TÜRK PEDİATRİ ARŞİVİ
TURKISH ARCHIVES of PEDIATRICS

Review / Derleme

What does a hemogram say to us?

Hemogram bize neler söyler?

Tülin Tiraje Celkan

Division of Pediatric Hematology-Oncology, Department of Pediatrics, İstanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty, İstanbul, Turkey

Cite this article as: Celkan TT. What does a hemogram say to us? Turk Pediatri Ars 2020; 55(2): 103–116.

Abstract

The most commonly performed blood test is complete blood cell count. This test includes hemoglobin, white blood cell count, platelet count, and detailed red blood cell indices. Automated complete blood count also give information for "differential" which gives information about percentages and absolute numbers of different subgroups of white blood cells. This test is necessary in diagnosing anemia, hematological cancers, infections, acute hemorrhagic states, allergies, and immunodeficiencies. Also it is used for monitoring side effects of certain drugs. A pediatrician is frequently challenged for evaluating complete blood count as a part patient's assessment. An enhanced and complete understanding of this laboratory test is essential for providing quality care of sick and normal children. Here in this paper, we want to share key laboratory interpretation strategies for complete blood count and some clues for differentiating normal from deviations and true problems.

Keywords: Abnormal hemogram values, child, hemogram, normal hemogram values

Öz

En sık istenilen kan testi tam kan sayımıdır. Bu test hemoglobin, lökosit, trombosit sayılarını ve ayrıntılı olarak eritrosit parametrelerini içerir. Otomotik kan sayım cihazlarının verdiği sonuçlar artık lökosit alt gruplarının yüzdeleri ve sayıları konusunda da bilgi vermektedir. Kan sayımı; anemi, hematolojik kanserler, enfeksiyonlar, akut kanamalar, alerjik hastalıklar ve immün yetmezliklerin tanısı için gerekli bir testtir. Ayrıca bazı ilaçların yan etkilerinin izlemi için de gereklidir. Bir çocuk hekiminin hastasını değerlendirmesi genelde tam kan sayımı okumasını ve yorumlamasını da gerektirir. Bu testin nitelikli olarak değerlendirilmesi hasta ve normal çocuk izleminde önemlidir. Bu yazıda hemogram değerlendirmesinde önemli noktalara değinilerek normal ve sorunlu durumların ayrılabilmesi için ipuçları paylaşılmıştır.

Anahtar sözcükler: Çocuk, hemogram, normal kan sayımı, patolojik kan sayımı

Introduction

Although important diagnostic clues are obtained with history and physical examination, the definite diagnosis of hematologic diseases is made as a result of laboratory examinations. Blood is composed of formed elements suspended in the fluid medium called plasma. Formed blood elements involve red blood cells (RBC), white blood cells (WBC) and platelets (Plt). Plasma constitutes 55–66% of total blood volume and is obtained by centrifugation of blood that is prevented from clotting. If blood is placed in a tube and allowed to coagulate by itself, its serum is separated. In contrast to plasma, blood serum lacks coagulation factors such as fibrinogen, and factor V and VIII (1–9).

Complete blood count (CBC) is easily requested and interpreted. As it is not expensive, it constitutes a significant portion of routine examinations. In CBCs, automation gives clearer and more accurate results. In the last 20 years, use of automated devices completely eliminated classic methods. However, performing CBCs in individuals with no symptoms is not advantageous, the group that truly needs investigation constitutes only 1%, though 11% of the results are found to be abnormal. Information about the counts, sizes, diameters, and percentages of different types of RBCs, WBCs, and Plts can be obtained with a small amount of blood (100 μL) in a time period of 1 minute with an error probability of less than 1% by way of automation. Currently, Plt counting using phase-contrast, which is one of the classic methods, is being used as a rare confirmation method (10–13).

Fresh venous, capillary or arterial blood samples can be used for CBCs. Blood samples can be obtained at any time

Corresponding Author/Sorumlu Yazar: Tülin Tiraje Celkan E-mail/E-posta: tirajecelkan@yahoo.com
Received/Geliş Tarihi: 17.06.2019 **Accepted/Kabul Tarihi:** 28.06.2019

DOI: 10.14744/TurkPediatriArs.2019.76301

Table 1. Conditions which influence blood counter devices

Condition	Parameters influenced	Explanation
Blood sample that was kept waiting	MCV H, MPV H, PLT L	RBC and PLT swell when they are kept waiting, blood sample should be kept waiting for 3 hours for testing and for 1 hour at most for peripheral blood smear
Leukemia treatment period	WBC L, PLT H	Fragmented WBCs are counted as PLT
WBC >50 000	Hb H, RBC H	In clouded plasma Hb is read incorrectly, WBC are also seen in RBC histogram.
Platelet cluster and satellitism	PLT D, WBC H	PLT clusters are counted as WBC; In case of unexpectedly low PLT, confirmation with peripheral blood smear should definitely be performed (with blood sample without EDTA)
Erythroblast	WBC H	Especially during hemolytic events, the normoblast count is calculated when 100 WBCs are counted and substracted from the WBC count
Microcyte	RBC L	Left shift in RBC histogram
RBC resistant to lytic agents	WBC Y, Hb H	Lysis-resistant Hb S, C, F
Blood sample with hemolysis	RBC L, Htc L, MCHC H	
Lipemic and icteric blood	Hb H, MCH H, MCHC H	Clouded plasma Hb H
Cold agglutinin	RBC L, MCV H, MCHC H	Right shift in RBC histogram

L: Low; Hb: Hemoglobin; Htc: Hematocrit; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; MPV: Mean platelet volume; PLT: Platelets; RBC: RBC count; H: High; WBC: WBC count

of the day. In fact, small variations may occur in the values during the day, thus it is recommended that blood samples should be obtained in the morning. Another question is if blood samples should be collected on an empty stomach; such an enforcement is usually not required for children. After the blood sample is placed into a purple-top tube up to the black mark, it is allowed to mix with EDTA by rotating the tube up and down 3–4 times; coagulum should not form. Shaking is not recommended because it may disrupt formed blood cells. The site of blood sampling is important in the assessment of the results. This parameter should be watched especially in newborns and when monitoring hematocrit values following liver or kidney biopsy. Otherwise, unnecessary transfusion may be performed thinking erroneously that hemorrhage is present, or a probable hemorrhage may be overlooked. As there is compressed plasma in the manual hematocrit value, a higher value is obtained compared with values obtained with automated methods. It has been reported that capillary values might be 15–20% higher compared with venous values. WBC counts have been shown to be lower in arterial blood samples, which are used more frequently in newborns. The appropriate amount of anticoagulant is 1.5 mg for EDTA for 1 mL blood. Divalent or trivalent potassium salt of EDTA may be used. Reliable results will be obtained even if the blood mixed with EDTA is stored in laboratory for 24 hours [especially WBC, hemoglobin (Hb), hematocrit (Hct), RBC count, and Plt count]. However, the mean platelet volume (MPV) gives an accurate result only if it is counted in the first 2–6 hours after a blood sample is obtained. In addition, it is known that mean erythrocyte hemoglobin concentrations (MCHC) may be found erroneously increased in blood samples stored in laboratory for a certain period (14).

With complete blood count, Hct, Hb, RBC, MCV, mean erythrocyte hemoglobin (MCH), MCHC, red cell distribution width (RDW), which shows the discrepancy in red cell diameters (anisocytosis), WBC, numbers and percentages of different types of WBCs (lymphocytes, segments, monocytes, eosinophils, bazophils), Plt count, and MPV can be evaluated (10, 11).

The ranges in which all these parameters can be counted safely are as follows: WBC: 0.1–99x10^9/mm^3; RBC: 0.4–8.0x10^{12}/mm^3; Hb: 2–25 g/dL; Plt: 10–999x10^9/mm^3. Generally, a 2% deviation from normal may be observed at extreme values, but this deviation may increase up to 5% in platelet counts at values of 50,000 and below. Under such conditions, the results obtained should be confirmed with a peripheral blood smear. Besides the data obtained by automation, assessment of peripheral blood smears in each patient may enable making many diagnoses in addition to having visual confirmation of the results. A pediatrician should assess each patient's peripheral blood smear at least once. Devices used in recent years can give warning messages by separating abnormal blood counts from normal counts and WBC differentials to-

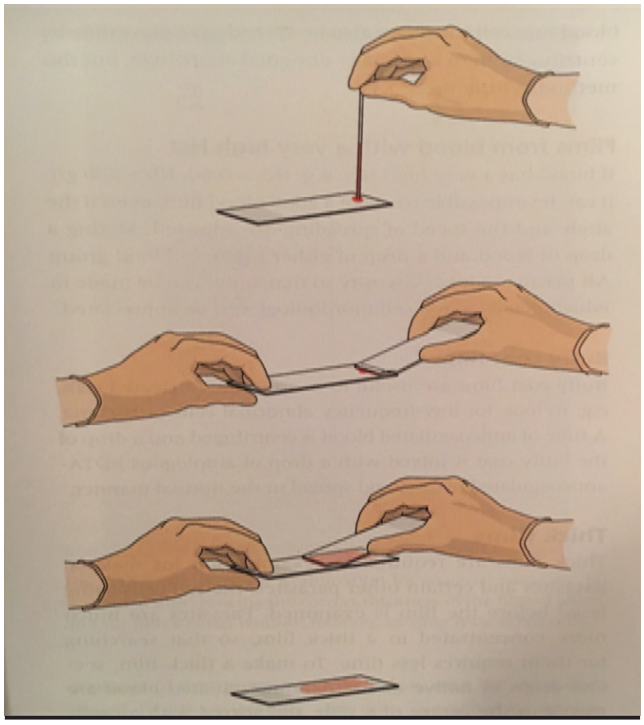

Figure 1. Technique for preparing qualified smear

Figure 2. A smear prepared in a qualified manner (8)

gether with three (neutrophils, monocytes, lymphocytes; basophils and eosinophils together with monocytes) and sometimes five (neutrophils, monocytes, lymphocytes, basophils, eosinophils) parameters. However, it should be kept in mind that no hemogram data can give information that could be obtained from peripheral smears (15).

Interpretation of results is easier if simple rules are followed when evaluating CBC results. According to the rule of three, for example, the hemoglobin value is equal to three times the RBC count and the hematoctrit value is equal to three times the hemoglobin value. If there are contradictory results, either there is an artifact or there is a condition which should be investigated. If the Hct value is 34% when the Hb value is 9 g/dL, for example (34>3x9=27),

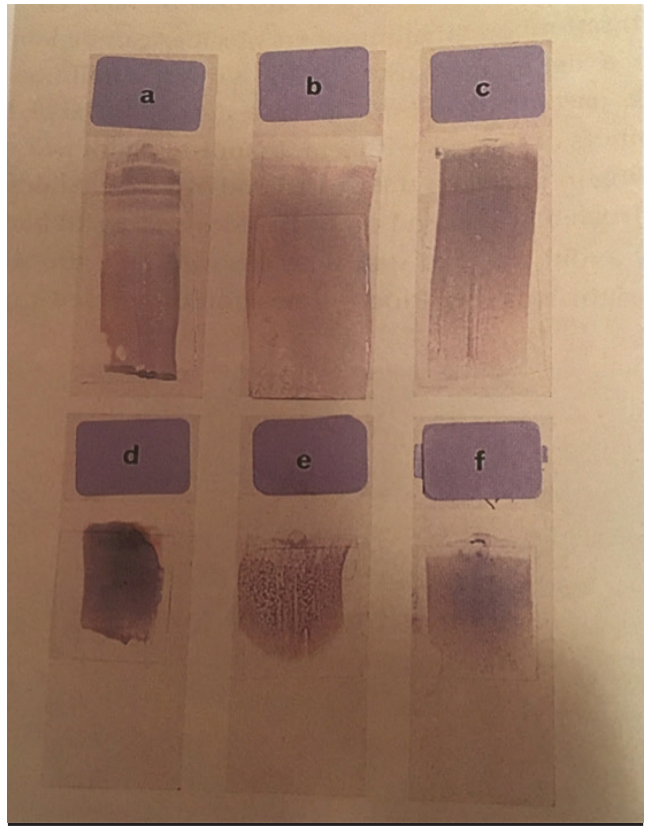

Figure 3. Examples of smears prepared poorly (8)

this shows that the plasma of the blood is decreased, and may be a clue that the patient is dehydrated because of diarrhea or vomiting. If the RBC count is 5.5 million when the Hb value is 9 g/dL, this indicates the presence of high RBC numbers but insufficiency of Hb, and this is usually observed in thalassemia trait (TT). Therefore, the presence of an RBC count above 5 million in childhood is generally known as the mini Mentzer sign. In conditions that cause cell lysis (uremia), hyperosmolar plasma is formed. This may lead to a pseudo-increase in Hct and MCV. A pseudo-increase in Hb, Hct, RBC, and MCV may be found also in cases of hyperleukocytosis. In the presence of cold agglutinin, RBCs may be found to be lower than normal, and MCV and MCH may be found to be higher than normal, because red cells adhere to each other. The device may erroneously evaluate nucleated red cells (normoblasts) as lymphocytes and fragmented red cells as platelets by their sizes. In hyperbilirubinemia, Hb may be found erroneously high. Therefore, Hb may suddenly be found to be much lower than expected in babies with hyperbilirubinemia who recover from jaundice (Table 1).

Peripheral blood smear

Peripheral blood smears are simple and inexpensive technical methods with high diagnostic value. The microscope slide method is the most frequently used method. It is performed with a drop of a blood sample obtained from

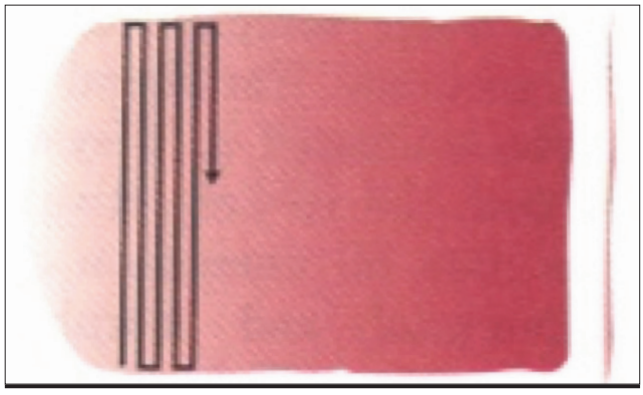

Figure 4. The screening method to be pursued during smear counting with the objective of not counting the same cells repeatedly (8)

the fingertip, heel or Hct tube and put on a slide (3 mm in diameter) (Fig. 1). Another slide is held with a mean slope of 30–45 degrees, the blood drop is allowed to spread across the slide and the smear is performed in one move without applying too much pressure. A figure appearing like a finger should be obtained (Fig. 2). Assessment cannot be performed with too short and too thick smears (Fig. 3). Wright or May-Grünwald-Giemsa can be used as stain. During counting, a certain technical method can be used so that the same areas are not counted repeatedly (Fig. 4).

Examination of peripheral smears is generally initiated with x10 magnification. At this magnification, generally, technical evaluation of the smear is performed rather than the cell details. On poorly smeared slides, formed elements may collect in one area, and they may not be observed in other areas. In that case, the counting will be inaccurate. Smears are generally evaluated with x100 magnification. All three formed blood element series should be morphologically investigated.

Complete blood counts and peripheral blood assessments are composed of three phases: RBCs, WBCs, and Plts.

I. Red blood cells

Red blood cells are biconcave disk-shaped anucleated cells with a diameter ranging between 6 and 9 microns. A greater surface area as compared with volume, enables the red cells to change shape easily, pass through capillary vessels easily, and facilitates exhange of gas and other substances. A normal RBC is composed of a concentrated hemoglobin part, and a membrane encircling other proteins and enzymes.

It is difficult to count the number of red cells manually. Net RBC count values, which are used when calculating red cell indexes, can be reached by counting about ten thousand red cells with optic scatter technology using blood cell counting devices. Mature red cells are biconcave cells with a diameter ranging between 7 and 8 μm,

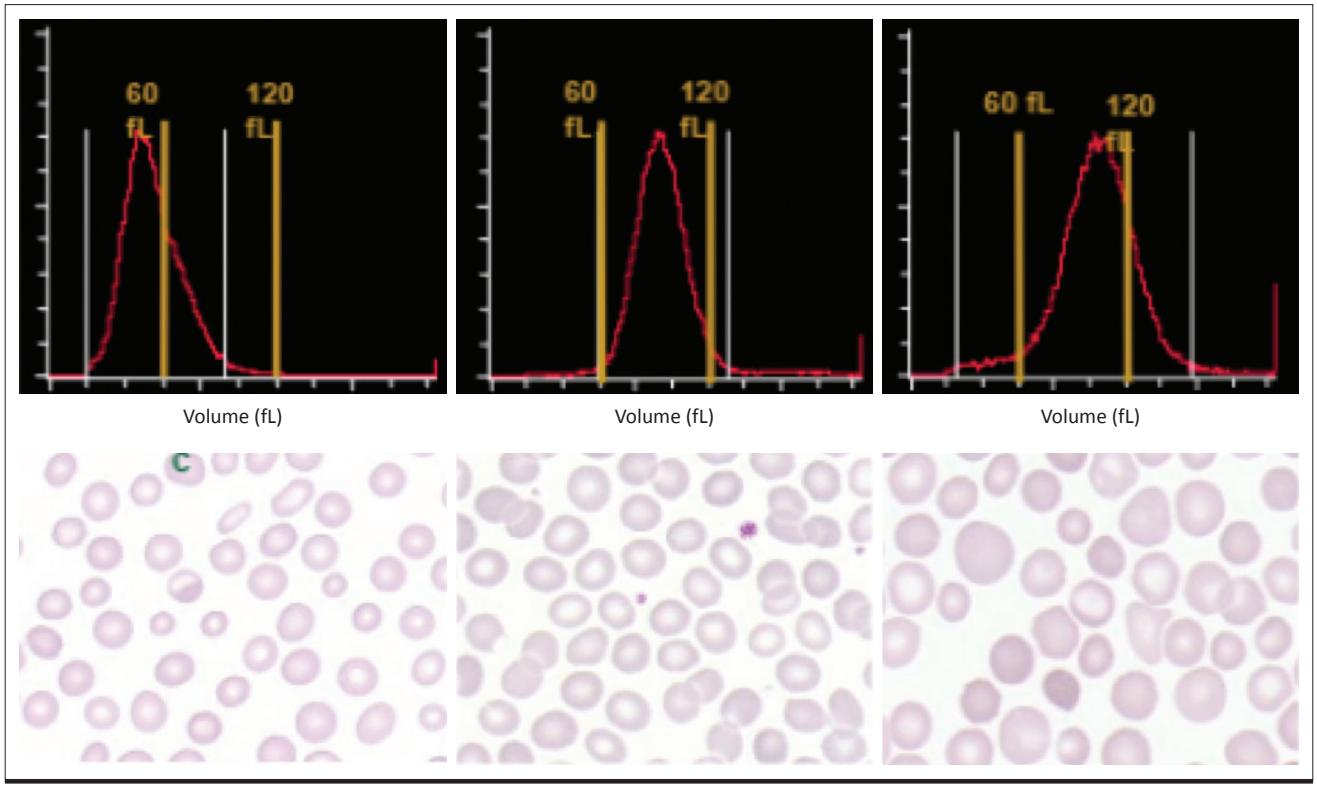

Figure 5. Demonstration of RBC sizes in histrogram and evaluation in peripheral blood smear

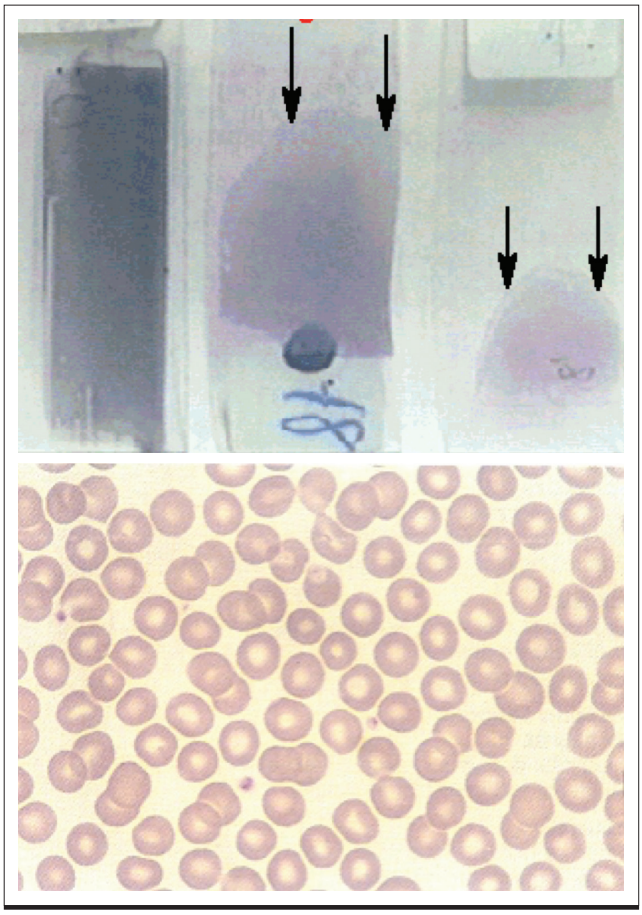

Figure 6. The areas where the RBCs fall one-by-one should be evaluated on a blood smear to be counted

with a mean number of 4–5.5 million. The presence of different sizes of red cells is called anisocytosis, the presence of red cells that are larger than normal size (9 μm) is called macrosytosis, and the presence of red cells with smaller than normal size (6 μm) is called microcytosis. This assessment can be made in the following way: if red cells are larger than normal lymphocytes, this is called macrocytosis, and if the red cells are smaller than normal lymphocytes, this is called microcytosis. If red cells with different shapes are found together, this is called poikilocytosis (Fig. 5). Examination of RBCs gains importance in patients with anemia in particular. Counting should be performed in areas where red cells are found one by one; the most eligible area is just the inernal part of the border where blood smear ends on the slide. The morphologic changes and sizes of red cells should be carefully examined (Fig. 5–7). If morphologic modification is suspected, this anomaly should be detected and confirmed in different regions of the smear and in different smears on different slides. Spherocytes, target cells, and stomatocytes may indicate an illness or may be caused by artifacts. If spherocytes are larger than normal red cells and lack central pallor, for example, these spherocytes are artifacts.

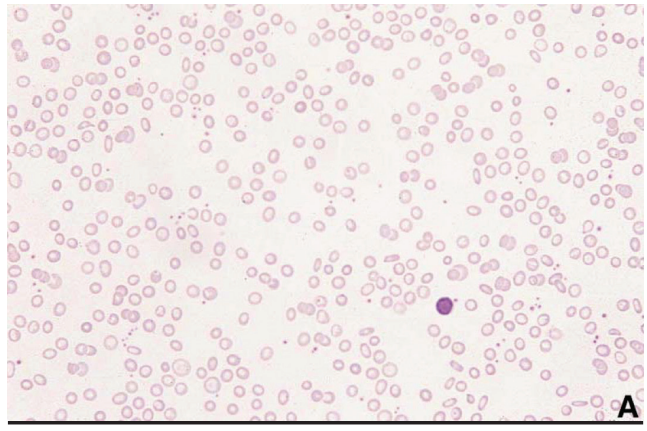

Figure 7. Presence of anisocytosis and poikilocytosis; hypochromic, microcytic RBCs

True spherocytes are formed as a result of a reduction in the surface/volume ratio. Generally, the border parts of smears may contain these types of red cells. Therefore, rather than the border areas, spherocytes observed in the middle areas of slides are important. There are illnesses that can be diagnosed only by recognizing red cell shapes. For example, the presence of schistocytes and helmet cells should suggest microangiopathic hemolytic anemia; the presence of spherocytes should suggest hereditary spherocytosis, ABO incompatibility or autoimmune hemolytic anemia; the presence of spiculated RBCs or akantocytes should suggest piruvate kinase deficiency; the presence of bite cells or blister cells should suggest glucose-6-phosphate dehydrogenase (G6PD) deficiency and mushroom-shaped red cells should suggest band 3 deficiency. Target cells are found in conditions such as iron deficiency anemia (IDA), hemoglobinopathy (C, D, E), thalassemia, and liver diseases, and following splenectomy. They are formed as a result of an increase in the red cell surface area/volume ratio. The colors of RBCs gain importance especially in conditions where the reticulocyte count test cannot be performed. Reticulocytes are precursors of RBCs (see the "reticulocyte" part). They appear a little more purple and larger as compared with RBCs. We can encounter these data as hemoglobin concentration distribution width (HDW) in some new blood counter devices.

In a patient with anemia, normoblasts, which are precursors of RBCs, must be investigated when evaluating RBCs. The presence of normoblasts on a peripheral smear is a very important finding in terms of reflecting hemolytic anemia or the active status of bone marrow following bleeding. Sometimes, normoblasts may appear in the peripheral blood together with blasts in conditions where the bone marrow is active, and rarely in leukemia. Attention should also be paid to abnormal formations in red cells in addition to their shapes, colors, and sizes. Intracellular parasites are found in malaria and extracellular par-

Table 2. Mean values and lower limits of the hematologic parameters by age and sex according to Dallman criteria

	Hemoglobin (g/dL)		Hemotocrit (%)		MCV (fL)	
	Mean	-2SD	Mean	-2SD	Mean	-2SD
Age						
1–5 days					108	**95**
2 months					96	**77**
0,5–2	12.5	11	37	33	77	**70**
2–512.5	11	38	34	79	73	
5–913	11.5	39	35	81	75	
9–12	13.5	12	40	36	83	76
12–14						
Female	13.5	12	41	36	85	**78**
Male	14	12.5	43	37	84	77
14–18						
Female	14	12	41	36	87	79
Male	15	13	46	38	86	78

MCV: Mean corpuscular volume

asites are found in trypanosomiasis and filariasis. Howell-Jolly bodies are nuclear remnants and indicate splenic hypofunction. Basophilic stippling indicates ribozomal remnants in the RBCs, and this is found in conditions such as thalassemia and lead poisoning. Rouleaux formation indicates presence of plasma protein, which causes elimination of the negative electrical charge on the red cell surface; it is generally found in association with increased erythrocyte sedimentation rate (ESR), and may be observed in mycoplasma infections.

Hemoglobin (Hb)

Hemoglobin is measured photometrically using the cyanmethemoglobin method. As it is a parameter based on measurement, it gives more accurate results in diagnosing anemia compared with the hematocrit value, which is calculated using two different parameters with automated devices. The upper and lower limits vary by age (Table 2, 3).

Hematocrit

When blood samples mixed with anticoagulant rae centrifuged, the ratio of the red cells to the whole blood volume is called hematocrit percentage (%). It can be measured manually or with a CBC device. Manually obtained values are higher because plasma is trapped between red cells during the centrifugation process. If the percentages of sickle cells or spherocytes are increased among red cells and reticulocytes, however, Hct will be found erroneously higher because the trapped plasma will be increased. Nevertheless, the microhematocrit centrifugation method is the most reliable one among the manual methods.

$$\text{Htc: } \frac{\text{RBC (mil/µL)xMCV (fL)}}{10}$$

Table 3. Classification of anemias by MCV and RDW (anemias with increased RDW and decreased or increased MCV can be remembered easily as nutritional anemias)

Anemia	MCV low	MCV normal	MCV increased
Normal RDW	Homogenously microcytic	Homogenously normocytic	Homogeneously macrocytic
	Thalassemia	Normal	Aplastic anemia
	Chronic disease	Chronic disease	Preleukemia
		Chronic liver disease	Hypothyroidism
		Chemotherapy	
		Non-anemic hemoglobinopathy	
		Chronic myelocytic leukemia	
		Bleeding	
		Hereditary spherocytosis	
Increased RDW	Heterogenously microcytic	Heterogeneously normocytic	Heterogeneously macrocytic
	Iron deficiency	Early-phase of iron and folate deficiency	Folate and viramin B12 deficiency
	S Beta thalassemia	Hemoglobinopathies (SS, SC)	Immune hemolytic anemia
	Hemoglobin H disease	Myelofibrosis	Presence of cold agglutinin
	RBC destruction	Sideroblastic anemia	

Nutritional anemias

MCV: Mean corpuscular volume; RDW: Red blood cel distribution width

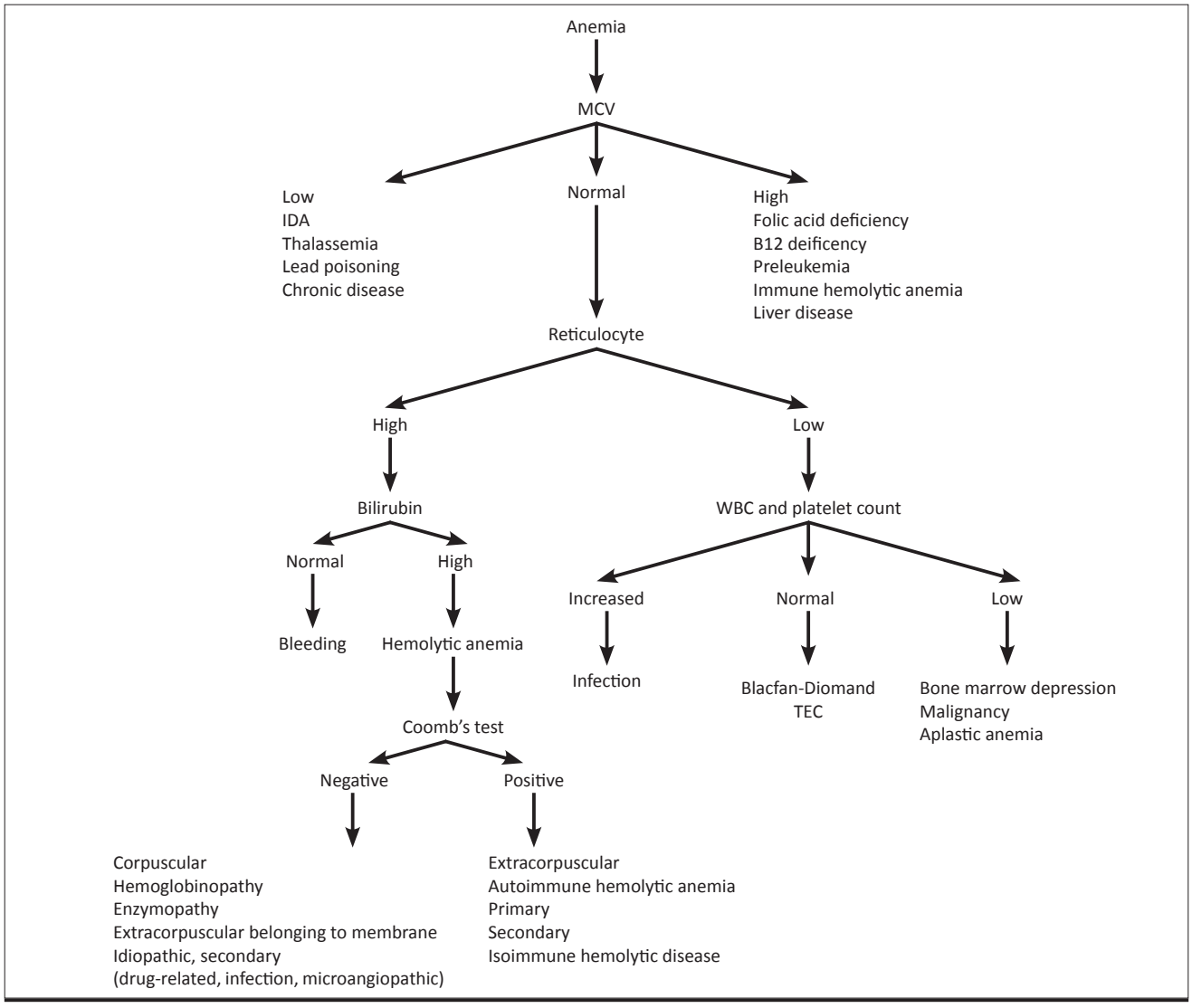

Figure 8. Differential diagnosis of anemia

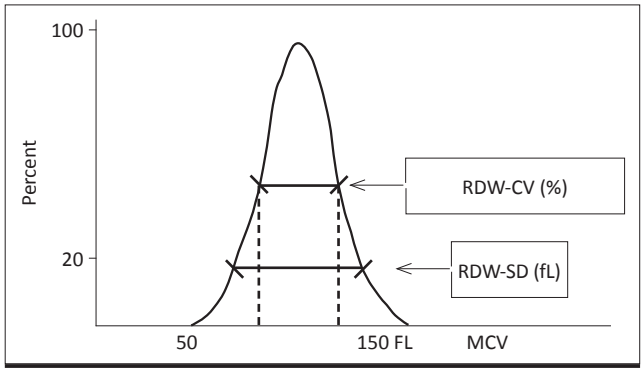

Figure 9. Appearance of two different ways of using RDW in histogram

Mean corpuscular volume

Mean corpuscular volume indicates the mean volume of an RBC. In childhood, it gradually increases by age. The normal values in adults range between 80 and 100 fL.

MCV can be calculated with the following formula:
MCV: hematocrit (%)x10
Red cell count (in millions).

The result is expressed in femtoliters (fL).
In children aged below 1 year:
70+age (years)= the lower normal limit of MCV
84+0.6 x age (years)= the upper normal limit of MCV.
In the classification of anemias, MCV is a very frequently used parameter (Table 3 and Fig. 8).

Microcytic anemias are generally associated with insufficient hemoglobin production. The Mentzer index may also be used when evaluating this type of anemia. MCV is divided by the red cell count to calculate this index; if the result is <12 the possibility of thalassemia is high, and if it is >13, the possibility of IDA is high. Many parameters can be used with this objective. However, none have been

Table 4. RBC parameters and formules used in the differentiation of thalassemia trait and iron deficiency (16)

MCV/RBC
MCVxRDW/RBC
MCVxMCVxMCH/100
MCH/RBC
MCVxMCVxRDW/Hbx100
MCV-RBC-(3xHb)
MCV-(10xRBC)
MCV-(5xHb)-RBC-3.4
RDW/RBC
(MCH/MCV)xRBC
MCH/MCV

MCV: Mean corpuscular volunme; RBC: RBC count; RDW: Red cell distribution width; MCHC: Red cell hemoglobin concentration

proven to be superior to another and Mentzer index is the most cathcy and most widely used one (Table 4).

Mean corpuscular hemoglobin (MCH)

Mean corpuscular hemoglobin indicates the mean amount of hemoglobin contained in an RBC. The normal value ranges between 30 and 34 picograms (pg). It is generally parallel to MCV. In other words, microcytic RBCs are mostly hypochromic.

MCH: $\frac{\text{Hb (g/dL)}}{\text{RBC (mil/μL)}}$ X 10

This parameter is not used widely in daily practice. However, MCV and MCHC vary with heat and over time. If reticulocytes are present, MCV increases and MCHC decreases; MCH is not influenced by these factors. MCH reaches a value with completion of Hb production during reticulocyte formation and becomes fixed. Therefore, it has started to be interpreted as the most valuable parameter. When evaluating anemia, watching for the compatibility of MCV and MCH is also important in addition to examining MCV and RDW (17). If MCV is incompatibly high, but MCH is low, for example in Hb E disease, Hb 'constant sparing' or vitamin B12-folic acid deficiency and simultaneous IDA or TT may be considered. If microcytic hyperchromic red cells are present, hereditary xerocytosis may be considered. In fact, reduced MCH in association with reduced MCV is the most frequent finding in TT and IDA. Increased MCH values (>32 pg) may be a finding of stress erythropoeisis, if laboratory calibration is accurate.

Mean corpuscular hemoglobin concentration (MCHC)

MCHC is an expression of hemoglobin contained in the RBC as a percentage. The amount of hemoglobin in a RBCs is generally 30–36%, independent of its size.

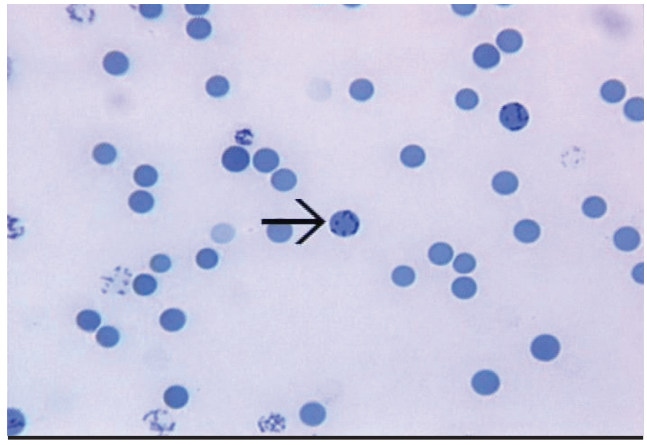

Figure 10. Demonstration of reticulocyte with supravital stain

Table 5. Normal reticulocyte values in children

Age	Reticulocyte (%)
1–3 days	3–7 (may be higher in anoxic and small preterms)
3–7 days	1–3
7 days-2 months	0–1
>2 months	0.5–1.5

This parameter is generally used as a control parameter for devices. It is one of the parameters that remains the most stable from the intrauterine life. If it is found to be increased (<36%) in association with increased RDW, the diagnosis is hereditary spherocytosis. However, MCHC may be falsely found to be increased in lipemic and icteric serum samples, and in the presence of cold agglutinin.

MCHC: $\frac{\text{Hb (g/dL)}}{\text{Htc (\%)}}$ X 100

Red cell distribution width (RDW)

Red cell distribution width is obtained from red cell histograms. It indicates anisocytosis. Before anemia emerges, increased RDW is found primarily. In practice, it is the most frequently used in the differentiation of IDA and TT. Red cell distribution width is normal in individuals with thalassemia trait, and is increased in IDA. It is calculated with two different methods (Fig. 9): the normal value ranges between 14.5% and 15% in the method that gives the result in pecentage, and the normal value is 45 fL in the other method. Red cell distribution width is found as 15–25 in IDA, 12–15 in TT, and 25–35 in thalassemia major. Our clinical experiences indicate that hereditary spherocytosis should be considered if RDW is 17–20 in association with increased MCHC. However, congenital diserythropoetic anemias (CDA) should be considered, if RDW is >20–25. In persistent severe microcytic anemias, sideroblastic anemia may be considered in the differential diagnosis, if RDW is >25.

Table 6. WBC distribution by age (11)

	Total WBC		Neutrophils			Lymphocytes			Monocytes		Eosinophils	
Age	Mean	Min.–Max.	Mean	Min.–Max.	%	Mean	Min.–Max.	%	Mean	%	Mean	%
Birth	18.1	9.0–30.0	11	6–26	61	5.5	2–11	31	1.1	6	0.4	2
12 hours	22.8	13.0–38.0	15.5	6–28	68	5.5	2–11	24	1.2	5	0.5	2
24 hours	18.9	9.4–34.0	11.5	5–21	61	5.8	2–11.5	31	1.1	6	0.5	2
1 week	12.2	5.0–21.0	5.5	1.5–10	45	5	2–17	41	1.1	9	0.5	4
2 week	11.4	5.0–20.0	4.5	1–9.5	40	5.5	2–17	48	1	9	0.4	3
1 month	10.8	5.0–19.5	3.8	1–9	35	6	2.5–16.5	56	0.7	7	0.3	3
6 months	11.9	6.0–17.5	3.8	1–8.5	32	7.3	4–13.5	61	0.6	5	0.3	3
1 year	11.4	6.0–17.5	3.5	1.5–8.5	31	7	4–10.5	61	0.6	5	0.3	3
2 years	10.6	6.0–17.0	3.5	1.5–8.5	33	6.3	3–9.5	59	0.5	5	0.3	3
4 years	9.1	5.5–15.5	3.8	1.5–8.5	42	4.5	2–8	50	0.5	5	0.3	3
6 years	8.5	5.0–14.5	4.3	1.5–8	51	3.5	1.5–7	42	0.4	5	0.2	3
8 years	8.3	4.5–13.5	4.4	1.5–8	53	3.3	1.5–6.8	39	0.4	4	0.2	2
10 years	8.1	4.5–13.5	4.4	1.8–8	54	3.1	1.5–6.5	38	0.4	4	0.2	2
16 years	7.8	4.5–13.0	4.4	1.8–8	57	2.8	1.2–5.2	35	0.4	5	0.2	3
21 years	7.4	4.5–11.0	4.4	1.8–7.7	59	2.5	1–4.8	34	0.3	4	0.2	3

Min.: Minimum; Max.: Maximum

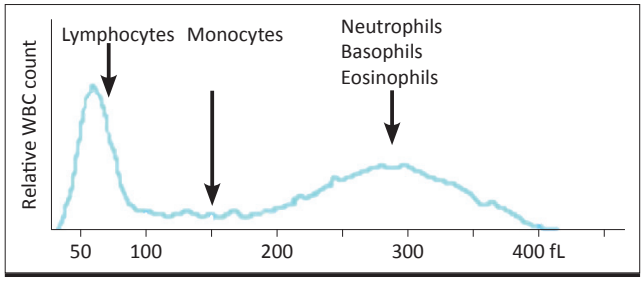

Figure 11. Histogram of WBCs

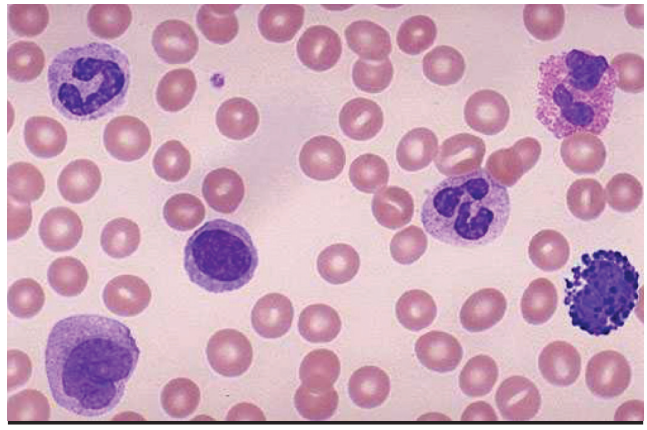

Figure 12. Presence of bands, neutrophils, lymphocytes, eosinophils, and basophils in peripheral blood smear

Reticulocyte

Reticulocyte is the final form of immature RBCs. When red cell precursors become reticulocytes, they spend 2 days in the bone marrow and circulate in the peripheral blood for a final 1–2 days before becoming mature red cells. Reticulocytes contain RNA remnants that can be stained with supravital stain (cresyl blue) in contrast to RBCs (Fig. 10). The reticulocyte count test can be performed using devices, or visually using a microscope. On microscopic counting, the reticulocytes found in the areas where 1000 RBCs are counted, are specified as percentage. Generally, ten 100x magnification areas where 100 RBCs are found, are selected, and the red cells in which nuclear remnants are observed, are counted in these ten areas; the average of these ten areas is calculated, and the reticulocyte percentage is found. When this percentage is multiplied by the red cell count, the absolute reticulocyte value is found. When the reticulocyte count test is performed using a nautomated counter, the result is obtained by counting at least 30 000 red cells (18). Reticulocytes must be examined in all cases of anemia because they give us information about bone marrow without performing bone marrow biopsy. If we roughly consider anemias as insufficient production or increased destruction, we can conclude that the bone marrow is well when the reticulocyte count is increased, and that there is a problem in the bone marrow or there is a deficiency in substances necessary for red cell production (nutritional?) when the reticulocyte count is low. The normal value is 1–2%. In patients with anemia, the corrected reticulocyte count should be celculated. The normal reticulocyte values in children are shown in Table 5. Corrected reticulocyte percentage:

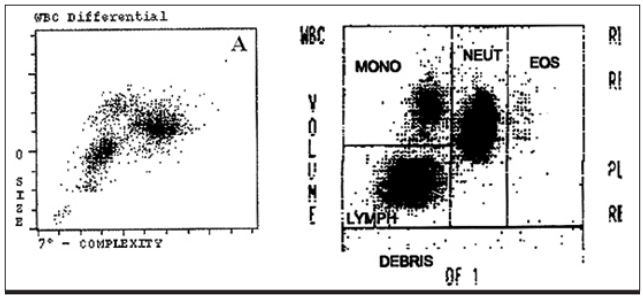

Figure 13. Localization of WBC subgroups on the X and Y axis in interpretation of histograms

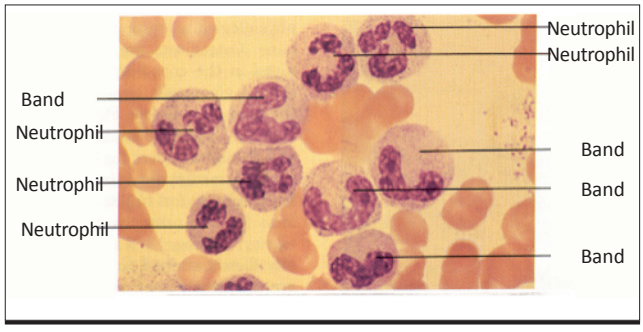

Figure 14. Bands and neutrophils

Ret. count x patient Hct/45

In addition, the absolute reticulocyte count should also be calculated. The normal value for the absolute reticulocyte count is 25 000–75 000/μL. A reticulocyte value of 1.5–2% or >80 000–100 000/mm^3 indicates reticulocytosis. In the presence of reticulocytosis, hemolysis or hemorrhage should be investigated primarily, and a bilirubin test should be requested for this objective. Generally, hemolysis should be investigated if the bilirubin level is increased, and hemorrhage should be investigated if the bilirubin level is normal.

Recently, reticulocyte precursors [immature reticulocyte fraction (IRF)] have started being expressed in some hemograms. An IRF value of >0.23 can be considered high. This value is an early marker indicating that there will be an increase in hemoglobin in a short time due to reticulocytes. It is most commonly used as an indicator of engraftment following stem cell transplantation (19).

2. White blood cells

The WBC count is generally 4500–11 000/μL (Table 6). Although an increase in WBC count is found to be associated with infection, factors such as age, body temperature, exercise and having seizure influence the WBC count. For example, values as high as 38 000 can be obtained after birth (Table 6). On the most left side in the WBC histogram, lymphocytes are distributed between 50 and 100 fL, monocytes, eosinophils and basophils are distributed between 100 and 200 fL, and neutrophils are distributed between

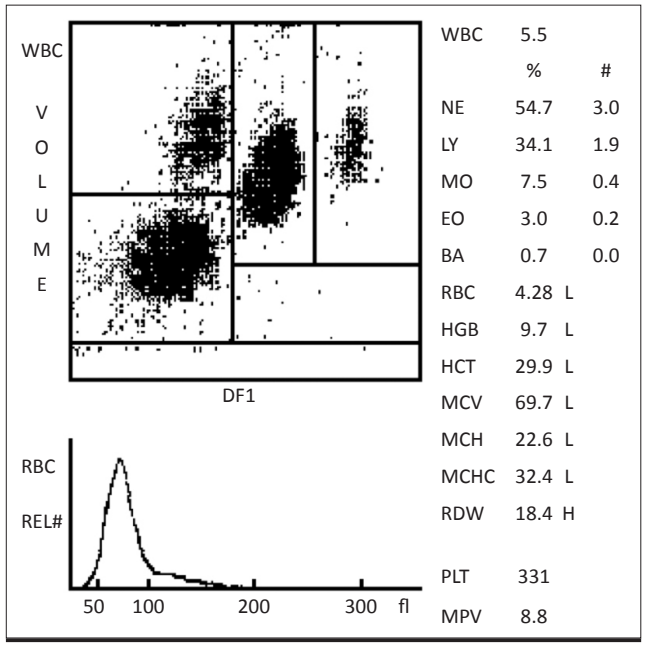

Figure 15. Hemogram printout of case 1

200 and 400 fL (Fig. 11). Physicians should get into the habit of examining histograms when examining WBC counts. A left shift of the lymphocyte peak supports the presence of probable normoblasts or clusters of platelets. When the WBC differential is not normal in automated CBCs (blast, immature granulocyte, left shift, abnormal lymphocyte), a warning is given. One hundred WBCs are counted and their types are given as percentages. Ordinarily, WBC subtypes are easily recognized on peripheral blood smears (Fig. 12). Generally, the values are also given as percentages together with counts. In the first 4–5 days, neutrophils are observed with a high rate on peripheral blood smears, similar to adulthood. Between four days and 4 years of age, when viral infections occur with a high rate, lymphocytes predominate (rule of four of hematology). Generally, neutrophilia may be present in the period before clinical findings develop in bacterial and viral infections.

Leukocytosis is an acute-phase response of the body against infection. For example, a WBC count of 15 000/μL in children with fever aged between 3 and 36 months is compatible with bacteriemia with a rate of 16%, and this percentage increases to 25% and 40%, respectively, when the WBC count reaches 20 000/μL and 30 000/μL.

Generally, histograms are also found below the data on CBC reports. Histograms are graphical representations in which WBC subgroups are listed by size on the Y axis and by granules on the X axis. Because lymphocytes are the smallest cells and contain the lowest granule counts, they are located in the lowest left area; above it, monocytes, which are larger in size, but contain few granules, are located. Granulocytes, which are larger in size and

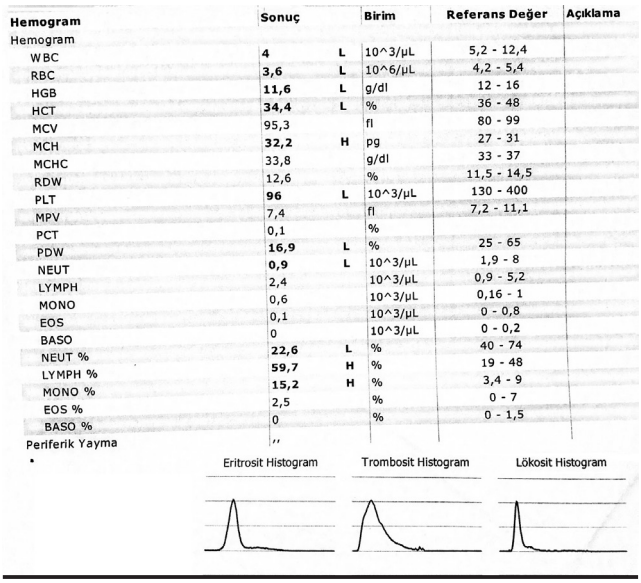

Figure 16. Hemogram printout of case 2

contain more granules, are located towards the right side. Eosinophils, which are both large and contain the highest number of granules, are located on the right of the granulocytes or polymorphonuclear leukocytes (PNLs) (Fig. 13).

The younger forms of neutrophils are called bands or stabs. Normally, this percentage constitutes less than 6% of all WBCs. After the neonatal period, a band cell count of >500/µL is considered to be associated with infection. In the neonatal period, the band/neutrophil ratio is frequently calculated in the differential diagnosis of sepsis. A shift to the left means that the number of these younger neutrophils has increased. If the ratio of the thinnest part to the thickest part in the nuclear structure inside the neutrophil is less than one-third, this cell is called band (Fig. 14). If this ratio is higher, the lobes in the nucleus are counted. Most lobes inside a WBC have three pieces. If there is a PNL with a lobe number of six, or if the percentage of PNLs with a lobe number higher than five is higher than 5%, this is called hypersegmentation. The most important causes of hypersegmentation are folic acid deficiency and vitamin B 12 deficiency, which also lead to megaloblastic anemia. If the number of WBCs with a lobe number less than two is high, Pelger-Huet anomaly or pseudo Pelger-Huet anomaly is mentioned.

When the ratio calculated as percentage on peripheral blood smear is multiplied by the WBC count, the absolute neutrophil count (ANC) is calculated. For example, the ANC is obtained when the WBC count found while assessing febrile neutropenia in patients who receive chemotherapy is multiplied by the neutrophil percentage found on a peripheral blood smear. For example, a patient with a PNL value of 2% on peripheral smear and a WBC count of 4000 µL has an ANC value of 80. If the ANC is <500/mm^3 or <1000 and tends to reduce, there is a risk in terms of bacteriemia. This is very important in patients who have received chemotherapy. When neutrophils decrease in the blood, monocytes and eosinophils that have the capacity for phagocytosis, increase. Eosinophilia and an increase in monocytes will be observed on a peripheral blood smear in a patient who has chronic neutropenia; the reason for lower-than-expected rates of infection in these patients is the fact that the total number of monocytes and neutrophils [absolute phagocytic count (APC)] is generally >500–1000/mm^3.

Leukopenia, as well as leukocytosis, may indicate the presence of an infection. Generally, leukopenia starts 1–2 days before the onset of viral infection and may last for weeks. It is most commonly found during infections with salmonella, staphylococci, and mycobacteriae.

Toxic granulation and the presence of vacuoles and Döhle bodies in neutrophils are also peripheral blood smear findings that support infection. Generally, toxic granulation is typical for Gram-positive infections, and left shift (increase in the percentage of bands on smear) is typical for more rapidly progressing Gram- negative infections. Especially, a band/neutrophil ratio of >0.18–0.20 is known as a finding of early sepsis including mainly Gram-negative bacteria in newborns. Blue cytoplasmic remnants in WBCs are called Döhle bodies, and are generally found in conditions such as severe infection, burns, pregnancy, and May-Hegglin anomaly. Dark granules in WBCs may be observed in Alder-Reilly anomaly, Chediak-Higashi syndrome, and mucopolysaccharidosis.

Eosinophilia is mostly associated with skin eruption, wheezing, allergic condition and parasitosis. Eosinophilia is present if the percentage of eosinophils is above 6–8% on a peripheral blood smear.

Lymphocytosis generally occurs in viral infections. If the lymphocyte count is >30 000/µL and the percentage of lymphocytes is 60–70%, pertussis should also be considered in the differential diagnosis.

Reactive lymphocytes generally have a 4–5-fold higher size compared with normal lymphocytes; they have a fragile structure with a dark and sometimes light cytoplasma, and may be confused with blasts by inexperienced physicians. The presence of more than 10% reactive lymphocytes on a peripheral blood smear supports Epstein-Barr virus (EBV), cytomegalovirus (CMV), and toxoplasmosis. They are most commonly confused with monocytes. To be more remindful, monocytes push the cells around and stand one by one (mono), but reactive lymphocytes are generally surrounded by RBCs, and their cytoplasm shapes are changed with the pressure of RBCs.

Leukoblastic reaction is the presence of early RBC and WBC precursors on peripheral blood smears; it may be found in leukemia, myelofibrosis, severe hemorrhage, and hemolysis.

The term 'leukemoid reaction' describes a WBC count above 50 000/μL and is used to define conditions other than leukemia.

We have mentioned that normoblasts are erroneously counted as lymphocytes on peripheral blood smears because they are nucleated cells. Fifty-eighty percent of WBCs may be normoblasts, especially in preterm babies (<24–25 gestational weeks) and in anoxic babies. In this case, 100/100+100 WBCs will be counted, and the normoblast value found, which is multiplied by the WBC value found in the CBC in order to find the actual WBC count.

A pediatrician should form the habit of noting the absolute values of both neutrophils and lymphocytes for each CBC even if the WBC count is within normal limits. A neutrophil value below 1000/mm^3 in a patient aged under one year and a neutrophil value below 1500/mm^3 aged over one year is considered neutropenia. The neutrophil value must be examined in patients with recurring nail bed infections, skin infections, and oral ulcers. Again, an absolute lymphocyte count below 3000/mm^3 may be a clue for an undiagnosed immune deficiency.

A peripheral blood smear must be examined in cases with CBCs showing higher-then-expected monocyte percentages considering that they may be blasts, because blasts appear like monocytes on peripheral blood smears.

3. Platelets

Platelets are the smallest formed elements in peripheral blood. They constitute the blood parameter that does not change by age in children. They have a size of 7–11 fL and a diameter of 1–3 μm. They are made of cytoplasmic fragments and do not contain a cell nucleus. Generally, they are found to be larger if destruction in the periphery is increased. In cases of bone marrow production disorder, however, they are found to be smaller. In Wiskott-Aldrich syndrome, they are found to have almost half the normal size, and they look like dust particles. It is generally thought that large Plts are young Plts. This is true in one sense, but increased MPV and increased numbers of large Plts, are also the findings of increased thrombopoesis. The normal life span of Plts is 7–10 days. Under normal conditions, one-third of Plts are found in the spleen. An approximate number of Plts may be estimated by multiplying the average number per field on microscopic examination at x100 magnification by 15–20 000. In individuals with anemia, however, this method is not very reliable (20). In daily life, problems related to the number and function of Plts are generally experienced. Complete blood counts do not give information in terms of Plt functions. However, anomalies in the number of Plts have started being confronted very frequently with the introduction of CBCs into routine examinations. A Plt count below 150 000/μL is called thrombocytopenia and a Plt count above 600 000/μL is called thrombocytosis. As subjects who have a Plt count of 100–150 000/mm^3 but who do not have symptoms have been found in recent years, individuals with a Plt count below 150 000/mm^3 should be monitored closely, and physicians should decide if this is a finding of an illness or normal blood count. Although thrombocytosis is frequently observed in childhood, it rarely becomes a problem, because polycytemia vera and essential thrombocytosis occur very rarely, and have a more favourable prognosis compared with adulthood. The majority of pediatric thrombocytosis cases are reactive. Infections are the most common cause. Platelets increase as an acute-phase reaction. In addition, IDA, hemolytic anemias, vitamin E deficiency, hemorrhages, collagen tissue diseases, Kawasaki disease (generally 2–3 weeks before clinical findings), nephrotic syndrome, inflammatory bowel diseases, postoperative recovery period, trauma, tumors (hepatoblastome and neuroblastoma), histiocytosis, myeloproliferative diseases and various drugs (e.g. epinephrine, corticosteroids, vinka alkaloids) may lead to thrombocytosis. In childhood, treatment is generally not necessary. Platelet morphology and bleeding time are generally normal. Splenomegaly is mostly absent and recovers in a short time. Thrombocytosis is also found in the presence of functional or anatomic asplenia, because one-third of Plts are normally stored in the spleen. Thus, the number of Plts decrease in the periphery without a reduction in the total Plt count, because the number of Plts stored in the spleen will increase in conditions where the spleen is enlarged; this is called thrombocytopenia associated with hypersplenism. In this group of patients, the Plt count is generally found as 20 000–80 000/mm^3. When thrombocytopenia is detected, it should be primarily noted if thrombocytopenia is isolated or other cells (RBCs and WBCs) are also involved. In the presence of pancytopenia in which all three series are involved, the condition generally originates from the bone marrow. However, it should be kept in mind that vitamin B12 deficiency may also be a cause and MCV and hypersegmentation (one neutrophil with six lobes or more than five lobes in more than 5% of neutrophils) on peripheral blood smears should be investigated. If findings of infection are not found in the presence of hepatosplenomegaly and lymphadenomegaly in association with thrombocytopenia, the most common cause is leukemia [acute lymphoblastic leukemia (ALL) or acute myeloblastic leukemia (AML)]. In the presence of thrombocytopenia without hepatosplenomegaly and lymphadenomegaly, the most probable diagnosis is immune thrombocytopenia (ITP) if there is a history of viral infection or vaccination in the last 10–15 days. However, the

accuracy of the Plt count should be investigated primarily; pseudothrombocytopenia may occur as a result of Plt coagulation in the presence of insufficient anticoagulant or interaction with antibody in the plasma when EDTA is used as anticoagulant, especially when clinical findings (petechiae, ecchymoses) are absent. Under these circumstances, the best way to solve this problem is to evaluate the Plt clusters and Plt count on blood smears prepared directly from a drop of finger-stick. When the Plt count is reduced below 80 000/μL, the bleeding time is found above 9 minutes. However, intracranial hemorrhage, which is generally fearsome in patients with thrombocytopenia, develops at a Plt level below 20 000/μL. In these cases, MPV is directive. Mean platelet volume should be examined in all patients with thrombocytopenia. It has been stated that the risk of hemorrhage is lower, if MPV is >8 fL. Familial macrothrombocyte disorders should be considered if the MPV value is >11 fL. In particular, the presence of any family member with early-onset cataract, hearing loss, and renal failure should be interrogated. Some studies emphasized that the MPV values were associated with prognosis, especially in patients hospitalized in intensive care units, and in some patients with cardiac disease (21). It should be kept in mind that use of any drug may lead to thrombocytopenia, albeit much more rarely in children. In clinical practice, we observe that most commonly antiepileptic drugs cause thrombocytopenia aside from chemotherapeutic drugs. When the drug is discontinued, the Plt count generally returns to normal in 2–4 weeks. However, in our clinical experience, we have had patients who had permanent or long-term (8–9 months) thrombocytopenia. Recently, some blood cell counting devices have started to give platelet large cell ratios (Plcr). If this value is high, especially in the thrombocytopenia recovery period, it means that the Plt count will increase in a short time.

Keep in mind:

1. **Are the patient's name and CBC compatible?**
2. **Is there an unexpected finding? If you have suspicion, repeat CBC!**
3. **Is the blood sample less or more than normal, was the blood sample obtained with difficulty, is there coagulum inside the tube?**
4. **Do not evaluate hemogram before examining peripheral blood smear, if possible.**
5. **If thrombocytopenia is present, prepare blood smear directly from a drop of finger-stick and examine.**
6. **Can the anomaly you observed on a smear be found on all smears? Prepare new smears and reevaluate.**
7. **"Eine is keine": Do not pursue anomalies that you have observed in only a single area.**

Let us interpret and evaluate two hemogram printouts in accordance with the above-mentioned points:

Case 1: Complete blood count and histogram of a patient with anemia

Case 1: The CBC of an 8-year-old male patient who presented with symptoms of falling ill frequently and tiring quickly, is shown in Figure 15. Anemia, and especially microcytic anemia, is not expected in an 8-year-old patient. Therefore, a detailed nutritional and socioeconomic level history should be obtained. As the RBC value is >5 million, the patient's hometown should be interrogated in terms of TT. The patient is aged 8 years, so his MCV value should be at least 78 fL. However, it is found as 69 fL; microcytic anemias should be considered in the differential diagnosis. When we find the MCV to be low, the second red cell parameter to be examined is RDW. An RDW value of >15 in association with low MCV most probably supports IDA. As IDA is most commonly confused with TT, the Mentzer index will be directive: 69.7/4.28=16.28, which is >13; this should suggest IDA. If the nutritional history is good, however, conditions that could lead to chronic blood loss or absorption disorder (e.g. parasitosis, anal fissure, celiac disease, chronic renal disease, rheumatic disease should be investigated. Here, the patient's percentile values and growth and developmental status will be a clue. Pediatricians are physicians of measurements: they should initiate examinations with measurements such as body weight, height, head circumference and abdominal circumference, chest circumference, and arm span, when necessary, for each patient. It would be appropriate to order iron, total iron binding capacity, and ferritin tests for this patient to support our diagnosis. Ferritin is an acute-phase reactant and may not always give us clear information because infection occurs commonly in children. If we find ferritin to be increased in such patients in whom hypochromic microcytic anemia is observed on peripheral blood smear, the iron/total iron binding capacity ratio may be helpful. This ratio is called the transferrin saturation index, and an index value of <15% is a strong finding for IDA (generally, blood iron level is <60 mg/dL and total iron binding capacity is >400 μg/dL in IDA). The other point to be noted in this hemogram, is the finding that the lymphocyte count is <3000. In fact, children usually present to hospital because of infections. This patient also had a history of frequent infections, though it is not very correct to make an evaluation according to a single count. Thus, this lymphocyte count should be considered and the patient should also be examined immunologically if low lymphocyte values persist during follow-up of treatment for anemia. The habit of noting neutrophil and lymphocyte values in each CBC should be adopted.

Case 2: A 4-year-old female patient had been followed up for 1 year in our Well-Child Outpatient Clinic because of borderline thrombocytopenia. The Plt count ranged be-

tween 87 000 and 110 000/mm^3. The patient had no history of active bleeding. She only had eccyhmoses below the knee. Her Plt count is 96 000/mm^3 (Fig. 16). The status of the other cells should primarily be evaluated if we are sure of the pre-test steps (obtaining blood sample, counting and waiting). In other words, how is WBC and Hb? The Hb level (11.6 g/dL) is good for the patient's age, but the WBC value is 4000, which is the lower limit. Thus, we can see that the patient has thrombocytopenia, but she actually has bicytopenia. She does not have anemia, but the MCV value, which is a very important parameter, is 95.3 fL. The patient is aged 4 years; the lower limit is 74 and the upper limit is 84+age(4) x 0.6=86.4 fL. This shows us a macrocytic status. How is RDW? Could it be vitamin B 12 deficiency? The RDW is 12.6 which is normal. In this case, bone marrow failure should be considered if we are not considering hypothyroidism in the differential diagnosis. Stress-induced hematopoesis occurs in bone marrow failure. Here, the body tries to return to its status in the intrauterine period, fetal cells and thus HbF production increase, and MCV increases. Fanconi anemia may be considered in the differential diagnosis for this patient. Thus, the presence of skin findings such as hyperpigmentation, hypopigmentation and cafe-au-lait spots, pointed chin, typical eye findings, and duplicated collecting system on abdominal ultrasonography support our diagnosis of Fanconi anemia, and a DEB test and genetic testing for Fanconi anemia are requested. The diagnosis of thrombocytopenia was made by evaluating the entire CBC. Again, another clue here is the MPV value, which we should note in each case of thrombocytopenia; an MPV value of <8 fL indicates deficiency of production and a MPV value of >8–10 fl indicates increased destruction.

Hakem Değerlendirmesi: Dış bağımsız.

Çıkar Çatışması: Yazar çıkar çatışması bildirmemiştir.

Mali Destek: Yazar bu çalışma için mali destek almadığını beyan etmiştir.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The author have no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

Kaynaklar

1. Bennett ST, Lehman CM, Rodgers GM. Laboratory hemostasis. 2nd ed. Heidelberg: Springer; 2015.p.68-108.
2. Loscalzo J, Schafer AI. Thrombosis and Hemorrhage. 3rd ed. Lippincott Williams-Wilkins; 2003.p.315–30.
3. Beutler E, Lichtman MA, Coller BS, Kipps TJ, Seligsohn U. Hematology. 6th ed. McGraw Hill; 2001.p.1471.
4. Jacobs DS, DeMott WR, Oxley DK. Laboratory Test Handbook. 5th ed. Lexi-Comp; 2001.p.326–58.
5. Desai SP, Isa-Pratt S. Clinician's guide to Laboratory Medicine: A Practical Approach. 2nd ed. Lexi-Comp; 2002.
6. Murphy MF, Pamphilon DH, Heddle NM. Practical Transfusion Medicine. Oxford, England: Wiley-Blackwell Science Ltd; 2001.
7. Mazza JJ. Manual of clinical hematology. 3rd ed. Philadelphia, USA: Lippincott Williams&Wilkins; 2002.
8. Rodak BF. Diagnostic hematology. 1st ed. Philadelphia: WB Saunders Company Pennsylvania; 1996.
9. Lanzkowsky P. Manual of pediatric hematology and oncology. 6th ed. San Diego: Academic Press; 2016.
10. Jakubik LD, Cockerham J, Altmann AR, Grossman MB. The ABCs of pediatric laboratory interpretation: understanding the CBC with differential and LFTs. Pediatr Nurs 2003; 29: 97–103.
11. Buttarello M, Plebani M. Automated blood cell counts: state of the art. Am J Clin Pathol 2008; 130: 104–16.
12. Martin SA. The ABCs of pediatric LFTs. Pediatr Nurs 1992; 18: 445–9.
13. Buttarello M. Quality specification in haematology: the automated blood cell count. Clin Chim Acta 2004; 346: 45–54.
14. Mahmoodi M, Hajizadeh M, Rashidinejad H, et al. Survey of changes in complete blood count and red cell indices of whole blood incubated in vitro at different temperatures up to 48 hours. J Ayub Med Coll Abbottabad 2006; 18: 14–6.
15. Bain BJ. Diagnosis from the blood smear. N Engl J Med 2005; 353: 498–507.
16. Vehapoglu A, Ozgurhan G, Demir AD, et al. Hematological indices for differential diagnosis of Beta thalassemia trait and iron deficiency anemia. Anemia 2014; 2014: 576738.
17. Simel DL. Is the RDW-MCV classification of anaemia useful? Clin Lab Haematol 1987; 9: 349–59.
18. d'Onofrio G, Chirillo R, Zini G, Caenaro G, Tommasi M, Micciulli G. Simultaneous measurement of reticulocyte and red blood cell indices in healthy subjects and patients with microcytic and macrocytic anemia. Blood 1995; 85: 818–23.
19. Davis BH. Immature reticulocyte fraction (IRF): by any name, a useful clinical parameter of erythropoietic activity. Lab Hematol 1996; 2: 2–8.
20. Segal HC, Briggs C, Kunka S, et al. Accuracy of platelet counting haematology analysers in severe thrombocytopenia and potential impact on platelet transfusion. Br J Haematol 2005; 128: 520–5.
21. Endler G, Klimesch A, Sunder-Plassmann H, et al. Mean platelet volume is an independent risk factor for myocardial infarction but not for coronary artery disease. Br J Haematol 2002; 117: 399–404.