs) where large numbers of leukocytes are categorized by flow cytometric techniques according to their size and cytoplasmic and nuclear characteristics. DIFF results generated by these analyzers from evaluation of at least 10,000 leukocytes include both the percentages and absolute numbers of neutrophils, lymphocytes, monocytes, eosinophils, and basophils, and flags for the potential presence of immature/abnormal/atypical white cells. Some automated instruments like the Sysmex hematology analyzer (Sysmex Corporation, Mundelein, IL) additionally measures IGs in percent and absolute number.[12,25] The IG count is performed in differential channels of the analyzer. A specific surfactant induces hemolysis of erythrocytes and the formation of ultramicroscopic pores in the leukocyte cell membrane. The increased permeability of leukocytes allows a polymethine dye with high affinity for nucleic acid to enter the cells. Subsequently, the cells are analyzed by nucleic acid fluorescence and side scatter. When excited by a 633-nm laser beam, the stained cells emit fluorescence proportional to their content of nucleic acid (ribonucleic acid [RNA] or DNA). IGs with an increased DNA content show an intense fluorescence that permits their separation from mature neutrophils. Of note, Sysmex is the only instrument, in our knowledge

that provides the IG count.

Quantitation of Immature Granulocytes: Comparison of automated counts with manual differentials

A manual differential count, once seen as the best method to review a patient's peripheral blood cells and provide the necessary numbers, is now challenged by the accuracy, precision, and turnaround time of automated differentials. Flow cytometry based methods are considered most sensitive and a gold standard for enumerating IGs.[20] Normal ranges for automated immature granulocyte counts (IG%) are described in adults and children as <1%, maximal IG concentrations of 0.5% or 0.03 × 10E9/L.[15,29] Studies comparing automated IG counts to manual 400 cell counts have found good correlation. Studies performed to see the degree of correlation between the automated and manual IG percentages when the latter is obtained from the conventional 100-cell manual differential[24] show that automated IG% and corresponding manual IG% group means are similar when the IG% are below 10. However, the difference between the automated and manual IG% grows larger when the manual IG% exceeds 10. CBC specimens that have a large number of hyper segmented neutrophils or cells with toxic granulation may give falsely increased IG values. Given these limitations and since increased IG% can raise suspicion for infection, one should consider scanning a smear to manually verify the count prior to reporting values when IG% exceeds a certain level. Automated IG counts at low levels (<5% in our experience) are reproducible, and have the potential to decrease the turnaround time of reporting CBCs. There is no consensus on the number at which a smear review is generated, therefore it is recommended that laboratories perform their own validation studies to compare automated vs manual counts for IGs. When combined with validated/verified auto verification protocols, automated differentials have the advantage that a blood smear is not needed, and technologist time is not required to perform a microscopic analysis, thereby conserving materials and reducing technologist time.

IG use in sepsis

Automated IG counts are useful in detecting infection and sepsis.[14,16,17] Ansari-Lari et al showed that the percentage of IGs correlates better with infection and positive blood culture results than the WBC count and is comparable to the ANC. The larger WBC sampling size and consistent parameters used to discriminate between cell populations used in automated differential counts allow for very accurate IG values even in the very low numbers. All of these parameters have low sensitivity and, therefore, are not individually useful as screening tests for infection. High cutoff levels for the percentage of immature granulocytes (in their study it is reported at >3%) may predict positive blood culture results. This may result in lower sensitivity but increases the specificity. IGs can be used to aid the diagnosis of sepsis in the adult population and infants >3 months based on as reference ranges that are established by manufactures and validated by laboratories based on current CAP guidelines.

Challenges in infants

Enumerating ANC and IG% in the infants poses some special challenges. It is difficult to determine reference ranges for immature granulocytes (IGs) in neonates due to rapid fluctuations during the first 5 days of postnatal life. Obtaining adequate samples to establish reference ranges can also be a challenge given that sample volume is usually limited and obtaining samples specifically for reference ranges is difficult in this population. It can also be difficult to establish a reference range in this population since IGs and ANC values in the first 48 hours of life are commonly increased for many reasons, including nonspecific stressors related to the delivery and steroid use, which is common in the setting of prematurity. Wiland EL et al[13] showed that IG counts in newborns (48 hours of life) appeared to be higher than reported for other age groups. IG% ranged from 0%-8.4% (95th percentile 5.2%).

At <12 h, 70% of samples had IG% >1%. IG% appears to decrease over time. Earlier hour of life and higher birth weight were independently associated with higher IG%. The study by MacQueen and Christensen et al, 2016[11], looked at 10,000 CBCs performed on neonates at 10 different institutions, compared automated vs manual leukocyte differential counts and established IGs reference ranges. They showed that the upper reference limit for absolute IG count during the first 48 hours was 1,460 per µL and thereafter (48 hours to 90 days) was 613 per µL. Lower reference limits were essentially zero in all cases. In the first 48 hours of life IG% were between 0%-6.2% and post 48 hours (48 hours to 90 days of life) the IG% were between 0%-4.2%. Further the ability of IT ratios and IG% to identify infants with both blood culture confirmed and clinically suspected sepsis were compared. Using the 95th percentile of IG% as the upper limit of normal (6.2% for newborns $\leq$ 48 hours of age, and 4.2% for those > than 48th of age) they found that IT ratios and IG% were similarly predictive. Some laboratories are using these numbers as guidelines. IG% greater than 6.2% in the first 48 hours of life and greater than 4.2% after 48 hours of age are likely to have similar information regarding the possibility of sepsis as an IT ratio of 0.30. As always, decision making regarding the possibility of sepsis should not be dependent solely upon information available from a CBC but instead must incorporate relevant historical and clinical information. Finally, use of adult and pediatric reference ranges for IG% would not be appropriate for newborns, particularly in those being evaluated for early-onset sepsis, and laboratories should establish age-specific reference ranges.

Neutrophilia: Causes and differential diagnosis

Neutrophilia or the increase of neutrophils in the blood is the most common form of leukocytosis. Neutrophilia is defined as an increased absolute neutrophil count (ANC) in PB above 2SD of the mean value for healthy individuals, ie, above 7.7 × 10E9/L. Mild neutrophilia may sometimes still be within spectrum of normal for an individual as we should remember that the neutrophil count in 2.5% of the general population is >2 standard deviations above the mean. Neutrophilia can be secondary to demargination or physiologic/shift /pseudo-neutrophilia when the neutrophils move from the marginated/storage pools to the circulating pools. This may happen in pregnancy, labor, newborns, strenuous exercise, emotional states, and vomiting. In this condition the total number of granulocytes does not increase. In true neutrophilia the granulocyte count increases as a result of increased release of neutrophils from the bone marrow or increase in the production of neutrophils in the bone marrow. There are several causes of true neutrophilia such as acute bacterial infection, inflammation that results from burns, post-operative, autoimmune conditions, acute myocardial infarction, trauma or tissue damage that induces an inflammatory reaction, conditions of abnormal metabolism such as diabetic ketoacidosis, uremia and eclampsia, acute hemorrhage, sepsis, cigarette smoking, and stress. Drugs such as steroids cause elevation of neutrophil count. Malignancy such as carcinoma, sarcoma, etc, can cause reactive neutrophilia. Finally primary hematologic malignancies can result in neutrophilia. **Left shift** or increase in circulating precursors is often seen in neutrophilia. **Toxic granulation** described as dark blue to purple lysosomal granules containing myleoperoxidase can be seen in these conditions and indicate a shortened maturation and activation or early release from the neutrophilic storage pool. This can be seen in neutrophils in leukemoid reactions, acute inflammation, as well as when granulocyte colony stimulating factors (G-CSF) has been administered. **Döhle bodies**, also seen in neutrophilia are RNA containing structures derived from endoplasmic reticulum. They are oval to round bodies that range in size from barely visible to 2 mcm. These are generally found in patients with infections, burns, trauma, pregnancy, and administration of G-CSF. They are also seen in a benign inherited condition called May-Hegglin anomaly.

Leukemoid reaction describes an increased WBC count, or leukocytosis, which is a physiological response to stress or infection or other medical conditions as mentioned above, except a primary blood malignancy such as acute or chronic leukemia. There can be presence of immature cells such as myeloblasts, promyelocytes, myelocyte or metamyelocytes along with bands and neutrophils showing toxic granulation or Döhle bodies in the peripheral blood. Nucleated red blood cells/erythroid precursors can also be seen. It is important to distinguish a leukemoid reaction from leukocytosis due to malignancy such as chronic myeloid leukemia or Chronic Neutrophilic leukemia.

CML is a malignancy characterized by an increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the peripheral blood.[18] Myeloid cells arise from stem cells that give rise to granulocytes, erythrocytes, or platelets. This clonal disorder primarily manifests as a proliferation of maturing granulocytes (neutrophils, eosinophils, and basophils and precursors). In most patients it presents with an insidious onset and approximately 50% will be aymptomatic and is discovered when the WBC performed in routine medical examination is found to be abnormal. In contrast, most times, a patient with leukemoid reaction will present with signs and symptoms of infection, sepsis, or inflammatory disorders. Other presentations include fatigue, malaise, weight loss, or symptoms of anemia; about 50% will present with massive splenomegaly. CML typically presents in chronic phase and may progress to accelerated phase or blast phase. Rarely, the initial presentation can be accelerated or blast phase. When the disease is progressive the signs and symptoms include bleeding, petechiae, and ecchymosis, bone pain and fever (transformation to acute leukemia-blast phase), increasing anemia, thrombocytopenia, basophilia, and a rapidly enlarging spleen in blast crisis. The diagnosis of CML is suggested based on peripheral blood counts and smear review. It is confirmed and staged with bone marrow biopsy histology and demonstration of t(9;22) and/or *BCR/ABL1* fusion by molecular and cytogenetic analysis. CBC with differential on the peripheral blood smear often shows a total WBC count 20,000-60,000 cells/µL, with mildly increased basophils and eosinophils. There can be mild to moderate anemia. The platelet counts can be low, normal, or markedly increased. Peripheral blood leukocytosis shows a prominent left shift, including granulocyte precursors (eg, myeloblasts, myelocytes, metamyelocytes with a relative increase in myelocytes is seen along with nucleated red blood cells. Toxic granulation or Döhle bodies are usually not appreciated. In chronic phase, blasts are not significantly increased as a percentage of WBCs in peripheral blood or bone marrow, but are often higher than seen in reactive conditions. Bone marrow analysis shows an increased cellular marrow with an increased granulocyte: erythroid ratio. The granulocytes usually display a left shift with increase in myelocytes. Erythroid precursors are relatively decreased. Megakaryocytes include small abnormal hypolobated forms (so called “dwarf” megakaryocytes). The confirmatory analysis is the presence of the Philadelphia (Ph) chromosome in bone marrow cells by conventional cytogenetic karyotyping or demonstration of *BCR/ABL1* fusion by fluorescent in situ hybridization (FISH) or polymerase chain reaction (PCR) analysis. This abnormality is associated with the chromosomal translocation t (9; 22) (q34.1; q11.2), which results in the formation of the Philadelphia (Ph) chromosome. Conventional karyotyping detects 90%-95% of the cases Philadelphia Chromosome (fusion between Chromosome 9 and 22). Cryptic translocations can further be delineated by FISH. Multiplex PCR tests that detect multiple fusion partners are often used at diagnosis to determine which specific fusion is present, and quantitative RT-PCR is routinely performed to effectiveness of ongoing therapy. FISH and PCR can be performed on peripheral blood specimens, while cytogenetic karyotyping is performed on bone marrow. These tests are used in various combinations at different points in the clinical

course for initial diagnosis, therapeutic monitoring, and evaluation for progression and/or resistance. The fusion protein exhibit deregulated protein tyrosine kinase (PTK) activity compared to normal ABL. As a result, there is excessive tyrosine phosphorylation of many intracellular proteins including the BCR-ABL protein itself. The abnormal tyrosine kinase receptor is constitutively expressed and causes abnormal cell growth and proliferation. In the 1990s, imatinib, a specific inhibitor of the unique BCR-ABL tyrosine kinase receptor was designed, and indeed was the first drug treatment targeted to molecular abnormality responsible for the pathogenesis of disease.[18]

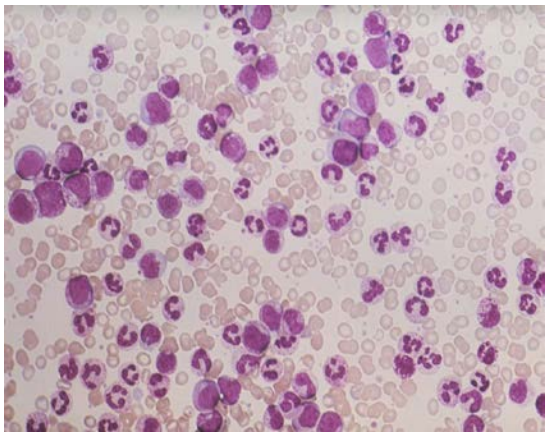
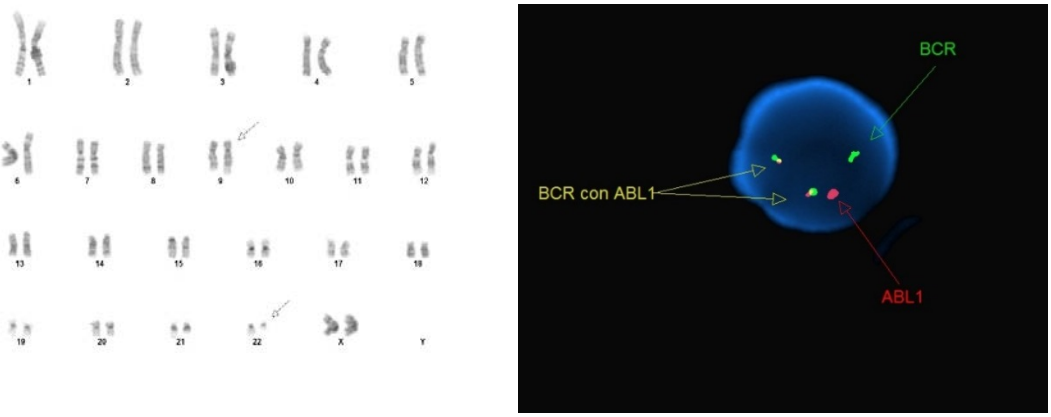


Figure 2: Peripheral blood showing leukocytosis with granulocyte left shift (oil 500x).



Left panel: Cytogenetic/karyotypic analysis that shows translocation of genetic material between the chromosome 9 and 22.

The right panel: FISH studies (fluorescently labeled- in-situ- the genes of interest. Here BCR gene is labeled green and ABL gene is labeled red; when there is translocation that brings genetic material from 2 separate genes together, in this example BCR and ABL; the overlap signal shows as yellow that confirms the presence of the translocation.

Acknowledgement: The Karyotype and FISH pictures are kindly provided by Azim Mohammad, MS, CG(ASCP) , supervisor, Cytogenetics Laboratory, Pathology, Dartmouth-Hitchcock Medical Center.

Chronic Neutrophilic Leukemia is another rare myeloproliferative neoplasm that presents with neutrophilia. Clinically, patients maybe asymptomatic or present with fatigue, easy bruising, or bone pain. Hepatosplenomegaly maybe seen. There is leukocytosis with counts of ≥25 x 10E9/L. The peripheral blood shows increased

segmented neutrophils/bands which comprise > 80% of the leukocytes. This is often referred to as “right” shifted. IGs usually account for <10% of leukocytes with myeloblasts <1% of leukocytes. The bone marrow is hyper cellular with an increased number and percentage of neutrophilic granulocytes. Again, myeloblasts account for <5% of the marrow cellularity. A normal neutrophilic maturation pattern is seen. Cytogenetics can show abnormalities in 10% of the cases with karyotype abnormalities that include deletions in Chromosome 20 or chromosome 11; gains in chromosome 8, 9, 21, and sometimes a complex karyotype. By definition there is no Philadelphia chromosome or *bcr/abl* fusion gene. All other causes of neutrophilia are ruled out such as infection or inflammatory process. There is no evidence of another myeloproliferative disease or myelodysplastic disease. This morphology can be seen in plasma cell neoplasm in a significant subset of cases; therefore plasma cell neoplasm should be excluded as the cause of neutrophilia or clonality must be established in the myeloid cells to consider the diagnosis of concurrent 2 neoplasms (plasma cell neoplasm and CNL) in the same specimen. CNL is now known to be strongly associated with CSF3R (receptor for Colony-Stimulating Factor 3) mutation, SETBP1 (SET Binding Protein 1) mutations, ASXL1(Additional Sex Combs-like 1) mutations, or JAK2 (Janus Kinase 2) mutations. The knowledge of these mutations is useful not only diagnostic but also for therapeutic purposes. For example CSF3R has been shown to signal through downstream SRC family and JAK-kinase pathways and makes these attractive markers for treatment with tyrosine kinase inhibitors such as Desatinib or JAK inhibitor Ruxolitinib. There is ongoing investigation on the use of these tyrosine kinase inhibitors for the treatment of patients with neutrophilic leukemia with CSF3R mutations.[18]

KEY POINTS

1. Automated differentials have better precision and accuracy of WBCs due to the large number of cells (at least 10,000) counted than the traditional visual 100-cell differential, which shows high imprecision due to the small number of cells counted, low reproducibility and a relatively wide inter-examiner variability.

2. As automation continues to increase and more hematology vendors provide IG counts, the use of automated IG counts instead of manual IGs will increase. The enumeration of band counts as an indicator of left shift should cease altogether. This will decrease the workload on the hematology technologists.

SUMMARY

Neutrophilia can be seen in several medical conditions. Clinical and laboratory correlation helps in the final delineation of the disease. Optimal use of automated technology can assist in optimal use of technologist time for more productive activities.