GUEST FORUM

Practical Use of Automated White Cell Differential Analysis

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Automated white cell differential analysis began with the 3-part differential and now 5-part differential is used in laboratories. The automated white cell differential has been recognized as useful for screening abnormal samples that need further microscopic examination to find actual morphological abnormalities. The 3-part differential is useful for diagnosing acute bacterial infection and the 5-part analysis is useful for diagnosing other inflammations. New hematology analyzers can provide hematological data and can assay C-reactive protein at the same time. These analyzers make diagnosis of inflammation easy and reliable.

1 Introduction

No physicians would deny the fact that the hematology test, as well as urine analysis, is a part of basic daily examination. In general the term “hematology test” is often used as a synonym for the complete blood count (CBC). When automatic blood analysis systems were first introduced, a CBC encompassed only the red blood cell (RBC) count and the white blood cell (WBC) count. As technology has been making remarkable advances, the hematocrit and hemoglobin analyses were added as parts of the CBC, and the platelet count followed. Now that an automated WBC differential analysis is readily available, more doctors are likely to include an automated white cell differential test in their essential examination in addition to blood cell counts, hematocrit, and hemoglobin analyses. To provide a better understanding of the blood test, this report describes how hematology tests have changed and explains the importance of the automatic white cell differential analysis from a clinical point of view.

2 Historical Background of the Hemogram Test

Dr. Ehrlich, the well-known German bacteriologist, discovered that there were varieties of white blood cells in the blood, while studying the white cells in peripheral blood. Although his discovery was made more than 100 years ago,[1)] some descriptions of polymorphonuclear neutrophils and basophils can be found in the records of the day.

In the 20th century, the white cell differential was used as an effective way to examine patients for sepsis, peritonitis, and visceral cleft. Often seen in medical records and archives from early in the century, the “Gibson Chart” first drew attention to the use of a white blood cell differential for the purpose of examining patients. The chart emphasized the number of white blood cells and the distribution ratio of neutrophils. The reference points for the two factors were set at 10000/μl for the leukocyte count and 75% for the neutrophil ratio, as shown in Fig.1. If a result exceeded the reference point by 5%, the patient who furnished the analyzed blood had an out-of-control inflammation.

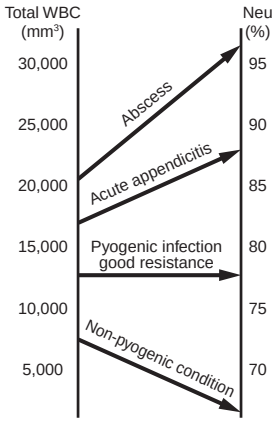


Fig.1 The change of leukocyte count and neutrophils ratio by the disease (Gibson chart).

German doctor J. Arneth focused on the segmented cell ratio on the assumption that it was closely related with the maturity and the function of white blood cells. Based on the segmented neutrophil ratio, he divided the variety of white blood cells into five classes, from class 1, having one segment, to class 5, having five segments. According to his report, the segmented neutrophil ratio for a healthy adult is as follows:

One segment: 5%, two segments: 35%, three segments: 41%, four segments: 17%, and five segments or more: 2% (see Fig.2).[2] Although it was too time-consuming for practical use, this analysis is notable because it defined decisive segmentation factors and helped lay the foundation for morphological diagnosis applied to vitamin B12 and folic acid deficiency.

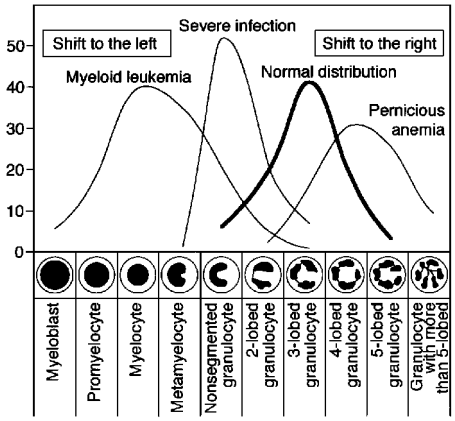


Fig.2 The distribution of neutrophils in which the degree of lobation differs in various diseases.

Dr. V. Schilling, the German hematologist, showed interest in the distribution of protoneutrophils. He reported that protoneutrophils were distributed unevenly over the right area in pernicious anemia and unevenly over the left area in inflammation. His report also mentioned the assortment of metamyelocytes, rod-form neutrophils, and segmented neutrophils in his statement on the toxi-granulocyte.[3] Schilling's studies have led to a chart that shows changes in WBC distribution as inflammatory reaction occurs (Fig.3).[4] This chart is frequently referred to by medical workers in Japan. The period illustrated in the chart consists of three stages: fighting phase (neutrophil growth phase), guarding phase (monocyte, eosinophil, and basophil growth phase), and healing phase (lymphocyte). Although the chart is old, it is so informative on the changes in cell differential that doctors can still find it useful when making diagnoses.

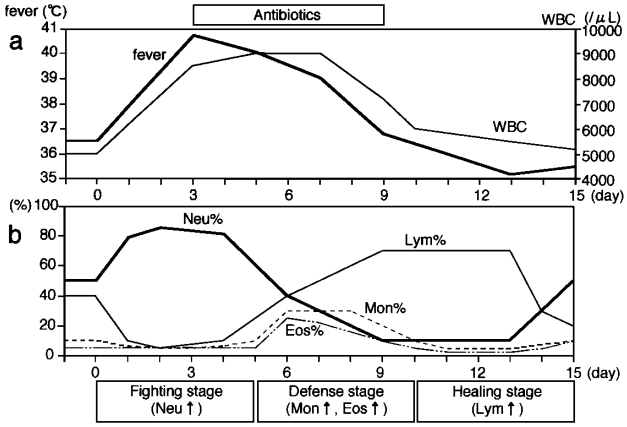


Fig.3 The transition of body temperature and WBC counts (a), and change of WBC distribution as inflammatory reaction occurs (b).

3 Is White Cell Differential Really Necessary?

The condition encountered most often in daily examination is inflammation. When a patient has a bacterial inflammation, white blood cells increase, a result of an increase in neutrophils. If a patient has a viral inflammation, the number of white blood cells remains unchanged or decreases. Bacterial inflammation is not the only factor that increases white blood cell numbers – leukemia and allergic reactions contribute to increases in leukocytes. What is clinically required to identify a disease in the somewhat confusing situations indicated earlier is the white cell differential.[5]

The morphological white cell differential has been used since Giemsa staining was developed, and doctors greatly rely upon the microscopic white cell differential. In contrast to their reliance upon the results, examinations including the white cell differential are rarely performed by doctors themselves due to the following reasons:

1) Use of a microscope is required.
2) Results of the examination depend on experience on in cell differential.
3) It takes a fair amount of time and work to analyze the characteristics of the leukocyte.
4) There may be a wide range of measurement deviation because the number of white blood cells subject to analysis is limited.

Such contradictory circumstances have encouraged the development of an automated means of performing a white cell differential analysis – a method that produces easier, faster, and accurate measurements.

The first step taken toward automatic analysis was an attempt to differentiate microscopic images of cells using computer analysis. In spite of the efforts put into deriving a conventional differential, the trial failed due to the slow processing speed, the limited number of cells analyzed, and the high price of the equipment. The automatic analyzer that has become the standard uses electrical or electro-optical technology to analyze blood. Such machines can perform a red blood cell count and a white cell differential at the same time. There are two types of automated white cell differential analyses, according to the degree of the differential: one is a 3-part differential and the other is a 5-part differential.

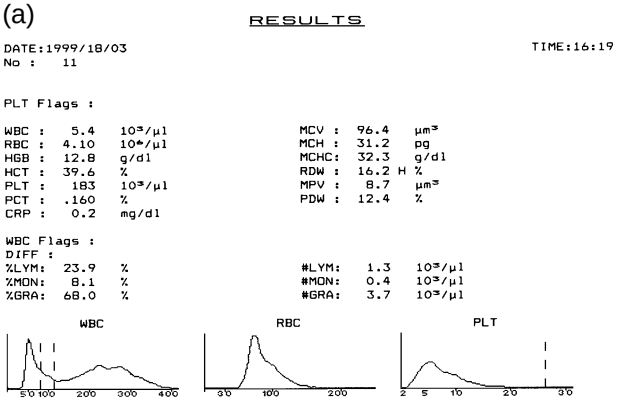
3.1 How Can CBC and 3-Part Differential Be Most Effectively Used?

The 3-part differential analyzer measures the volume of white blood cells electrically and classifies the cells, based on their size, into three groups: a small white blood cell group (lymphocytes), a medium-sized white blood cell group (monocytes, eosinophils, and basophils), and a large white blood cell group (neutrophils). Note that the monocyte is included in the medium-sized group, a classification that differs from that used by the conventional method of Giemsa staining.[6] As the operating principle for this type of analyzer is simple, it can be compact, uses fewer reagents and is thus more cost-effective, and can detect elevated numbers of neutrophils more accurately than the 5-part differential analyzer. The 3-part differential analyzer is suited for the use in emergency rooms and outpatient laboratories, where acute inflammations must be identified promptly and reliably, and small laboratories attached to wards or operating rooms.

This analyzer uses blood specimens treated with the anticoagulant EDTA and can be operated by an inexperienced person. The results of the CBC and the 3-part differential for WBC can be obtained within two minutes. When attached to a printer, the 3-part white cell differential chart (sorted by diameter) can be printed out. Once the chart becomes familiar, abnormalities in the white cell differential should be readily noticed just by looking at the chart (Fig.4-a, 4-b). Even if a sufficient amount of specimen cannot be obtained from the patient (common with infants and children), the analyzer can operate properly in the microanalysis mode.

Inflammations should be diagnosed to determine if they are of bacterial origin (increase in WBC, neutrophil ratio, and CRP), viral origin (normal or reduced number of WBC, increase in lymphocyte, and normal or high CRP), or accompanied by collagen disease (normal number of WBC and normal or slightly elevated CRP). Using CBC, 3-part differential, and CRP in accordance with the characteristics of the disease will help the clinician determine the cause of the inflammation.

(a)



(b)

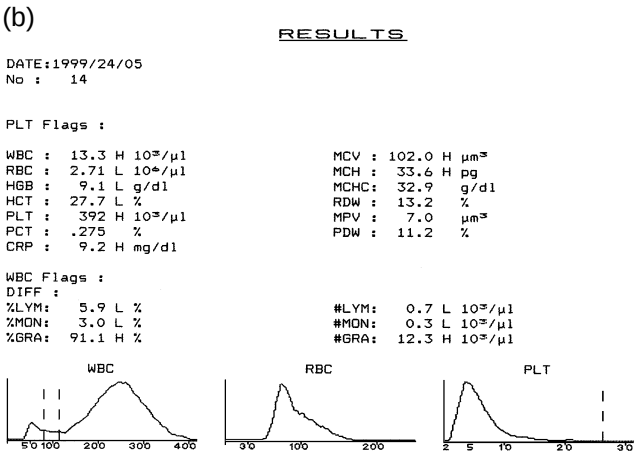
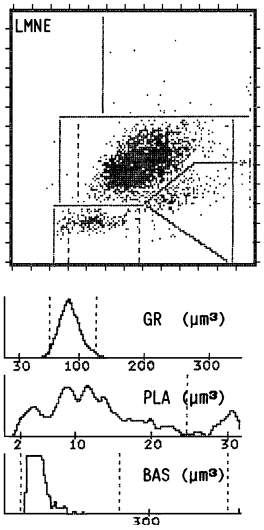


Fig.4 3-part differential WBC analysis example of the anemia patient.
(a)normal, (b)abnormal
(Automated Blood Cell Count and C-reactive Protein Measuring Analyzer LC-170 CRP made by Horiba, Ltd.)

3.2 How Can CBC and 5-Part Differential Be Most Effectively Used?

The 5-part white cell differential can provide accurate cell ratios for five varieties of white blood cells including neutrophils, lymphocytes, monocytes, eosinophils, and basophils. This precision gives the 5-part differential the same clinical significance as microscopic analyses.[7] The 5-part differential analyzer uses blood specimens treated with the anticoagulant EDTA and can analyze the specimen as fast as the 3-part differential analyzer. Although it requires a fair amount of experience to determine the nature of leukemia just by observing the chart, a printed WBC scatter diagram certainly offers advantages of visual diagnoses (Fig.5-a, 5-b).

(a)



(b)

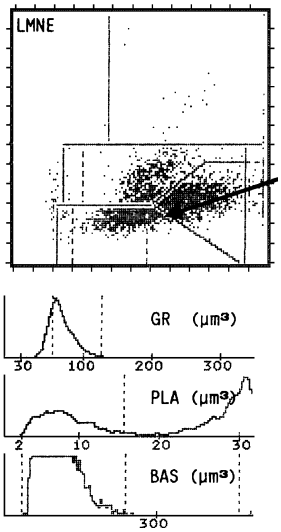


Fig.5 5-part differential WBC analysis example of the anemia patient.
(a)normal, (b)abnormal
(Automated Blood Cell Counter VEGA Retic made by ABX S.A.)

While it features high reliability and precision, the 5-part differential analyzer has room for improvement. Compared with the 3-part differential analyzer, it is larger, more expensive, costs more to maintain, uses more reagents, and requires a full-time medical engineer to keep the electro-optical system fine-tuned. The financial impact of these factors may prevent some laboratories from being equipped with the advanced analyzer.

After reading the description above, many clinicians may wonder if abnormal cells specific to certain diseases such as leukemia can really be identified. This is a common problem for both 3-part and 5-part differential analysis. Neither analyzer can guarantee 100 % identification of leukemia cells, but the internal computer is designed to display a warning message (Table 1) on the screen when the presence of leukemia cells is suspected. If the warning message appears, a microscopic analyses should be made to examine the specimen for hematological abnormalities. This double check will minimize the chance of overlooking abnormal conditions. Despite the fact that the automated white cell differential analysis may require a microscopic examination for certain diseases, the accuracy of measurements is an undeniable advantage offered by the automatic differential analyzer.

Pit	Ret
Thrombocytosis	Immatures
Thrombopenia	NRBC
Microcytosis	Reticulocytosis
Small cell	WBC

WBC	RGB
Leukocytosis	Erythroytosis
Leukopenia	Cold agglutinin
Lymphoytosis	Anemia
Neutrophilia	Microcytosis
Eosinophilia	Hypochromia
Myelemia	Poikilocytosis
Largeimmature cell	Pancytopenia
Atypic,lymphocyto	
Left shift	
Nucleated RBC	
Monocytosis	
Basophilia	
Pancytopenia	

Table 1 Message Table of the VEGA Retic.

4

Conclusion

The national budget for medical care has been reduced due to expanding health insurance costs. These financially unfavorable conditions force most medical institutions to choose cost-effective equipment for their facilities, and demands for small laboratories and compact test devices, also known as (point-of-care testing or POCT, are on the increase. From an international standpoint, medical institutions in developing countries and small hospitals tend to opt for CBC and 3-part differential analyzers while CBC and 5-part differential analyzers dominate the market targeted for medium- to large-scale hospitals and examination centers, and medical institutions in developed countries. If companies can supply quality products that meet social demands without delay, they will be able to increase their market share significantly in this growing medical field.

References

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