



Evaluation of leucocytes and differentiation

Leucocyte number

Reference 4.0-10.0 G/l

Neutrophile total (band+ seg)

Reference 1.40-8.00 G/l

Neutro. tot. ↑ Neutrophilie
Neutro. tot. ↓ Neutropenia

Neutro. tot < 0.5 G/l = severe neutropenia. The complete absence of neutrophils is called agranulocytosis.

Neutrophils band

Reference: < 25% of the neutrophils

Bands ↑ Left-shift

Eosinophils

Reference 0.00-0.70 G/l

Eosinophils ↑ Eosinophilia

Basophils

Reference 0.00-0.15 G/l

Basophils ↑ Basophilia

Monocytes

Reference 0.16-0.95 G/l

Monocytes ↑ Monocytosis
Monocytes ↓ Monopenia

Lymphocytes

Reference 1.50-4.00 G/l

Lymphocytes ↑ Lymphocytosis
Lymphocytes ↓ Lymphopenia

Introduction

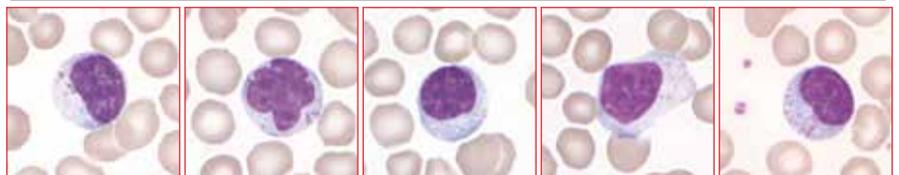
Microscopic examination of the white blood count is important even today in the age of automated hematology analysis (hematogram). Microscopy provides information on the percentage of individual cell populations, some of which are not separately counted by the Hematology Analyzer or not reported as numbers (e.g. rod-shaped polynuclear neutrophils, precursors of granulopoiesis etc.). Likewise, morphological features of cells can be seen by microscopic examination that provides important information regarding reactive events (e.g. infections) or neoplastic processes (e.g., leukemia). This information completes the picture of findings for the attending physician and frequently contributes to conduct further, targeted diagnostics.

Our current focus shows the step-wise generation of the white blood count findings using as example the proficiency testing survey specimen MQZH 2014-4 H3b. The blood picture is from a 54-year-old woman with Large Granular Lymphocyte (LGL) leukemia.

1.1. Evaluating the hematogram and leukocyte differentiation

1.1 Blood Count					
Leukocytes	12.07 ↑	4.0-10.0 G/l	MCV	85.2	81-100 fl
Thrombocytes	294	150-450 G/l	MCH	28.6	28.0-34.0 pg
Erythrocytes	4.49	f 4.2-5.4 T/l m 4.5-6.3 T/l	MCHC	336	300-360 g/l
Hemoglobin	129	f 120-160 g/l m 140-180 g/l	RDW	13.9	< 16 %
Hematocrit	0.38	f 0.37-0.47 l/l m 0.42-0.52 l/l			

1.2 Leukocyte differentiation					
First conduct the automated measurement; with indications of pathological findings or with specific questions, microscopic differentiation is carried out.					
automate (5 Part Diff)			microscopic		
Neutrophils total.#	1.64	1.40-8.00 G/l	Neutrophils total#	1.81	1.40-8.00 G/l
Neutrophils total%	14 ↓	40.0-74.0 %	Neutrophils total%	15.0 ↓	40.0-74.0 %
			Neutrophile band	2.0	0-20 %
			Neutrophile seg	13.0 ↓	30-50 %
Eosinophils #	0.04	0.00-0.70 G/l	Eosinophils #	0.06	0.00-0.70 G/l
Eosinophils %	0.5	0-7%	Eosinophils %	0.5	0-7%
Basophils #	0.07	0.00-0.15 G/l	Basophils #	0.18	0.00-0.15 G/l
Basophils %	1.0	0.00-1.5 %	Basophils %	1.5	0.00-1.5 %
Monocytes #	0.26	0.16-0.95 G/l	Monocytes #	0.48	0.16-0.95 G/l
Monocytes %	2.5	3-9%	Monocytes %	4.0	3-9%
Lymphocytes #	9.78 ↑	1.50-4.00 G/l	Lymphocytes #	9.54 ↑	1.50-4.00 G/l
Lymphocytes %	82.0 ↑	19.0-48.0 %	Lymphocytes %	79.0 ↑	19.0-48.0 %



1.3 Interpretation and evaluation of the leukocyte morphology	
Quantitative findings	<input checked="" type="checkbox"/> leukocytosis <input checked="" type="checkbox"/> lymphocytosis (relative % and absolute #) <input checked="" type="checkbox"/> Neutropenia (relative%) but not absolute!
Quantitative findings (Morphology)	Neutrophil G: granulation fine lymphocytes: typical +++ atypical - Comment: Around 65% of typical lymphocytes are larger lymphocytes with wider cytoplasm margins and sometimes coarse azur granulation (LGL cells). <input type="checkbox"/> presumably reactive <input checked="" type="checkbox"/> presumably neoplastic: proportion LGL cells > 10%



Evaluation of cell morphology

The evaluation of leukocyte morphology is performed while counting the leukocyte subtypes.

For this purpose a place on the smear is selected, where the erythrocytes are isolated. The central groove should be visible.

In each lymphocyte, both a definite nucleus as well cytoplasm border should be visible. If this is not the case, cell autolysis is present. These cells must not be counted in the differential blood count nor assessed morphologically.

Morphological changes in cells are semi-quantitatively assessed by the frequency of their occurrence.

3-10%	Low, discreet, few	+
10-20%	Significantly, many	++
20-50%	Pronounced significantly	+++
>50%	Severe, extreme	

Morphological assessment of leukocytes

The morphology of all leukocytes is evaluated. Special attention is directed to cell lineages that are quantitatively increased or decreased.

Neutrophils

- Granulation**
 - fine / medium — normal findings
 - coarse — toxic signs (e.g. bacterial infections, cytostatic therapies)
 - hypogranular — e.g. myelodysplastic syndromes
- Cytoplasm**
 - vacuoles — toxic signs (e.g. bacterial infections, cytostatic therapies)
 - basophilic streaks — toxic signs (e.g. bacterial infections, cytotoxic therapies) or administration of growth factors (G-CSF)
- Nuclei**
 - normal segmentation — normal findings
 - hyper segmentation — 6 or more nuclei segments, with DNA synthesis disorders such as vitamin B12 or folate deficiency.
 - Pelger forms — Isolated with viral infections or myelodysplastic syndromes. With over 80% of neutrophils occurring: Suspected Pelger-Huët nucleus anomaly (familial, hereditary)

Eosinophils
Basophils

- Vacuoles — are usually the result of cellular degranulation
- hypogranular — with disorders of formation or degranulation of the cell

Monocytes

- Vacuoles — are common, often also frequent and large

Lymphocytes

Standard erythrocyte: Cell diameter 7 µm

0 5 10 15 20 25 µm

Typical lymphocytes

- nuclei small, round to ovoid (approx. 7 µm)
- chromatin dense
- plasma light-basophilic, narrow to medium width
- fine azur granulation (LGL-cell, large granular lymphocyte 10-14 µm)

Atypical Lymphocytes

- Presumably reactive (usually with viral infections)**
 - Cell size 10-20 µm (particularly large cells of 18-20 µm occur for example with EBV infections)
 - Nuclei irregular, ovoid, sometimes indented
 - Cytoplasm of medium width, sometimes irregular extensions
 - Plasma color near the nucleus brighter, at the borders darkbasophilic
- Presumably neoplastic**
 - based on quantity: - LGL-cells >10% of all lymphocytes
 - based on morphology: - known morphologies such as hair cells are named - other atypical morphologies are described

About

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