Chapter

Neutropenia and Hematology Analyzer

Gulsum Feyza Turkes

Abstract

The automated hematology analyzer is used to analyze white blood cell (WBC) counts. WBC differentials (WBC-diff) can be measured using a diff-scattergram. This group includes neutrophil cells. Neutrophils, characterized by their multi-lobed nuclei and a diameter ranging from 12 to 15 μm, constitute about 50–70% of the leukocytes present in the bloodstream. They serve as the initial line of defense against infections in the body. Neutropenia is defined as a neutrophil count below the reference range. Neutropenia can be classified as either hematological causes or non-hematological causes. Non-hematologic causes include ethnicity, medical procedures, viral infections, autoimmune illnesses, sepsis, and congenital neutropenia. Hematological causes include myelodysplastic syndromes, acute leukemia, lymphoma, etc. The complete blood count CBC flags notify hematological analyzer operators of abnormal cell conditions, such as neutropenia. Neutropenia can manifest either independently or in conjunction with other flags. Neutropenia can be diagnosed through the utilization of peripheral blood smear tests. We can determine auto-verification rules with certain algorithms by performing a reflex test with these flags. Then, we can perform a peripheral smear analysis. The aim of this section is to explain the method that can provide early diagnosis to patients.

Keywords: hematology analyzer, WBC, neutropenia, auto-verification, flags in hematology analyzer, flagging interpretation, flag messages

1. Introduction

A complete blood count (CBC) is a hematology analysis that offers comprehensive details about all kinds of blood cells in the body. Developments in technology have enabled the use of automated instruments for measurement. It can be recognized as the primary diagnostic method for hematological diseases. Furthermore, it is a technique employed in the identification and monitoring of many different diseases. Leukocyte differential cell number is determined by creating a leukocyte differential distinction in the complete blood count, which in addition consists of the white blood cell (WBC) count, erythrocyte count, and platelet count. Due to the presence of suspicious factors during the CBC, the physician decides to perform additional tests and suggests a peripheral smear for the patient.

Previously, the examination of peripheral blood morphology was conducted using a microscope and a manual peripheral smear. Thanks to modern technology,

laboratory examination of peripheral smear morphology is currently possible with automated peripheral smear devices. Furthermore, doctors have the capability to digitally access and view morphological results on their personal computers. Within this digital setting, the physician not only assesses the morphologic findings but also expedites the diagnosis of patients. Subsequently, an accurate diagnosis can be acquired using flow cytometry using either a peripheral blood sample or a bone marrow sample.

With this digitalization in the field of hematology, it has become important to interpret the flag results on CBC devices. Developing algorithms and automatic peripheral smearing based on these flags can guide us in the early diagnosis of diseases. The neutropenia flag is one of them. It can be isolated or combined with other flags.

2. White blood cell count

White blood cells originate from pluripotent hematopoietic stem cells found in the bone marrow. Once they reach adulthood, they are then released into the peripheral bloodstream. Within the circulatory system, these cells can be classified into two primary groups: granulocytes and agranulocytes. Neutrophils, basophils, and eosinophils are categorized as granulocytes because they contain granules in their cytoplasm. Polymorphonuclear leukocytes (PMNL) are an alternative term for these cells. Lymphocytes and monocytes are categorized as agranulocytes because they lack granules in their cytoplasm. These cells are commonly known as mononuclear leukocytes. Leukocyte counts in modern automated complete blood count systems are calculated through the classification of cells based on their size and volume [1].

The electrical impedance approach, utilizing the Coulter principle, depends on the nonconductive characteristics of cells to ascertain their quantity and size. When quantifying WBC *via* this technique, the information can be shown in the form of a histogram. Cell size and concentration are measured [2].

Modern WBC analysis in analyzers often relies on laser light scattering techniques, such as those used in flow cytometry. WBCs are hydrodynamically focused through a flow cell and exposed to laser light, with the resulting scatter captured by photodetectors. The total WBC count is determined by the number of light interruptions. Forward scatter correlates with cell size, while 7° side scatter indicates internal cell complexity and 90° side scatter (lobularity) reflects cytoplasmic granularity [2].

The flow cytometry using a semiconductor laser counts and classifies cells by irradiating them with a 633 nm laser beam and analyzing their forward scattered light (FSC), side scattered light (SSC), and side fluorescent light (SFL). The intensity of the two types of scattered light (FSC and SSC) reflects cell surface structure, particle shape, nucleus form, refractive index, and reflectivity of the cells. In general, the FSC signal is stronger for larger cells, and the SSC signal becomes stronger as the intracellular structures become more complex. The intensity of the side fluorescent light mainly reflects the type and amount of nucleic acids and cell organelles. These three signals are used to differentiate and count white blood cells, nucleated red blood cells, reticulocytes, and platelets, and to detect abnormal cells and immature cells with the help of unique digital technology and algorithms [3]. The semiconductor laser flow cytometry approach involves conducting measurements over many channels. The WNR channel is utilized for the measurement of white blood cells (WBC). WBC quantification can be carried out by analyzing the

Age	Beckman Coulter DXH 900		Sysmex XN 3000	
	Female	Male	Female	Male
0 to <3 years	5.3–13.2	5.3–13.2	5.75–13.5	5.75–13.5
3 to <5 years	4.8–11.5	4.8–11.5	4.92–11.8	4.92–11.8
5 to <21 years	4.2–10.2	4.2–10.2	4.23–9.99	4.23–9.99

Table 1.
WBC reference range according to age group

forward scattered light and fluorescence intensity acquired from each cell using a two-dimensional scatter plot [4].

The reference ranges for WBC count in automated CBC devices, categorized by age, are displayed in **Table 1** [5, 6]. While the adult reference range for Turkey is 4.39–11.59 × 10^9/L, it was determined as 3.4–9.6 × 10^9/L in the Mayo Clinic Laboratory [7, 8].

Monoclonal antibodies and polyclonal antibodies are acceptable for validating measurement procedures. The term monoclonal antibody refers to one unique kind of antibody that is produced by a single lymphocyte cell. Monoclonal antibodies, which consist of antibody molecules that possess identical antigenic qualities, are very suitable for accurately measuring antigens. They find extensive use in various applications, including cell sorting. Typical blood consists of a combination of several antibody molecules generated by multiple B cells. Antibodies that consist of a mixture of various types of antibody molecules are referred to as polyclonal antibodies [9].

Many modern analyzers utilize fluorochrome-conjugated monoclonal antibodies, like those in flow cytometry, to enhance and broaden the differential cell count. The flow cytometry method utilizes fluorescent-labeled monoclonal antibodies (mAb). Monoclonal antibody (mAb) play a crucial role in diagnosing hematological illnesses and evaluating the immunophenotype for leukemia and lymphoma. The antigenic structures on the surface of human cells are referred to as clusters of differentiation (CD) to ensure standardized terminology. Through the selection of a suitable CD antibody, one can effectively and specifically mark the desired cells. Sysmex common antibodies, such as CD3, CD4, CD8, and CD34, are employed to identify T-cell subsets and immature cell populations [2].

2.1 White blood cell differential (WBC-Diff)

Differential counting of WBC types began approximately 120 years ago. Approximately 120 years ago, differential counting of leukocyte types began, marking a significant milestone in hematology. In 1891, Ehrlich developed a triacid stain for malarial parasites and differentiated neutrophilic cells, naming tissue cells with prominent granules' mast cells. Romanowsky, Giemsa, and Wright later modified the stain method to reveal subtle structures in nuclear chromatin and cytoplasmic granules. Gibson proposed the Gibson Chart, correlating leukocyte count with the severity of infection. Arneth classified neutrophils based on the number of lobes. Ehrlich's staining methods greatly advanced leukemia classification. Notably, Conan Doyle reported a case of leucocythemia in 1882, contributing to early awareness of blood disorders [10].

The history of blood counting and counting instruments is closely linked to the evolution of compound microscopes, which originated in the 1600s in the Netherlands. Microscopy-based cellular analysis began in the 1700s, with pioneers like von Leeuwenhoek. Paul Ehrlich played a crucial role in describing blood cell elements. Subsequently, Malassez and others utilized instruments like the hemocytometer for cell counts, and Neubauer refined manual enumeration techniques. In the twentieth century, centrifugation enabled the quantification of various blood fractions. Coulter's aperture impedance concept revolutionized hematology, leading to automated blood counting based on electric field suspension and cell separation. Multiparameter cell analyzers further advanced automated counting, culminating in modern hematology analyzers providing CBC [2].

Peripheral blood contains five distinct varieties of WBC-Diff. Differentiated leukocyte cells include monocytes, neutrophils, eosinophils, and basophils. In the peripheral bloodstream, neutrophils comprise the vast majority of WBC. Any variation in any of these factors has the possibility of impacting the WBC. Therefore, it is essential to carry out a WBC-diff analysis together with the WBC. White blood cell differential analysis can be performed either manually or with automated instruments. A manual WBC-diff analysis is conducted on a peripheral blood smear using microscopic imaging. For WBC-diff results, a total of 100 WBC are imaged and the different subtypes are quantified. WBC-diff analysis can be performed either manually or automatically. Automated imaging devices have replaced manual microscopy as the preferred method, although hand counting is still considered the most reliable. Clinicians can now use automated peripheral smear equipment to evaluate morphological data on their computers, thanks to modern technology. Automated imaging devices have replaced manual microscopy viewing with the advancement of technology, but manual counting is still considered the gold standard [11].

WBC enumeration and classification into different leukocyte types can be achieved through image analysis using proprietary software and algorithms. This method quantifies WBCs from a stained slide using a fixed volume of EDTA-anticoagulated blood. Stained cells are analyzed *via* automated digital microscopy employing an artificial neural network. The system allows manual review and sorting of unidentified cells before releasing results. These analysis systems show a strong correlation with other methods for the typical five-part differential, but there may be more variability in results for extended cell types, particularly immature granulocytes [2].

Automated CBC devices are also capable of conducting WBC-diff analysis. The 5-part or 7-part differentials are identified in the advanced hematology analyzer. An absolute quantity and a percentage are assigned to each cell type [12].

2.1.1 Neutrophile

Neutrophils are 12–15 μm in diameter and have multi-lobed nuclei. They are called polymorphonuclear neutrophils. Neutrophils comprise about 50–70% of circulating leukocytes and represent the body's initial line of defense. They are involved in the acute inflammatory response to bacterial infection and the removal of the bacteria by phagocytosis. Neutrophils, constituting 50–70% of circulating white blood cells, serve as the body's initial defense against infections. Primarily involved in the acute inflammatory response to bacterial infections, they remove bacteria through phagocytosis [13].

2.1.2 Neutrophil count

The result of the neutrophil count is of two types, like other WBC-diff. Both the absolute neutrophil count (NEU# - ANC) and the neutrophil percentage (NEU%) result should be included in the patient outcome report. While the ANC adult reference range for Turkey is 2.04–7.54 × 10^9/L, and the NEU% adult reference range is 40–74%, ANC was determined as 1.56–6.45 × 10^9/L in the Mayo Clinic Laboratory [7, 8]. ANC-validated reference ranges automated CBC devices according to age are shown in **Table 2**, while NEU%-validated reference ranges are shown in **Table 3** [5, 6].

2.1.3 Neutropenia

The term "neutropenia" is used to describe a reduction in blood neutrophil count below the reference range in CBC devices. The international consensus group for hematology review recommends a threshold value of 1 × 10^9/L for neutropenia in peripheral smear while the French Speaking Cellular Hematology Group (GFCH) recommends 1.5 × 10^9/L [14, 15].

Bone marrow hypoplasia-associated neutropenia may occur as either primary or secondary. The primary causes are rarely observed. The primary causes include leukocyte adhesion deficiency, Chediak-Higashi syndrome, hyper-IgE syndrome, recurrent infection syndrome, and chronic granulomatous illness. Secondary causes are prevalent and encompass cytotoxic medications, aplastic anemia, leukemia, drug hypersensitivity responses, and infections [16].

Apart from primary and secondary causes, neutropenia can be divided into two groups: hematological diseases and non-hematological conditions [14].

Non-hematologic causes: possible causes include ethnic factors, medical procedures, viral infections, autoimmune disorders, sepsis or congenital neutropenia.

10^9/L	Beckman Coulter DXH 900		Sysmex XN 3000	
Age	**Female**	**Male**	**Female**	**Male**
0 to <1 years	0.9–4.6	0.9–4.6	0.69–5.82	0.69–5.82
1 to <21 years	1.5–6.4	1.5–6.4	1.45–6.75	1.45–6.75

Table 2.
Absolute neutrophil count reference range.

%	Beckman Coulter DXH 900		Sysmex XN 3000	
Age	**Female**	**Male**	**Female**	**Male**
0 to <1 years	9.2–45.3	9.2–45.3	8.7–57.9	8.7–57.9
1 to <5 years	17.6–68.1	17.6–68.1	18.6–68.6	18.6–68.6
5 to <15 years	29.7–68.6	29.7–68.6	28.9–67.9	28.9–67.9
15 to <21 years	42.8–75.1	41.3–76.1	39.6–73.9	42.8–75.1

Table 3.
Neutrophil percentage reference range.

Congenital neutropenia encompasses a group of neutropenic disorders that can be persistent or intermittent, severe (<0.5 10^9/L) or mild (between 0.5 and 1.5 10^9/L), and can also affect other organ systems such as the pancreas, central nervous system, heart, muscles, and skin. Congenital neutropenia has two primary groups: those without extra-hematopoietic symptoms and those with. No additional organs are involved in congenital neutropenia without extra-hematopoietic symptoms. This category includes ELANE (ELA2), extracellular G-CSF receptor abnormalities, GFI1-associated neutropenia, and WAS gene mutation-induced lifelong congenital neutropenia. Congenital neutropenia with extra-hematopoietic symptoms involves various organs. Kostmann disease, HAX1 mutations, Shwachman-Diamond syndrome, G6PC3 mutations, Cohen syndrome, Poikiloderma, Clericuzio type, Barth syndrome, AP14 deficiency, and Hermansky Pudlak syndrome type 2 are congenital neutropenia diseases with extra-hematopoietic manifestations [17].

During viral infections, the automated hematology analyzer frequently identifies an "Atypical Lymphocytes" flag. This is typically caused by the presence of activated lymphocytes or the actual mononucleosis syndrome. Severe sepsis can manifest as a condition characterized by low levels of neutrophils and platelets, together with an indication of an abnormal increase in immature white blood cells, which warrants a study of a peripheral blood smear. In cases of autoimmune or iatrogenic neutropenia, the condition often involves a decrease in neutrophil count, and examining a blood smear has minimal influence on the treatment of the patient [14].

Hematological causes: possible causes include acute leukemia (AL), myelodysplastic syndromes (MDS), lymphoma malignancies, hairy cell leukemia (HCL), large granular lymphocytic leukemia (LGL) acute monoblastic leukemia (AML-M5a), acute erythroid leukemia (AML-M6), AML with myelodysplasia-related changes, myelodysplastic syndrome—refractory anemia with excess blasts (RAEB 2), acute myeloblastic leukemia without maturation (M1), acute promyelocytic leukemia (APL), multiple myelome (MM), B-cell lymphobhlastic leukemia (B-ALL), Pelger-Huet anomaly-2, etc. [14, 18, 19].

2.1.4 Flags in hematology analyzers

Auto-verification is the computer-based interpretation of clinical biochemistry analysis results used within the framework of certain rules algorithm determination or machine learning [20]. By reducing manual interpretation time, it prevents extra effort and unintentional errors made in thousands of patients. By focusing on truly problematic cases, staff can interpret the results more accurately [21].

Auto-verification rules include device flags, reference range, analytical measurement range, critical value, mathematical or logical error, clot, delta check, etc. Algorithms determined within the framework of these rules should be determined and verified using the Clinical and Laboratory Standards Institute (CLSI) guideline, AUTO10-A (Autoverification of Clinical Laboratory Test Results) and CLSI guideline, AUTO15-ED1:2019 (Autoverification of Medical Laboratory Results for Specific Disciplines) [18].

In the field of hematology, the term "flag" refers to signals that are displayed to operators to indicate the presence of abnormal cell conditions. The presence of abnormalities in the CBC can offer valuable flags for doing further examinations on patients. Neutropenia is a condition that falls within this category. Peripheral smear testing is primarily indicated for the diagnosis of neutropenia. Neutropenia can occur

Flags	Meaning
<	Low the analytical measurement range
>	High the analytical measurement range
L	Low the reference range
H	High the reference range
Abn. WBC Scattergram	Abnormal WBC Scattergram
Pancytopenia	Low WBC-Diff cells
Neutropenia	Low neutrophil count
Lymphopenia	Low lymphocyte count
Lymphocytosis	High lymphocyte count
Monocytosis	High monocyte count
Basophilia	High basophil count
Leukocytopenia	Low leukocyte count
NRBC present	High nucleated RBC count
IG present	Increased immature granulocyte
Blasts/Abn Lympho?	Possibility that blasts are present/ Possibility of abnormal lymphocytes
Blasts?	Possibility that blasts are present
Abn Lympho?	Possibility of abnormal lymphocytes
Left Shift?	Possibility of left shift
Atypical lympho?	Possibility of atypical lymphocytes
Variant Lymphs?	Possibility of variant lymphocytes

Table 4.
Flags in hematology analyzers for WBC and WBC-diff analysis

either on its own or in conjunction with other flags abnormal cells or morphological abnormalities associated with hematological disorders are rarely observed in peripheral blood smear results when isolated neutropenia is present. Nevertheless, peripheral smear results can be significant when there are additional flags of neutropenia [14].

There are many flags regarding WBC analysis that may accompany neutropenia in automated CBC analyzers. The flags are listed in **Table 4**.

2.1.5 Cases and flags

The following sample cases include the analysis results observed at the time of first admission to the hospital.

2.1.5.1 Acute promyelocytic leukemia (APL)

CBC flags: Abn. WBC scattergram, Blasts?, Abn Lymph/blast?, Immature Gran?, basophilia, leukocytosis, anemia, thrombocytopenia, L for ANC, L for NEU%, H for WBC, L for eosinophil, and H for basophil.

Peripheral blood morphology examination: Low segmented neutrophil count, low lymphocyte count, and blasts are present [18].

2.1.5.2 Multiple myeloma (MM)

CBC flags: *Pancytopenia*, Blasts?, Abn Lymph/blast?, Atypical Lymph?, L for ANC, L for NEU%, L for WBC, L for eosinophil, L for eosinophil%, H for lymphocyte%, and H for monocyte%.

Peripheral blood morphology examination: Low segmented neutrophil count, high lymphocyte count, low monocyte count, and plasma cells are present [18].

2.1.5.3 B-cell lymphoblastic leukemia (B-ALL)

CBC flags: Blasts?, Abn Lymph/blast?, Immature Gran?, L for ANC, L for NEU%, L for monocyte, L for monocyte%, H for eosinophil, and H for eosinophil%.

Peripheral blood morphology examination: *Low segmented neutrophil count*, low monocyte count, high eosinophil count, and myelocytes is present. Blasts and abnormal lymphocytes are present [18].

2.1.5.4 B-cell lymphoblastic leukemia (B-ALL)

CBC flags: Abn. WBC scattergram, Abn Lymph/blast?, Immature Gran?, lymphocytosis, *neutropenia*, leukocytosis, anemia, thrombocytopenia, L for ANC, L for NEU%, L for eosinophil, L for eosinophil%, H for WBC, H for lymphocyte, and L for monocyte%.

Peripheral blood morphology examination: Low segmented neutrophil count, blasts are present [18].

2.1.5.5 Mantle cell lymphoma (MCL)

CBC flags: Abn. WBC scattergram, Abn Lymph/blast?, Lymphocytosis, L for ANC, L for NEU%, H for WBC, H for lymphocyte, and H for lymphocyte%.

Peripheral blood morphology examination: *Low segmented neutrophil count*, low monocyte count, high lymphocyte count, blasts, reactive lymphocytes, and abnormal lymphocytes are present [18].

2.1.5.6 Pelger–Huët anomaly

CBC flags: *Pancytopenia*, lymphopenia, leukocytopenia, anemia, thrombocytopenia, L for ANC, H for NEU%, L for WBC, L for lymphocyte, L for monocyte, L for eosinophil, and L for eosinophil%.

Peripheral blood morphology examination: low segmented neutrophil count, high band neutrophil count, low monocyte count, and metamyelocytes are present [18].

2.1.5.7 Acute monoblastic leukemia (AML-M5a)

CBC flags: *Neutropenia,* monocytosis, blasts?, anemia, trombocytopenia.

Peripheral blood morphology examination: Low segmented neutrophil count, blasts, and monoblasts are present [19].

2.1.5.8 Acute erythroid leukemia (AML-M6)

CBC flags: *Neutropenia,* leukocytopenia, NRBC present, blasts?, anisocytosis, macrocytosis, anemia, fragments?

Peripheral blood morphology examination: Low segmented neutrophil count, myeloblasts, and NRBC are present [19].

2.1.5.9 AML with myelodysplasia-related changes

CBC flags: *Neutropenia*, Blasts?, anisocytosis, and anemia.
Peripheral blood morphology examination: Low segmented neutrophil count, myeloblasts , and NRBC is present [19].

2.1.5.10 Myelodysplastic syndrome—refractory anemia with excess blasts (RAEB-2)

CBC flags: *Neutropenia,* lymphopenia, leukocytopenia, NRBC present, blasts?, anisocytosis, macrocytosis, fragments?, PLT Abn distribution?, and thrombocytopenia.
Peripheral blood morphology examination: Low segmented neutrophil count, myeloblast , and NRBC is present [19].

2.1.5.11 Acute myeloid leukemia (AML-M0)

CBC flags: variant lymphs?, blasts?, NRBC present, L for ANC, L for NEU%, L for monocyte, L for monocyte%, and H for lymphocyte%.
Peripheral blood morphology examination: low segmented neutrophil count, blasts are present, and high lymphocyte count [22].

2.1.5.12 MDS with monosomy 7 presenting as aplastic anemia

CBC flags: NRBC present, L for ANC.
Peripheral blood morphology examination: NRBC and Pelger-Huët cells are present [22].

2.1.5.13 T-cell large granular lymphocyte leukemia

CBC flags: Variant lymphs?, NRBC present, L for ANC, L for NEU%, L for monocyte, H for lymphocyte, H for lymphocyte%, L for monocyte%, L for eosinophil%, L for basophil%
Peripheral blood morphology examination: low segmented neutrophil count, large granular lymphocytes, smudge cells, and some big platelets are present [22].

In many cases, such as the ones listed above, a conclusive diagnosis is established by further tests after the analysis of the CBC test, which serves as the initial screening examination. Upon analyzing the CBC results, the hemato-oncologist may recommend additional tests if they are judged appropriate. Nevertheless, contemporary algorithms have been devised as a substitute for this prolonged approach [23]. Most tests are added by automated analyzers, using algorithms devised by laboratory professionals. This process is known as "reflex testing" [24]. The reflex test allows for the performance of a peripheral smear test using the same sample following automated hematological analysis, which serves as the initial screening procedure. Hence, it is crucial to study and interpret the flag data obtained from the hematological analyzer. When neutropenia is present, the time it takes to get results known as the turnaround time (TAT) is reduced by using the peripheral smear test as a reflex test [23].

3. Conclusion

Complete blood count devices are used primarily to screen for hematology patients. Since automated peripheral smear devices have been developed, they can be considered as a screening test. Since additional diseases, especially malignancy, are observed in patients with neutropenia report results, neutropenia should be included in the auto-verification rules. In these patients, peripheral smear analysis should first be performed from the same sample with the reflex test rule. In this way, the advantage of more accurate and earlier diagnosis can be obtained for patients.

Conflict of interest

The author declares no conflict of interest.

Acronyms and abbreviations

CBC	complete blood cell
WBC	white blood cell
WBC-Diff	white blood cell differential
PMNL	polymorphonuclear leukocytes
EDTA	Etilendiamin tetraasetik asit
ANC	absolute neutrophil count
NEU%	neutrophil percentage
GFCH	the French Speaking Cellular Hematology Group
CLSI	Clinical and Laboratory Standards Institute
RBC	red blood cell

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