

ESAME MORFOLOGICO DEL SANGUE VENOSO PERIFERICO

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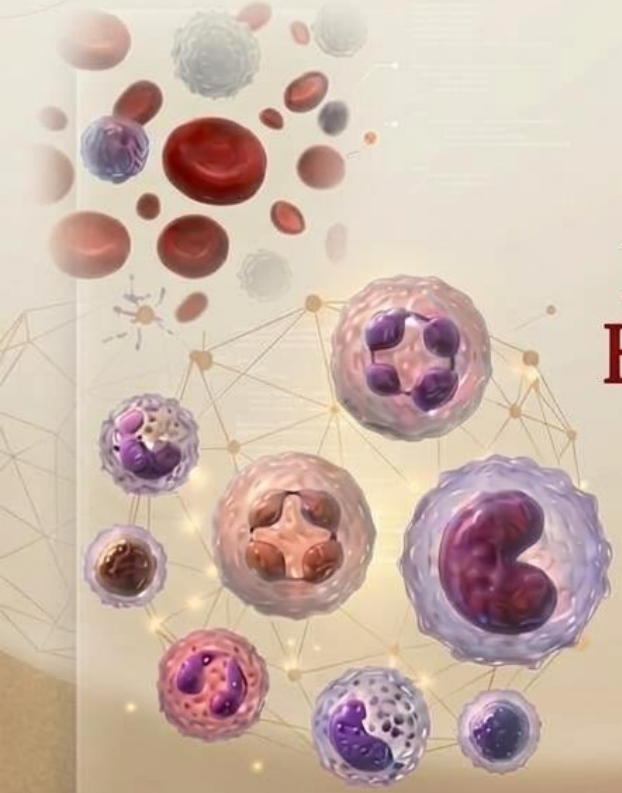
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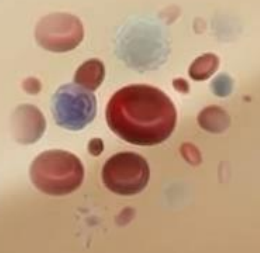
Disclosures of Name Surname

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
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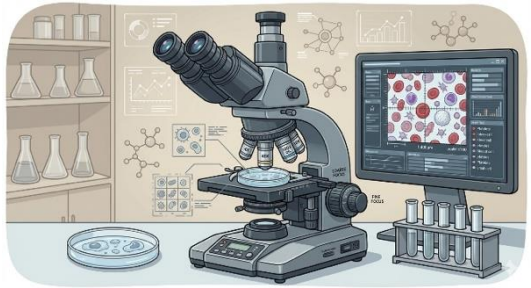
MORPHOLOGY



DOES IT STILL HAVE A ROLE IN HEMATOLOGICAL DIAGNOSTICS?





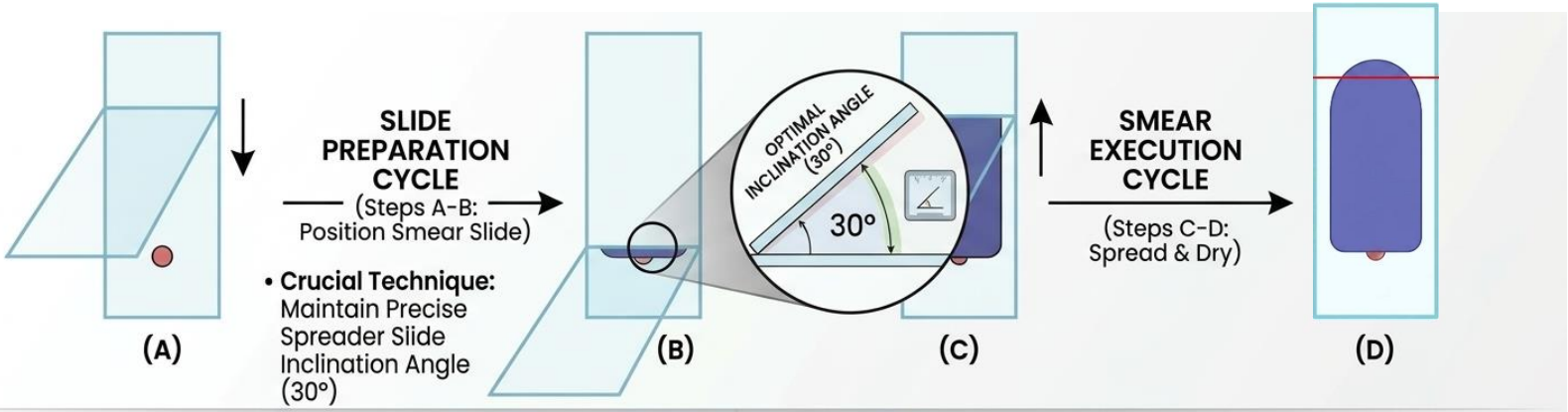


- The interpretation of peripheral blood smear morphology is a **cornerstone of the diagnostic work-up.** It remains a fundamental skill for practicing hematologists.

- The morphologic evaluation can reveal pathologic findings, which allow for **timely diagnosis and rapid treatment** for hemolytic anemias, suspected thrombotic microangiopathies (TMA), and leukemias.
- **200 white cell and at least 1,000 RBCs** should be evaluated to provide an accurate quantification of the morphological abnormalities
- Schistocytes should be counted and reported as a percentage

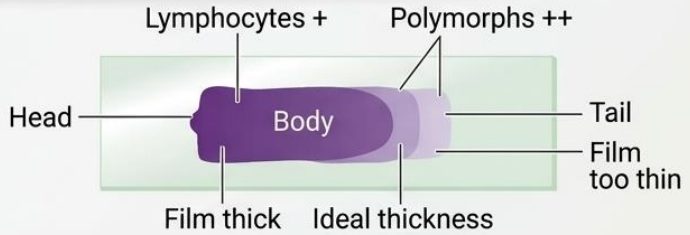
Chase ML et al, Consensus recommendations on peripheral blood smear review. Blood Advances 2023, 7; 3244-3252

HOW TO PREPARE A PERIPHERAL BLOOD SMEAR





Peripheral Blood Smear on a Microscope Slide



Peripheral Blood Film Zone Analysis & White Cell Distribution (Schematic View)

Figure 3.2 Schematic drawing of a blood film. Spread from left to right. An indication is given of the white blood cells are distributed. Cells are identified and quantified based on their characteristic locations: lymphocytes are concentrated in the 'Body' and intermediate zones, while polymorphs are found at the 'Head' and 'Tail' where the film thickness varies.

Dacie and Lewis, Practical Haematology 2011, 11th Edition. Hematology Lab Protocol – Staining & Distribution Guide.

HOW TO PREPARE A PERIPHERAL BLOOD SMEAR

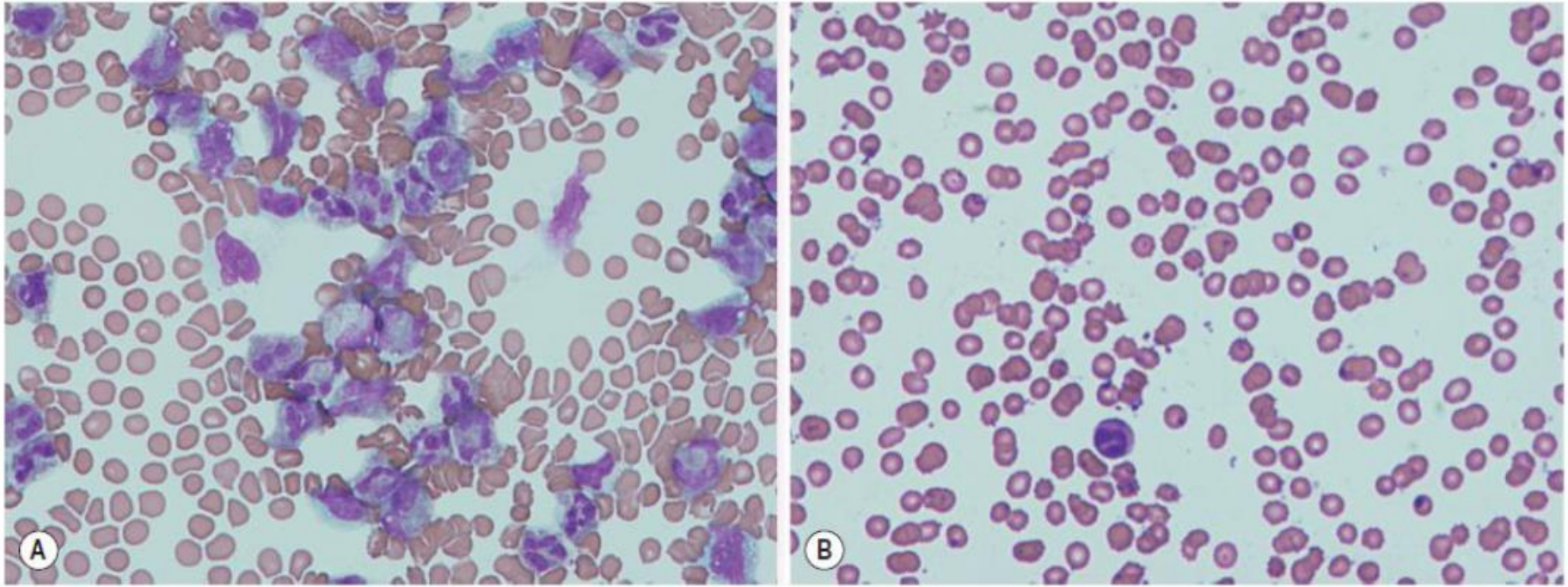


FIGURE 3-1 **Badly spread film.** Two areas of a badly spread film from a patient with a white blood cell count of 20×10^9/l showing (**A**) many leucocytes in the tail and (**B**) very few leucocytes in body of film.

Dacie and Lewis, Practical Haematology 2011, 11th Edition

HOW TO PREPARE A PERIPHERAL BLOOD SMEAR

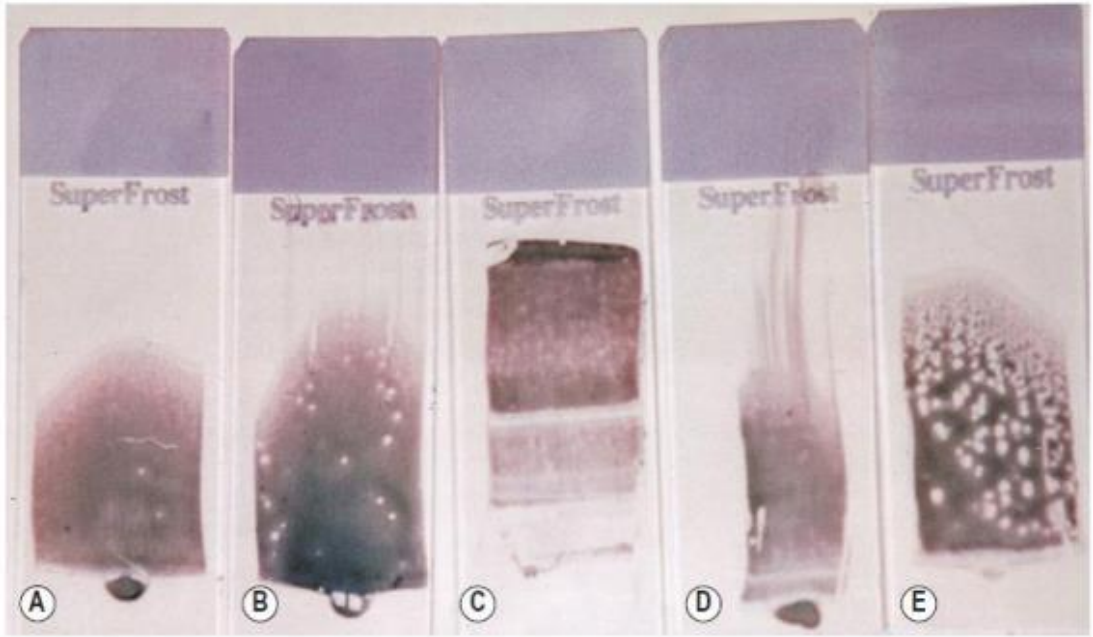


FIGURE 4-1 Blood films made on slides. (**A**) A well-made film. (**B**) An irregular patchy film on a dusty slide. (**C**) A film that is too thick. (**D**) A film that has been spread with inconsistent pressure and using an irregularly edged spreader, resulting in long tails. (**E**) A film made on a very greasy slide.

Dacie and Lewis, Practical Haematology 2011, 11th Edition

PERIPHERAL BLOOD MORPHOLOGY OVERVIEW

RED BLOOD CELLS



- Abnormalities of RBC size and/or color
- Abnormalities of RBC shape
- Inclusions in RBCs

WHITE BLOOD CELLS

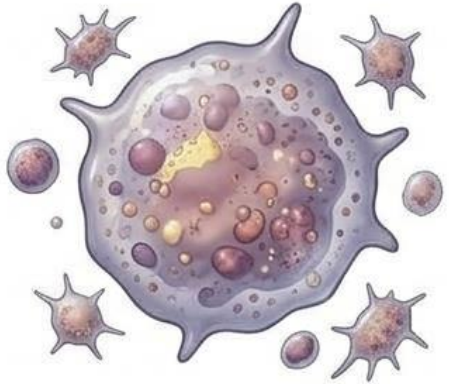


- Normal myeloid morphology
- Normal lymphocyte morphology
- Qualitative abnormalities in myeloid cells
- Qualitative abnormalities in lymphoid cells

PLATELETS



- Giant Platelets



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ORIGINAL ARTICLE
INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features

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SUMMARY

These guidelines provide information on how to reliably and consistently report abnormal red blood cells, white blood cells and platelets using manual microscopy. Grading of abnormal cells, nomenclature and a brief description of the cells are provided. It is important that all countries in the world use consistent reporting of blood cells. An international group of morphology experts have decided on these guidelines using consensus opinion. For some red blood cell abnormalities, it was decided that parameters produced by the automated haematology analyser might be more accurate and less subjective than grading using microscopy or automated image analysis and laboratories might like to investigate this further. A link is provided to show examples of many of the cells discussed in this guideline.

RECOMMENDED NOMENCLATURE AND COMMON RED CELL SYNONYMS

Table 2. Common red cell synonyms

Recommended Nomenclature	Synonym	Common clinical conditions associated with
Acanthocyte	acanthoid cell, astrocyte, burr cell, prickle cell, pyknocyte, star cell, spur cell, thorn cell	Liver disease, vitamin E deficiency, postsplenectomy, abetalipoproteinaemia, McLeod RBC phenotype
Basophilic stippling	punctate basophilia	Lead poisoning, haemoglobinopathies, thalassaemia, abnormal haem synthesis
Bite cell	keratocytes	G6PD deficiency
Blister cell	puddle cell, eccentrocyte	Oxidative haemolysis, G6PD deficiency
Echinocyte	berry cell, burr cell, crenated cell, mulberry cell, poikilocyte, pyknocyte, spiculated cell, spur cell, sputnik cell, star cell	Liver and renal disease, pyruvate kinase deficiency, storage artefact
Elliptocyte	bacillary cell, cigar or rod shaped cell, ovalocyte, pencil cell	Hereditary elliptocytosis, iron deficiency
Howell-Jolly body		Hyposplenism, postsplenectomy, haemolytic anaemia, megaloblastic anaemia
Hypochromic cell	anulocyte, pessary form, ring form	Iron deficiency, thalassaemia
Irregularly contracted cell		G6PD deficiency, haemoglobinopathies
Macrocyte	macronormocyte, megalocyte	B12/folate deficiency, liver disease, MDS
Microcyte	micronormocyte	Iron deficiency, thalassaemia
Ovalocyte	bacillary cell, cigar or rod shaped cell, elliptocyte	Hereditary elliptocytosis, iron deficiency
Pappenheimer bodies		Sideroblastic anaemia, haemoglobinopathies,, hyposplenism
Poikilocyte	burr cell, irregular shaped cell, irregularly contracted cell, pyknocyte, spur cell	
Polychromatic cell	polychromatophilic cell	Haemolytic anaemia, haematinic treatment
RBC	erythrocyte, normocyte, discocyte	
Schistocyte	burr cell, helmet cell, horn cell, keratoschistocyte, pincer cell, poikilocyte, prickle cell, red cell fragment, schizocyte, thorn cell, triangular cell	Microangiopathic haemolytic anaemia, TTP, HUS, DIC, renal disease
Sickle cell	drepanocyte, holly leaf cell	Sickle cell anaemia and other sickle cell diseases
Spherocyte	spherical cell	Hereditary spherocytosis, ABO and warm AIHA, Clostridium perfringens sepsis, burns
Stomatocyte	cup cell, knizocyte, slit cell	Alcoholic liver disease, hereditary stomatocytosis
Target cell	codocyte, leptocyte	Liver disease, haemoglobinopathies, thalassaemia
Teardrop cell	dacrocyte, pear-shaped cell	myelofibrosis

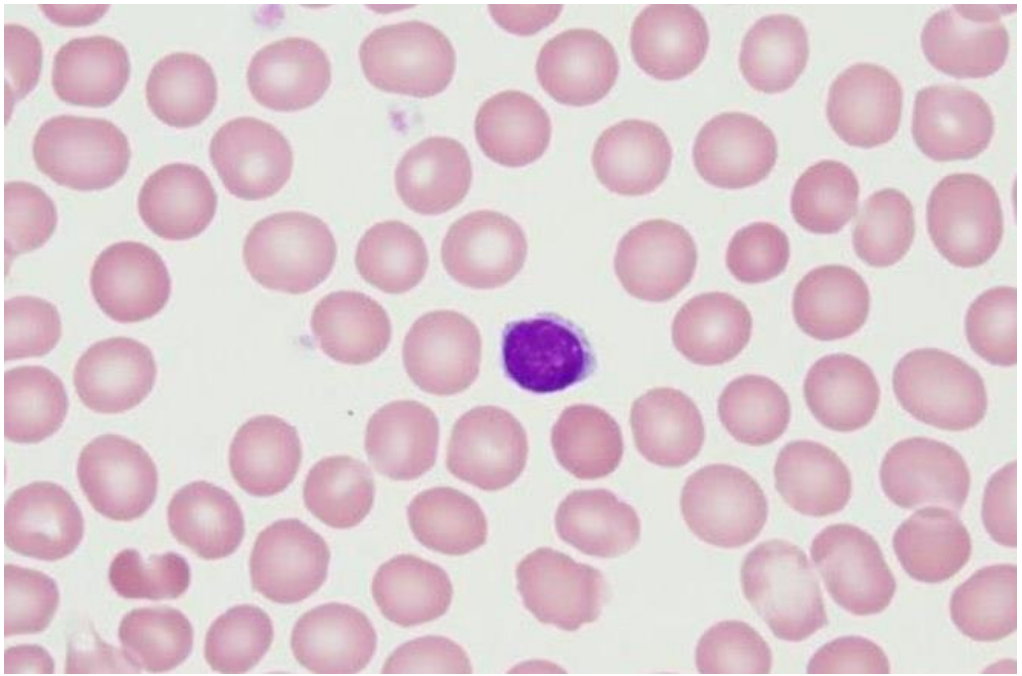
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

RED CELL MORPHOLOGY

Red blood cells are biconcave disks with a diameter of 7-8µm, similar to the size of the nucleus of a resting lymphocyte.

As a rough guide, **normal red cell size appears to be about the same as that of the nucleus of a small lymphocyte on the dried film.**

Optimal Examination Field: The ideal zone can usually be found toward the tail of the film, where there are no rouleaux and the red cells are touching but with little overlap.



Dacie and Lewis, Practical Haematology 2011, 11th Edition

ABNORMALITIES OF RBC SIZE AND/OR COLOUR

- **Microcytes (<7 µm; MCV <80 fL)**: frequently linked to hypochromia. Target cells can falsely lower the MCV. Interpretation must be **age-contextualized** (physiologically smaller in healthy children, larger in neonates).
- **Macrocytes (>8.5 µm; MCV >100 fL)**: They may be round or oval in shape which can have diagnostic significance. Red cells in premature, newborn babies and neonates are physiologically larger than in adults. Reticulocytosis may also cause an elevated MCV.

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

ABNORMALITIES OF RBC SIZE AND/OR COLOUR

- **Anisocytosis**: increased variability in RBC size. It is non specific and will be reflected in an increased red cell distribution width (RDW) in automated analyser counts.

- **Dimorphism**: coexistence of two distinct RBC populations, typically characterized by an increased RDW and clearly visible on the automated analyzer's red cell histogram. It requires explicit reporting and a detailed description of both populations.

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

ABNORMALITIES OF RBC SIZE AND/OR COLOUR

- **Hypochromia**: Erythrocytes with a central pallor exceeding one-third of the cell diameter, indicating a reduction in MCH/MCHC or thinner-than-normal cell architecture. The automated **MCH** is recommended over visual grading to evaluate hypochromia.
- **Polychromasia**: Immature, enlarged erythrocytes exhibiting a pinkish blue-grey hue due to residual ribosomal RNA; requires quantitative visual grading and clinical correlation with a **reticulocyte count**.

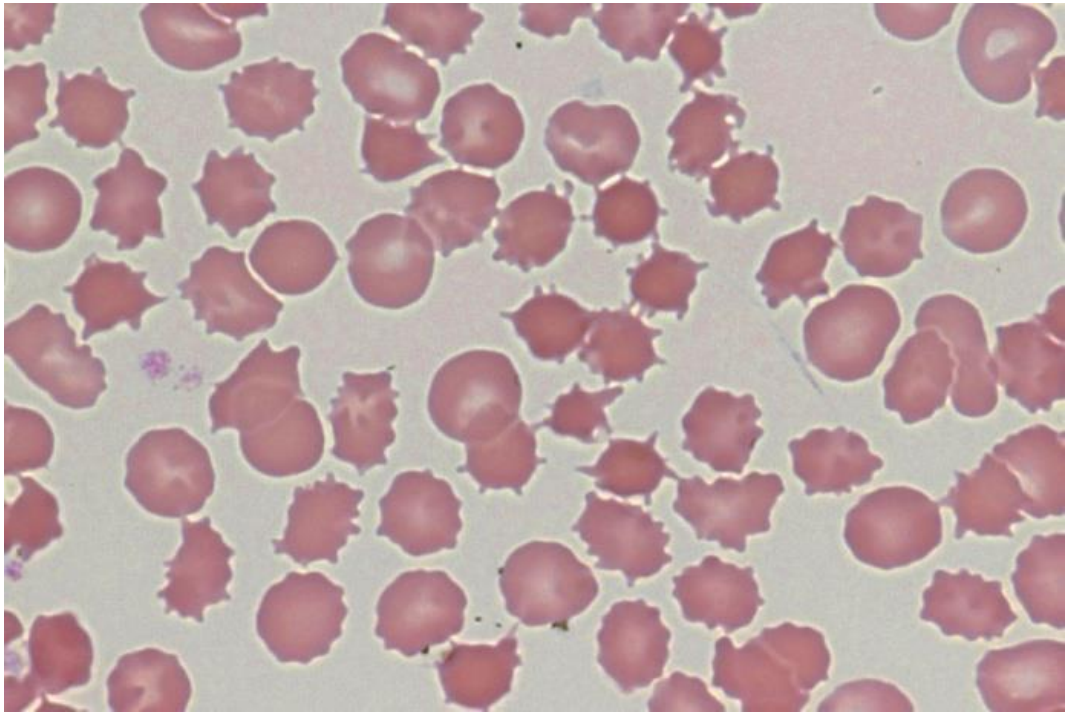
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

ABNORMALITIES OF RBC SHAPE

Acanthocytes

Acanthocytes are round, hyperchromic red cells with 2-20 irregularly spaced projections or spicules of variable length, thickness and shape. Some spicules have club-shaped rather than pointed ends.

Liver disease, vitamin E deficiency, postsplenectomy, abetalipoproteinaemia, McLeod RBC phenotype.



Abetalipoproteinaemia

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

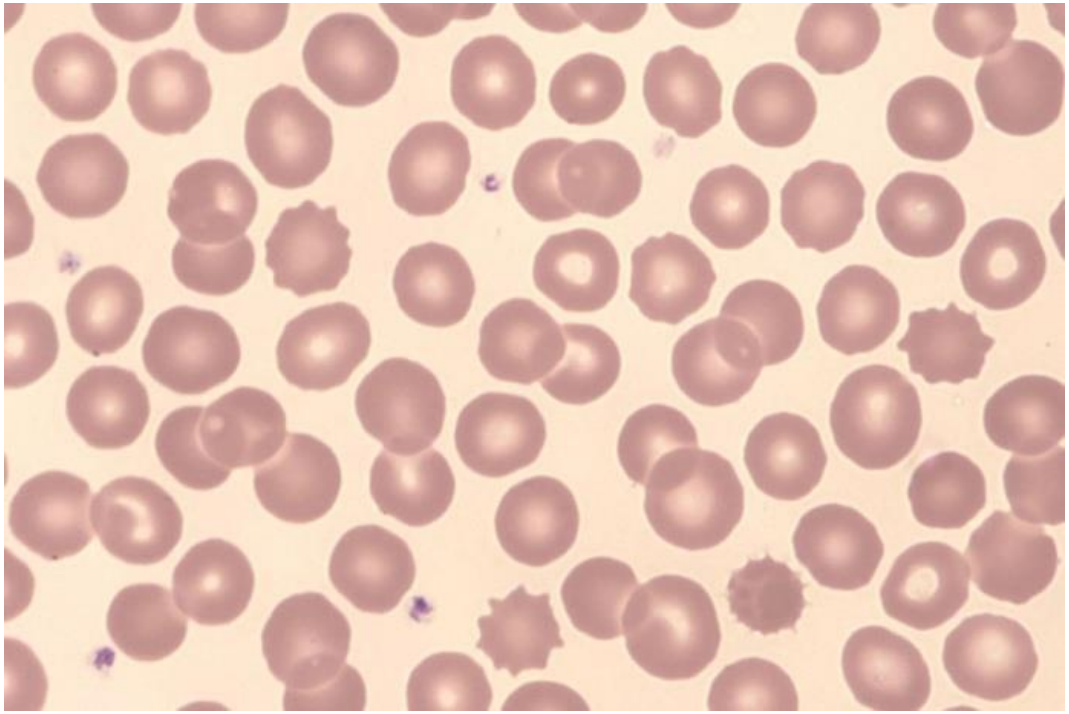
ABNORMALITIES OF RBC SHAPE

Echynocytes

Echinocytes are red cells that have lost their disc shape and are covered with 10-30 short blunt projections or spicules of fairly regular form.

Liver and renal disease, pyruvate kinase deficiency, storage artefact

Renal failure



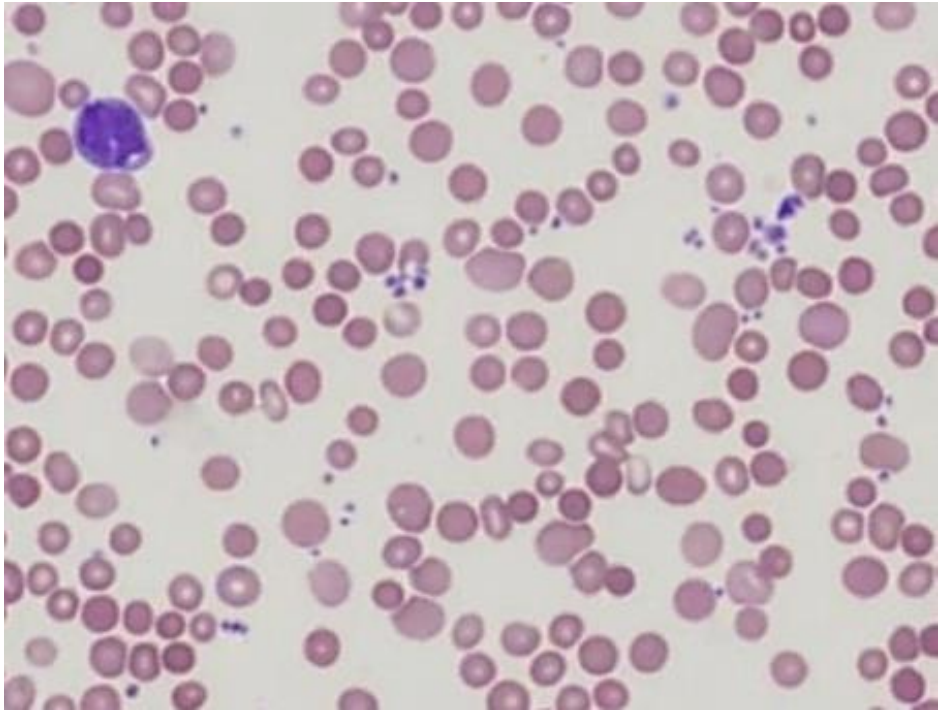
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

ABNORMALITIES OF RBC SHAPE

Spherocytes

Spherocytes are of small diameter (<6.5 µm) and has a spherical shape and absence of central pallor. They have a reduced surface area, are less deformable and more susceptible to destruction in the spleen. They may be formed as a consequence of an abnormality of the RBC cytoskeleton and membrane, but they can also be caused by immune-mediated mechanisms, as in autoimmune hemolytic anemia, or by direct microangiopatic damage.

Hereditary spherocytosis, ABO and warm AIHA, Clostridium perfringens sepsis, burns.



Hereditary spherocytosis

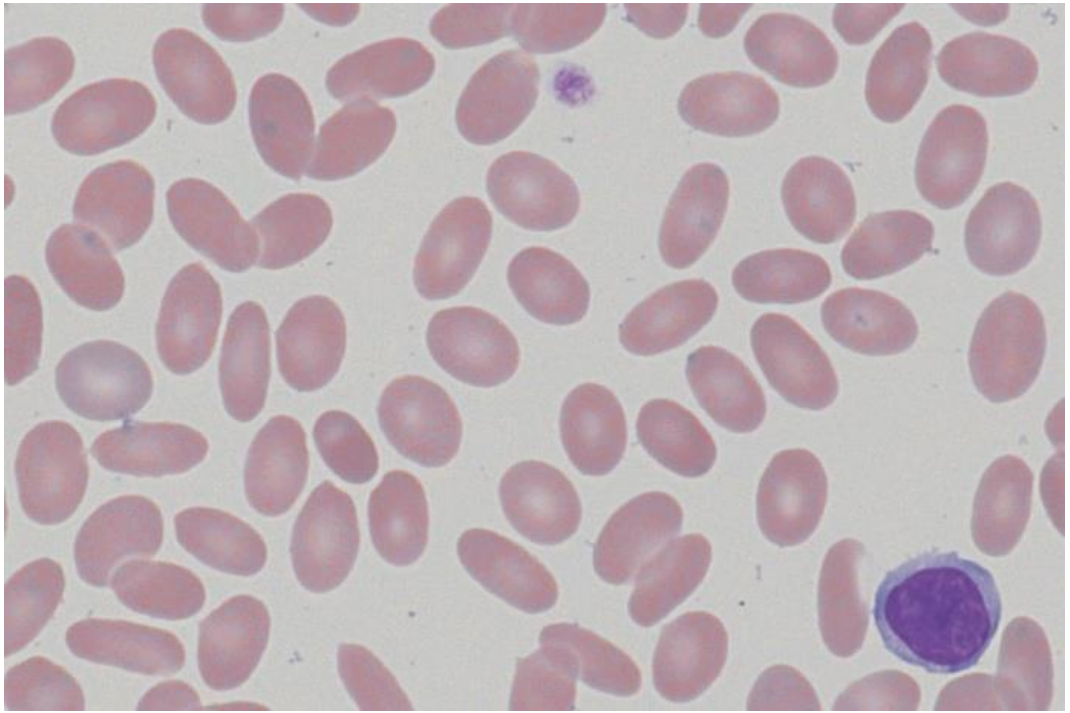
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

ABNORMALITIES OF RBC SHAPE

Elliptocytes and ovalocytes

Elliptocytes are cells with an elliptical shape (the long axis is more than twice the short axis), while ovalocytes have an oval shape (the long axis is less than twice the short axis).

Hereditary elliptocytosis, iron deficiency



Hereditary elliptocytosis

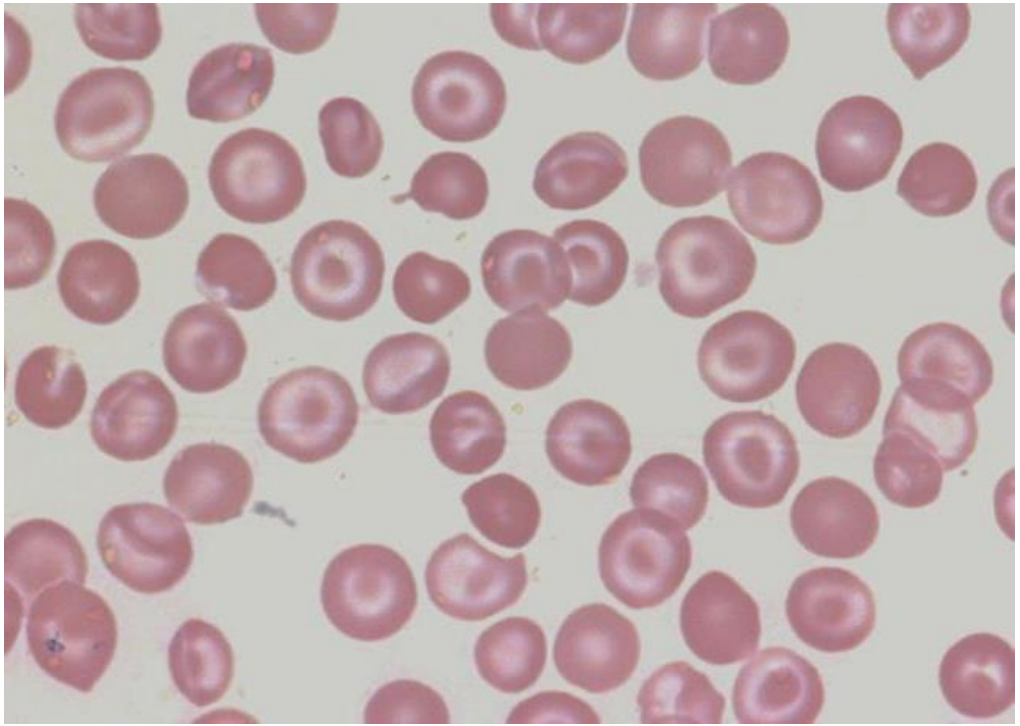
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

ABNORMALITIES OF RBC SHAPE

Target cells

The term target cell refers to a cell in which there is a central round stained area and a peripheral rim of haemoglobinized cytoplasm separated by non-staining or more lightly staining cytoplasm. Target cells result from cells having a surface that is disproportionately large compared with their volume. They may be normal in size, microcytic or macrocytic.

Liver disease, haemoglobinopathies, splenectomy.



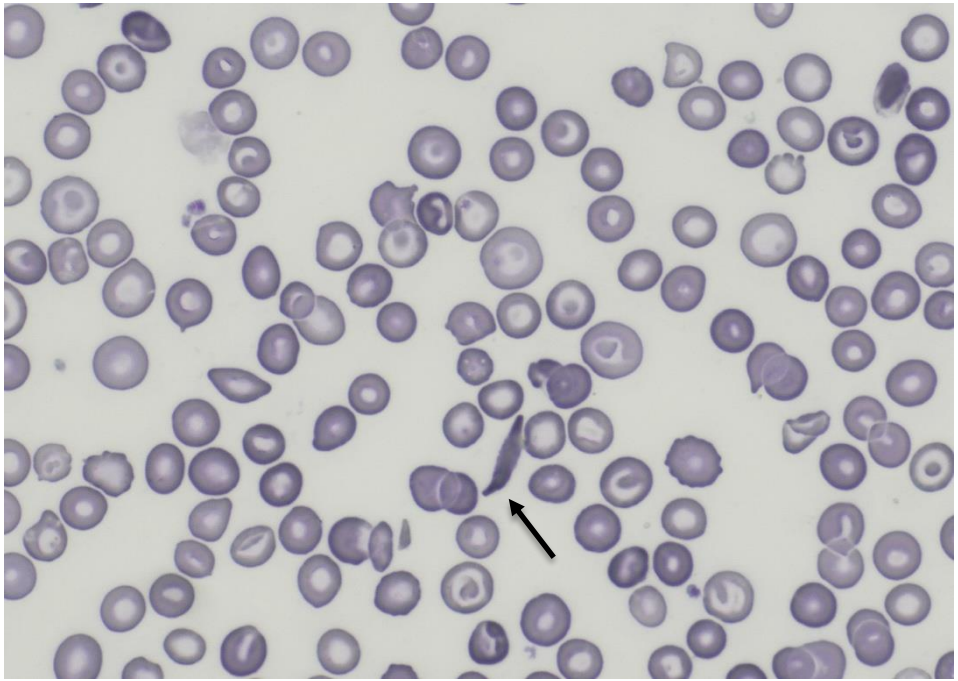
Haemoglobin C disease

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

ABNORMALITIES OF RBC SHAPE

Sickle cells

Sickle cells are red cells that become crescent or sickle-shaped with pointed ends as a result of polymerization of HbS. A sickle screen or haemoglobinopathy screen is recommended.



Sickle cell disease

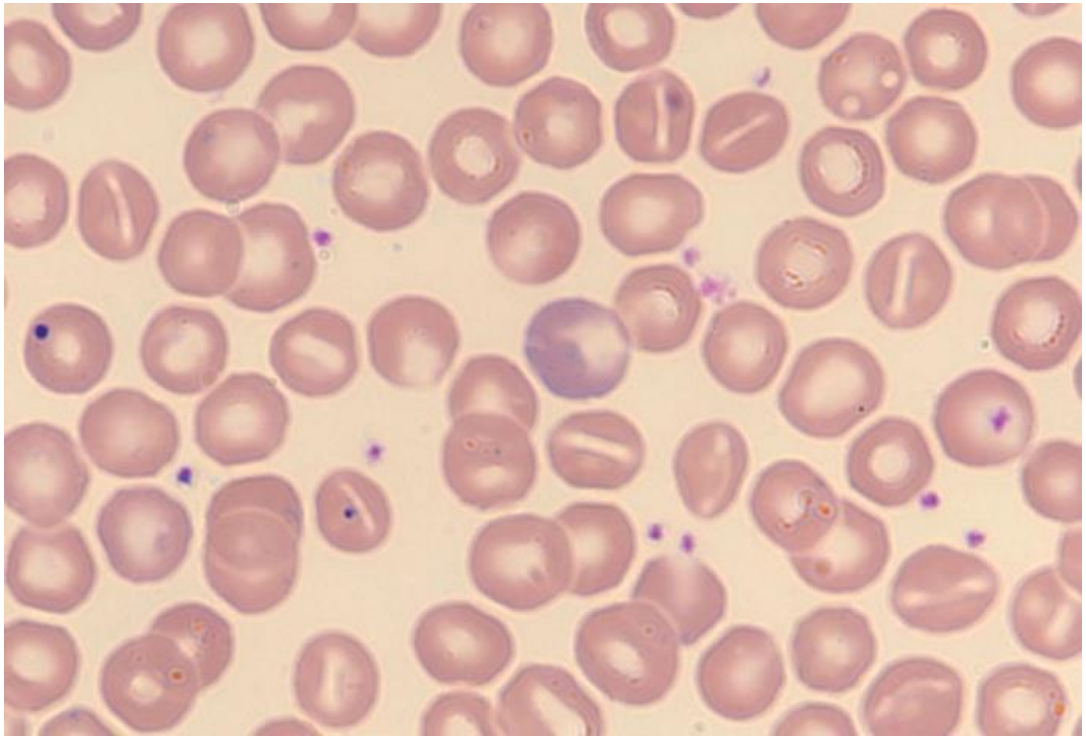
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

ABNORMALITIES OF RBC SHAPE

Stomatocytes

Stomatocytes are red cells in which the central biconcave area appears slit-like in dried films. In hereditary stomatocytosis abnormal permeability alters red blood hydratation. In acquired forms a specific ion transport defect is not necessarily present.

Hereditary stomatocytosis, Asian ovalocytosis; liver disease, alcoholism, occasionaly myelodysplastic neoplasms.



Hereditary stomatocytosis

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

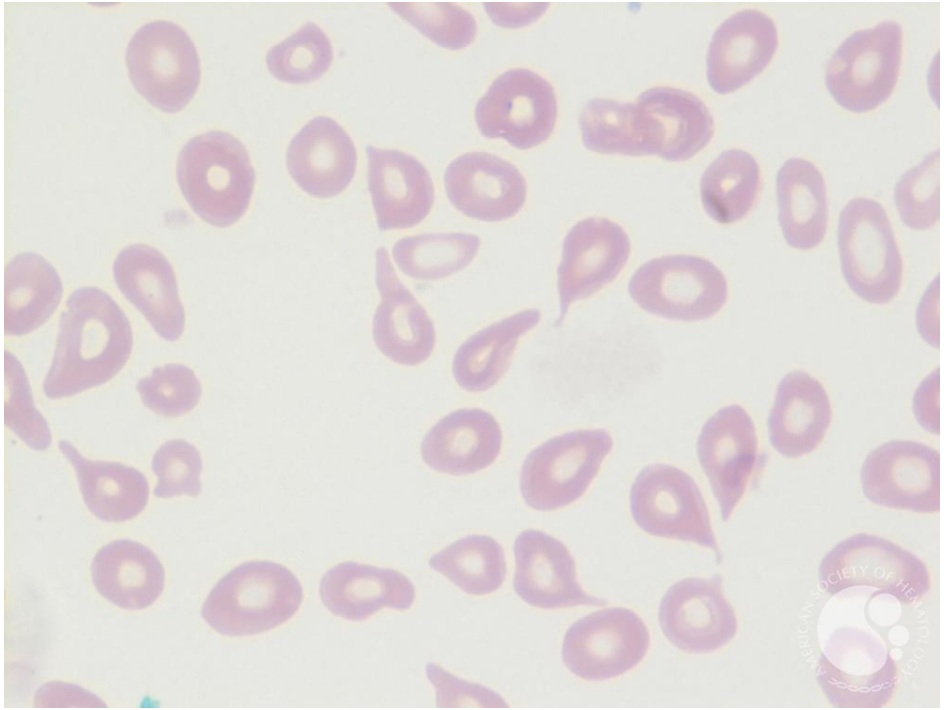
ABNORMALITIES OF RBC SHAPE

Teardrop cells

Pear-shaped erythrocyte with a single, blunt tail oriented in random directions across the field. If all tails point in the same direction, it is a technical artifact from slide stretching.

They formed as they pass through an abnormal bone marrow environment, such myelofibrosis or bone marrow infiltration.

Bone marrow infiltration ,severe hemoglobinopathies, vitamin B12 deficiency, Myelofibrosis.



Myelofibrosis - ASH Image Bank

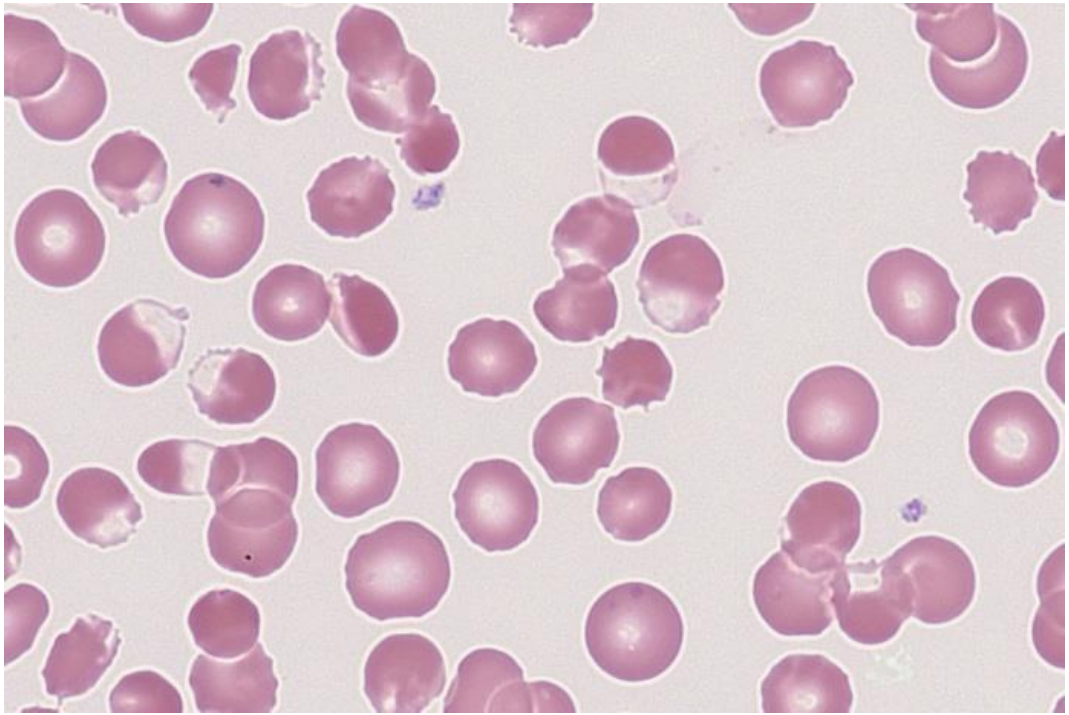
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

ABNORMALITIES OF RBC SHAPE

Blister cells

Red cells that undergo oxidative stress, which damages and denatured hemoglobin. The denatured hemoglobin formed aggregates on one side (Heinz bodies) causing formation of bubble-like area near the edge of red blood cells –> blister appearance.

Oxidative haemolysis, G6PD deficiency.



G6PD deficiency

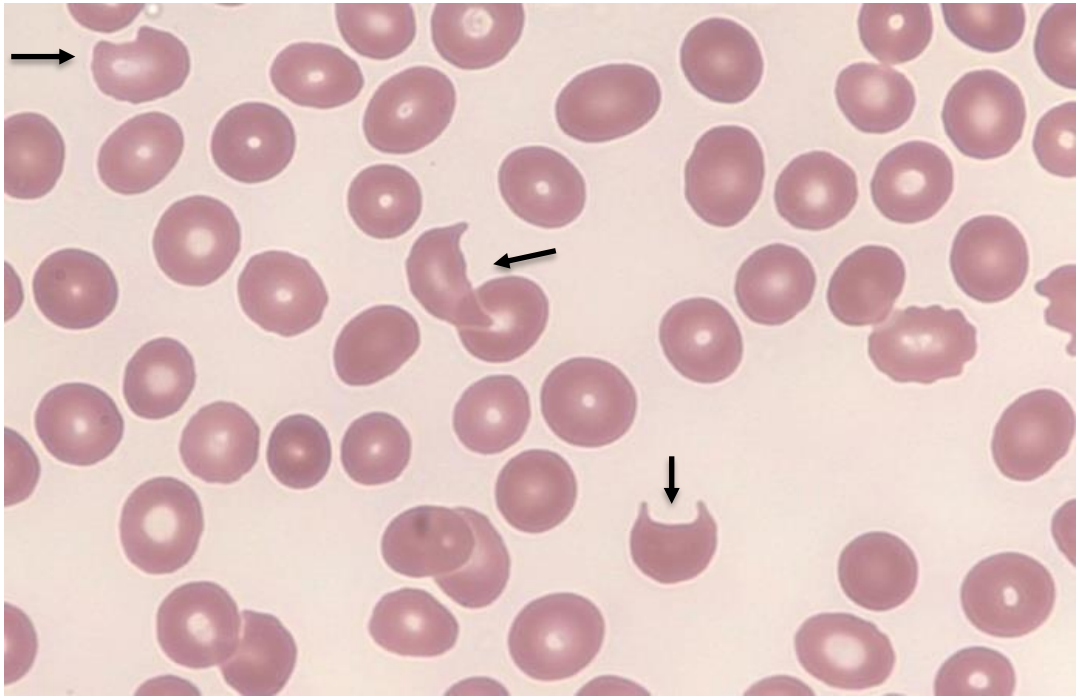
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

ABNORMALITIES OF RBC SHAPE

Bite cells/keratocytes

Bite cells are RBC with peripheral single or multiple arcuate defects (bites) caused by the removal of Heinz bodies by the spleen and are a feature of oxidant haemolysis.

Microangiopathic haemolytic anaemias and mechanical damage to the red cells may produce morphologically identical cells (keratocytes), which are formed by the rupture of peripheral pseudovacuoles and subsequent fusion of the red cell membrane.



G6PD deficiency and microangiopathic hemolytic anemias

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

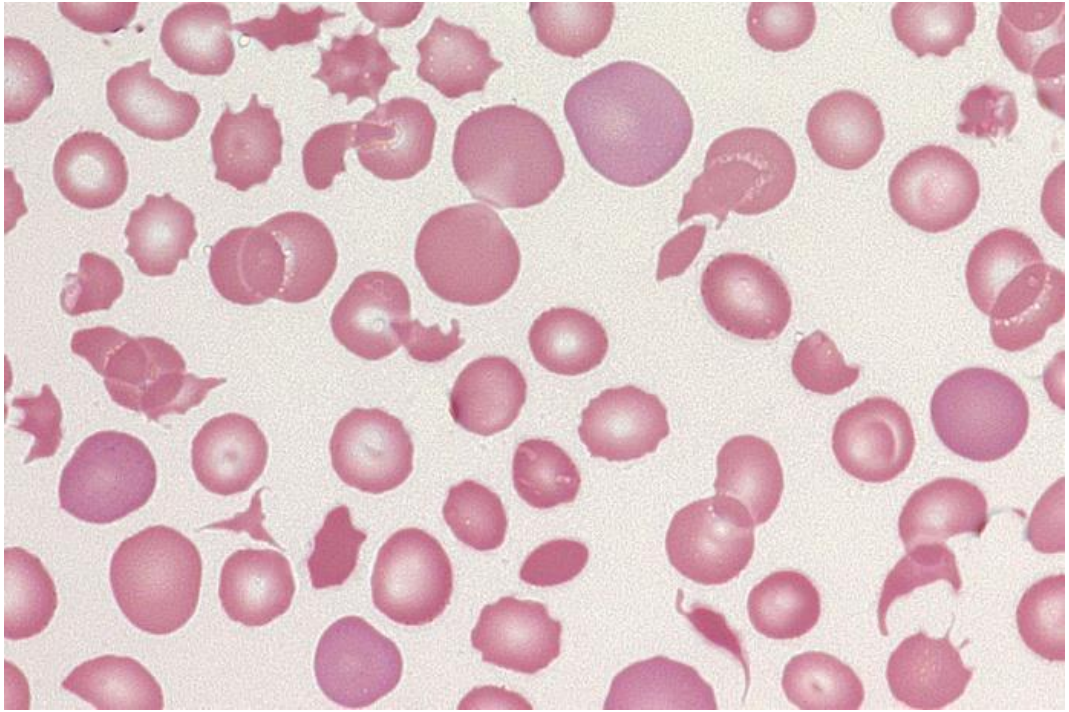
ABNORMALITIES OF RBC SHAPE

Schistocyte

Schistocytes are fragments of red blood cells produced by extrinsic mechanical damage within the circulation and are a diagnostic feature of microangiopathic haemolytic anaemia. Schistocytes are always smaller than intact red cells and can have the shape of fragments with sharp angles and straight borders, small crescents, helmet cells or keratocytes. Microspherocytes may also be a feature of MAHA.

Microangiopathic haemolytic anaemia, TTP, HUS, DIC, renal disease.

The recommendation is to grade schistocytes.



Thrombotic thrombocytopenic purpura

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

ABNORMALITIES OF RBC SHAPE: SCHISTOCYTES

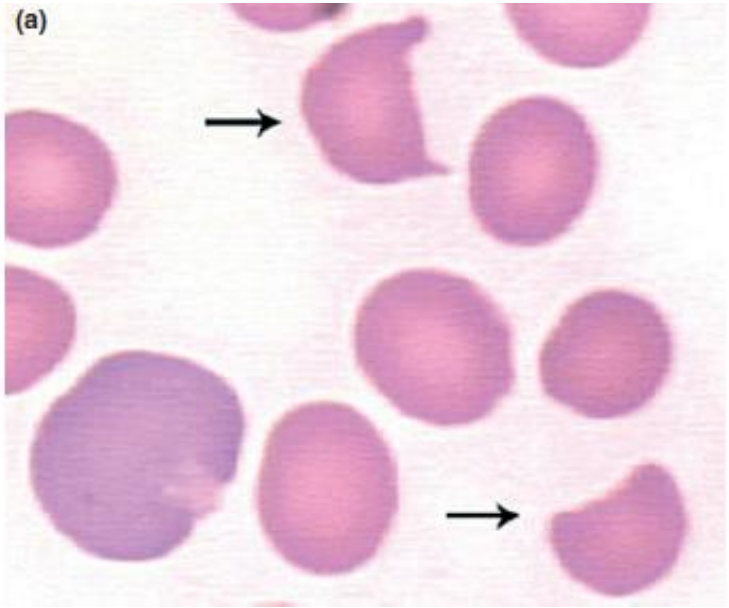
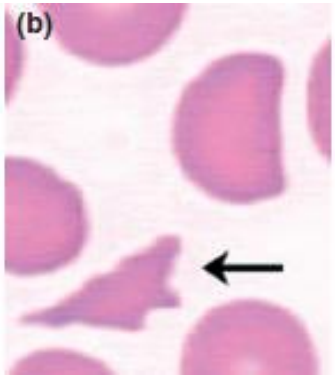
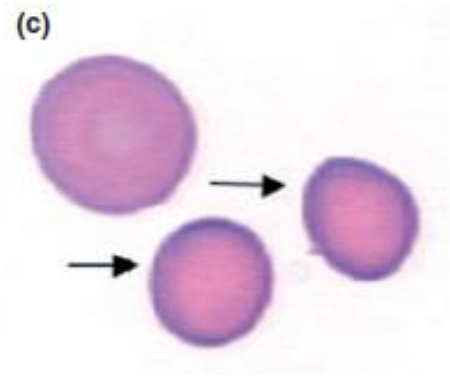


Figure 1. Typical shapes for specific identification of schistocytes. (a) keratocyte (upper arrow) and helmet cell (lower arrow), close to a polychromatophilic erythrocyte in the left lower corner; (b) a triangle schistocyte (arrow) with a helmet cell on the upper right; (c) two microspherocytes (arrows); they are derived, in a context of thrombotic microangiopathic anemia, from schistocytes.





Zini G, et al. ICSH recommendations for identification, diagnostic value and quantitation of schistocytes. Int J Lab Hematol. 2012;34(2):107–116. Update 2021: Int J Lab Hematol. 2021;43(6):1256–1262.

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ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

ICSH recommendations for identification, diagnostic value, and quantitation of schistocytes

G. ZINI*, G. D'ONOFRIO†, C. BRIGGS‡, W. ERBER§, J. M. JOU¶, S. H. LEE**, S. MCFADDEN††, J. L. VIVES-CORRONS¶, N. YUTAKA‡‡, J. F. LESESVE§§

REVIEW

ISLH International Journal of Laboratory Hematology WILEY

2021 update of the 2012 ICSH Recommendations for identification, diagnostic value, and quantitation of schistocytes: Impact and revisions

Gina Zini[1,2] | Giuseppe d'Onofrio[2] | Wendy N. Erber[3] | Szu-Hee Lee[4] | Yutaka Nagai[5] | Grzegorz W. Basak[6,7] | Jean-François Lesesve[8] | International Council for Standardization in Hematology (ICSH)

ABNORMALITIES OF RBC SHAPE: SCHISTOCYTES

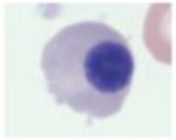
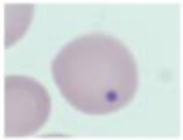
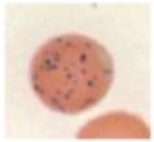
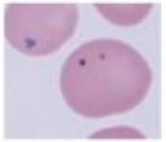
Diagnostic Thresholds suspicious for thrombotic microangiopathy:

- Adults & Term Infants: **> 1.0%**
- Preterm Infants: > **5.0%**.

manual count of at least **1,000 RBCs** under x600 or x1000 magnification; smear prepared within **3 hours** at room temperature.

Zini G, et al. ICSH recommendations for identification, diagnostic value and quantitation of schistocytes. Int J Lab Hematol. 2012;34(2):107–116.
Update 2021: Int J Lab Hematol. 2021;43(6):1256–1262.

INCLUSION IN RBC

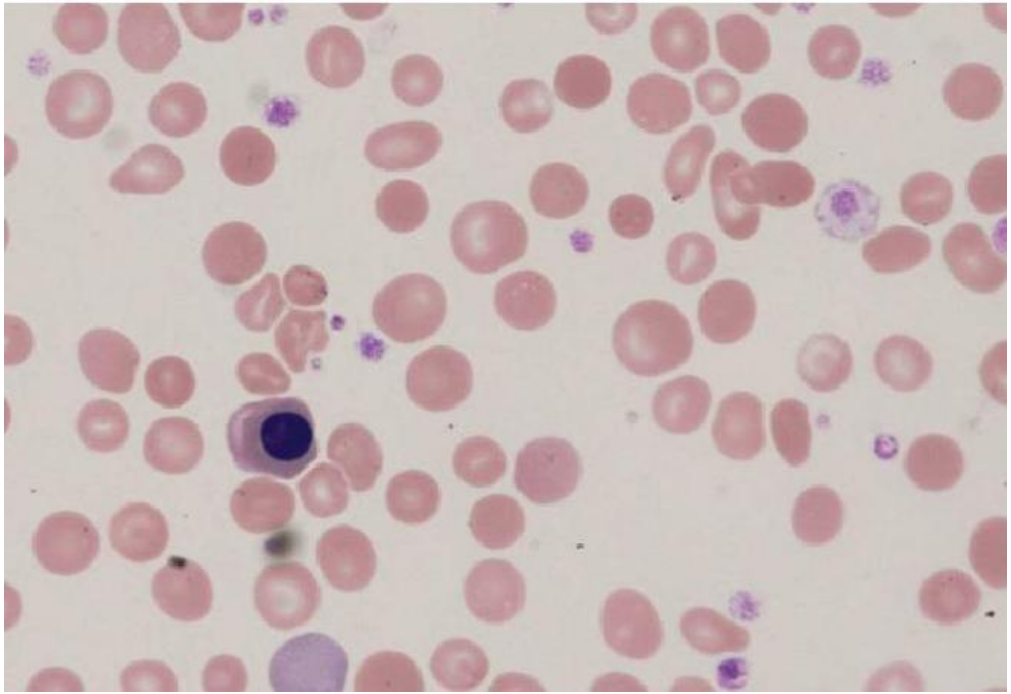
Nucleated RBC	Howell-Jolly body	Basophilic stippling	Pappenheimer body	Parasite
				
				
Pyknotic, shut-down nucleus. Reflects extreme increases in erythropoietic activity or failure of the blood filtration mechanisms.	Nuclear remnants, ordinarily removed from red cells by the spleen, thus seen in asplenia.	Many small coarse or fine blue granules, which are aggregates of ribosomes. Seen in cases of disturbed erythropoiesis.	Occupy only one region of the RBC vs more global distribution of granules with basophilic stippling; aggregates of ferritin, lysosomes, ribosomes.	Multitude of different inclusions seen in babesiosis and malaria.

INCLUSION IN RBC

Nucleated Red Blood Cell (NRBC)

A nucleated red blood cell is a red cell precursor and is used to describe an erythroblast in the peripheral circulation. **The presence of circulating NRBC is a strong indicator of underlying pathology.**

The recommendation is to report the number of NRBC per 100 WBC.



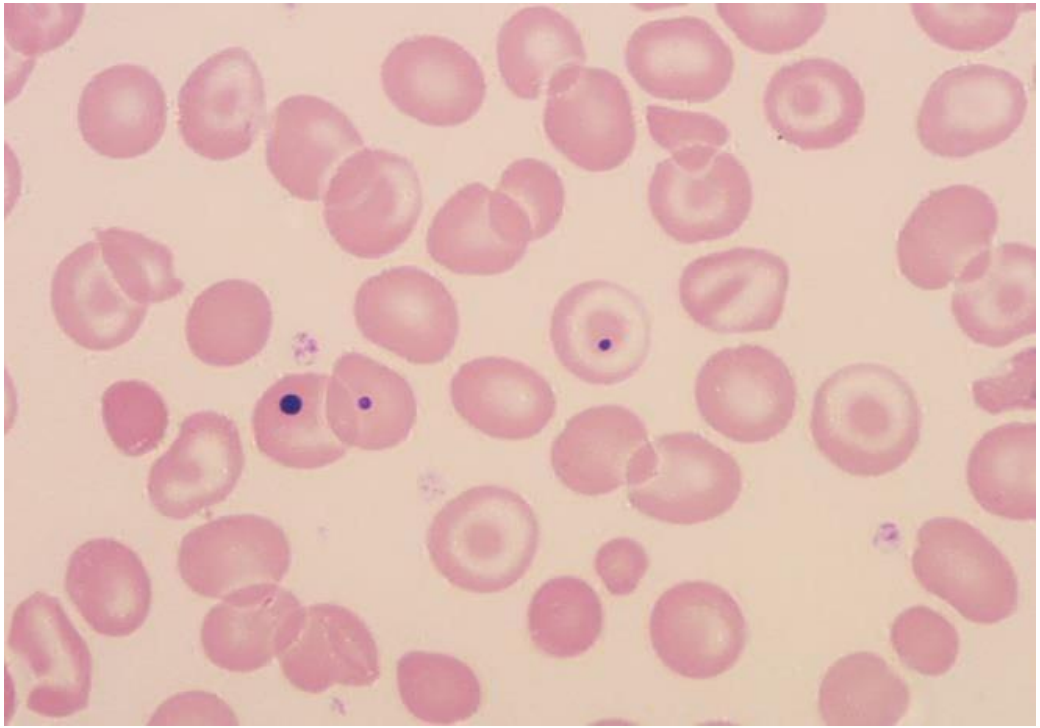
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

INCLUSION IN RBC

Howell-Jolly bodies

Howell-Jolly bodies are usually single, small (1 µm), dense, perfectly round basophilic inclusions that are fragments of nuclear material (**DNA**). They are regularly present after splenectomy and when there is splenic atrophy.

Hyposplenism, postsplenectomy, haemolytic anaemia, megaloblastic anaemia.



Splenectomised patient

ASH Image Bank

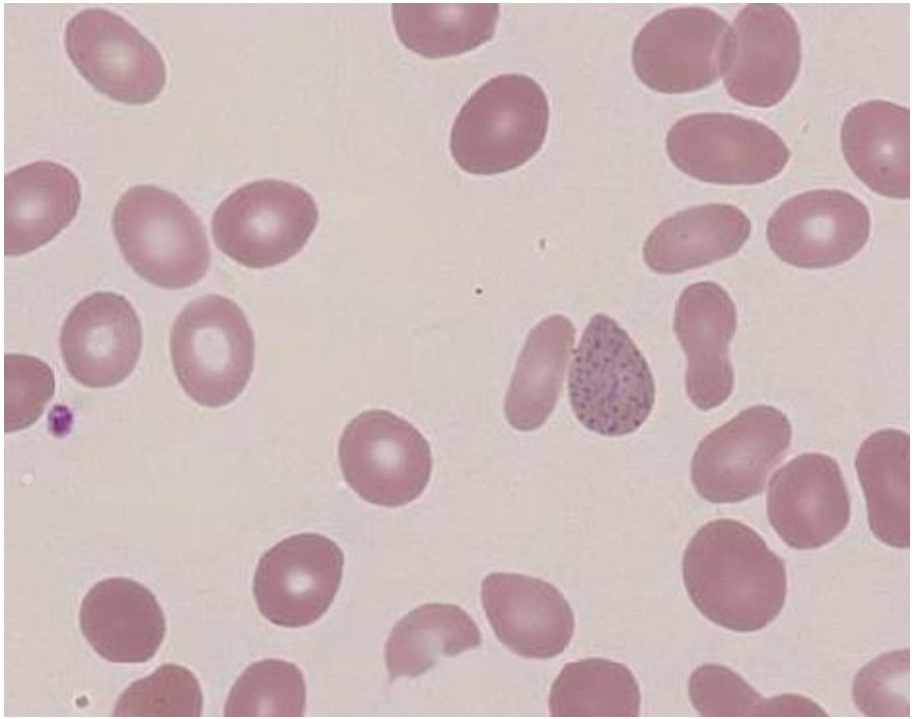
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

INCLUSION IN RBC

Basophilic stippling

Basophilic stippling describes the occurrence of fine, medium, or coarse blue granules due to abnormally aggregated **ribosomes**, uniformly distributed throughout the RBC.

Lead poisoning, haemoglobinopathies, thalassaemia, abnormal haem synthesis.



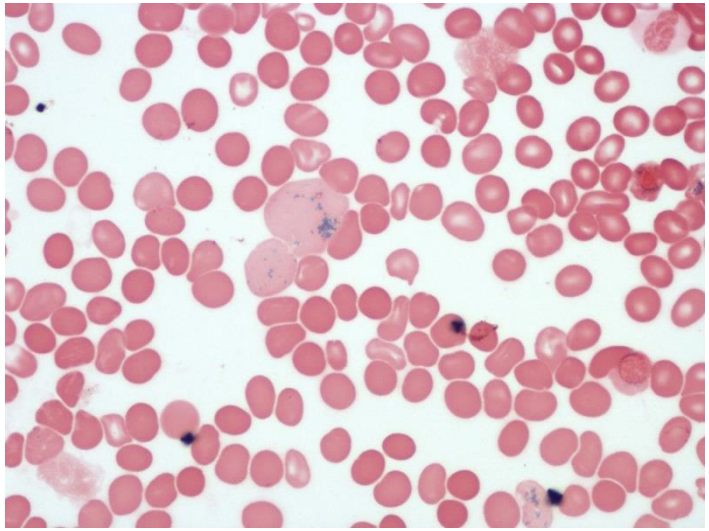
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

INCLUSION IN RBC

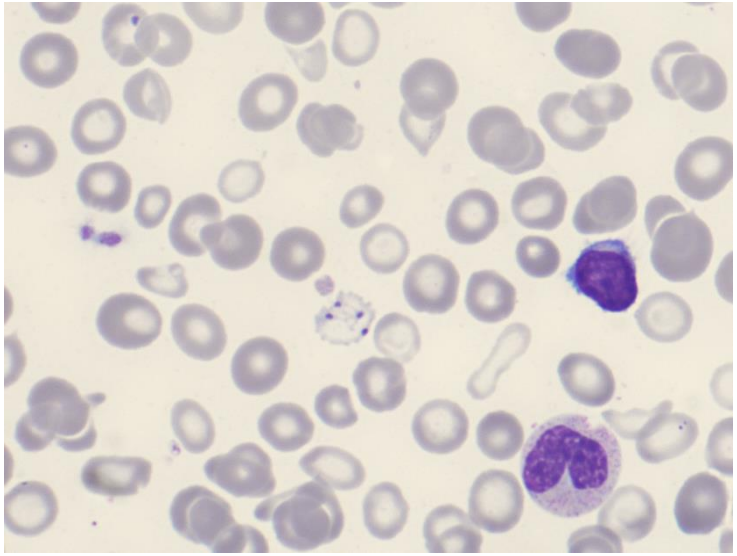
Pappenheimer bodies

Pappenheimer bodies are small peripherally sited basophilic (almost black) erythrocyte inclusions. They are smaller than Howell–Jolly bodies. Usually only a small number are present in a cell. They are composed of **haemosiderin** and their presence is related to sideroblastic erythropoiesis and hyposplenism.
They stain positively for Iron (Perls blue reaction).

Sideroblastic anaemia, haemoglobinopathies, hyposplenism



Perls' staining.
Courtesy Dr. Susanna Fenu



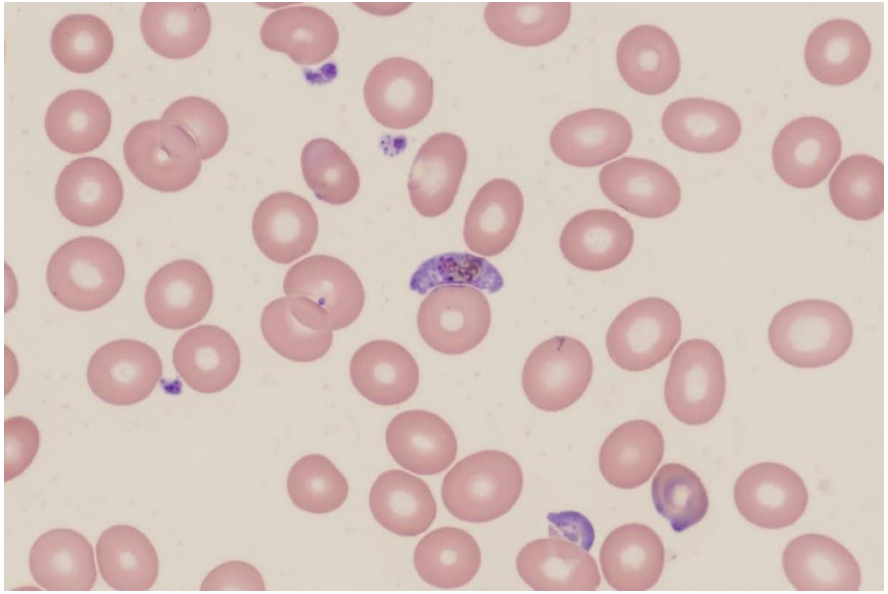
Congenital sideroblastic anemia

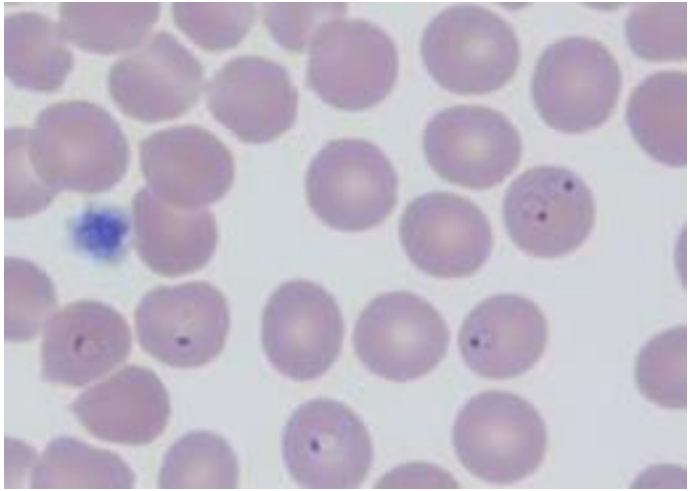
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

INCLUSION IN RBC

Micro-organisms in RBC

Micro-organisms may be seen free between or within RBC in patients with bacterial, fungal, protozoan or parasitic infections. Malarial species identification should be made and reported.





Plasmodium Falciparum

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

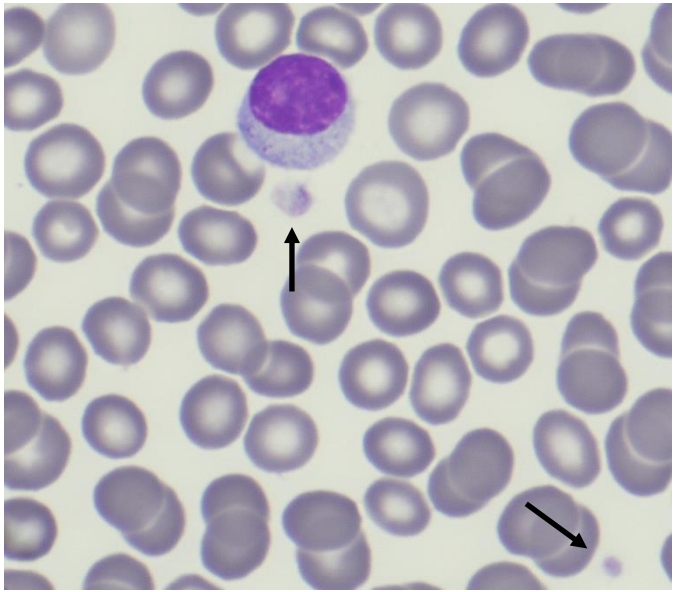
PLATELET MORPHOLOGY

- **A normal platelet** measures 1.5–3 µm in diameter.
- **Large platelets** measure 3–7 µm (roughly the diameter of a normal sized red cell), whilst **giant platelets**, are larger than normal sized red cells at 10–20 µm in diameter. Usually less than 5% of the platelets appear large.
- **Large platelets** are seen in the blood when platelet production is increased (e.g immune thrombocytopenia) and in hyposplenism. **Very high platelet counts with extreme platelet anisocytosis** may be associated to a myeloproliferative neoplasm, with some platelets being as large as red cells and often with some agranular or hypogranular platelets.
- **Platelet Estimation:** monolayer area (**x100 oil immersion objective**). Assess platelet numbers across 10 representative fields (**normal reference range: 8–20 platelets/field**). Estimate plt/µL= mean field count x 15,000 (or 20,000).
- **In pediatric patients, reactive thrombocytosis due to inflammatory stress, infections, or iron deficiency is highly commo**n, even exceeding 1000 x 10^9/L while remaining a benign process.

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

PLATELET MORPHOLOGY

Characteristic morphologic features are seen in various uncommon inherited platelet disorders associated with bleeding. These include the **Bernard–Soulier syndrome**, in which there are giant platelets with a defective ristocetin response, and **the grey platelet syndrome**, in which the platelets have decreased/absence alpha-granules and have a ghost-like appearance on the stained blood film. Thrombocytopenia with large platelets is also a feature of the **May–Hegglin anomaly.**



Grey platelet Syndrome



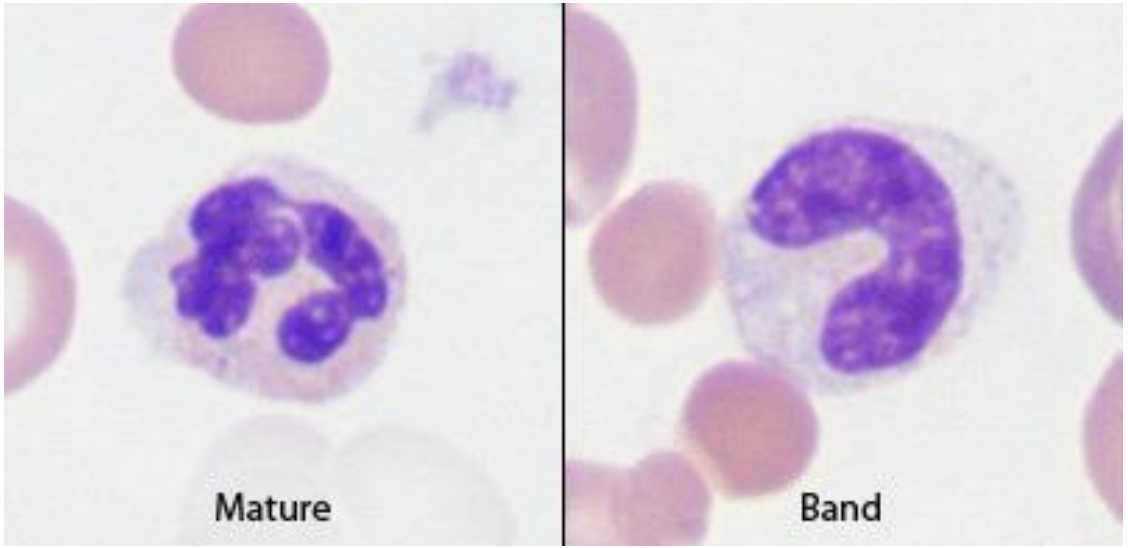
Bernard-Soulier Syndrome

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

NORMAL MYELOID MORPHOLOGY

Band neutrophil

Band neutrophils are 10–14 µm in diameter and have a nucleus that is non segmented or has rudimentary lobes that are connected by a thick band rather than a thread. Cytoplasm is abundant, pink and contains many small violet-pink neutrophilic or secondary granules distributed evenly throughout the cell.



Segmented neutrophil

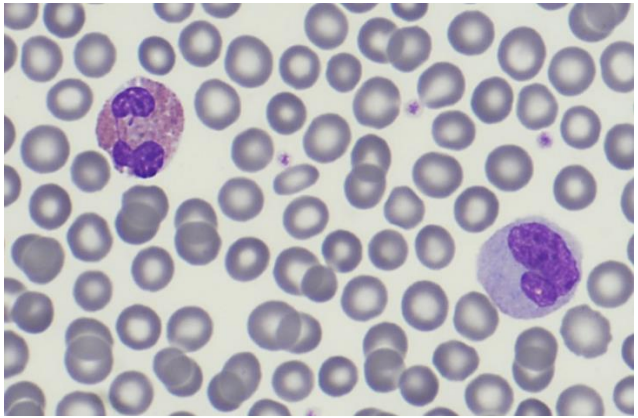
A granulocyte that is 10–14 µm in diameter with a lobulated nucleus (usually 3–4 lobes, but small numbers of 2 and 5 lobed neutrophils may also be seen) connected by a thin thread of chromatin. The chromatin is coarse, stains violet and is arranged in clumps. Small nuclear appendages may be seen. There is abundant pink cytoplasm with many small secondary granules.

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

NORMAL MYELOID MORPHOLOGY

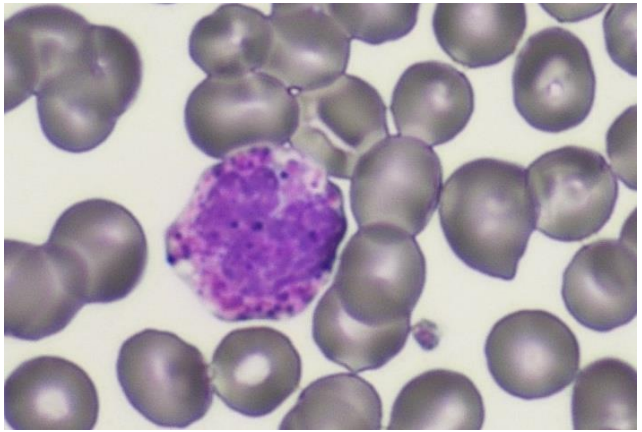
Eosinophil

The diameter of the eosinophil is 12–17 µm. The nucleus usually only has 2 lobes with coarsely clumped, violet-staining chromatin. There is abundant cytoplasm containing many eosinophilic (orange) secondary granules that are larger than neutrophil granules and more uniform in size.



Monocyte

Monocytes are the largest cell in the peripheral blood, variable in size but usually 15–22 µm in diameter. The nucleus is irregular in outline (often kidney shaped), and the chromatin is arranged in fine strands with sharply defined margins. The cytoplasm is light blue-grey and contains numerous fine dust-like granules. Some cells may contain a small number of red violet granules. Vacuolation may be present.



Basophil

A basophil is 10–16 µm in diameter with pale blue cytoplasm containing purple-black secondary granules. These granules are water soluble and may dissolve on staining leaving clear areas in the cytoplasm. The nucleus is segmented but is often obscured by basophilic granules which may vary in number, size and shape.

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

NORMAL LYMPHOCYTE MORPHOLOGY

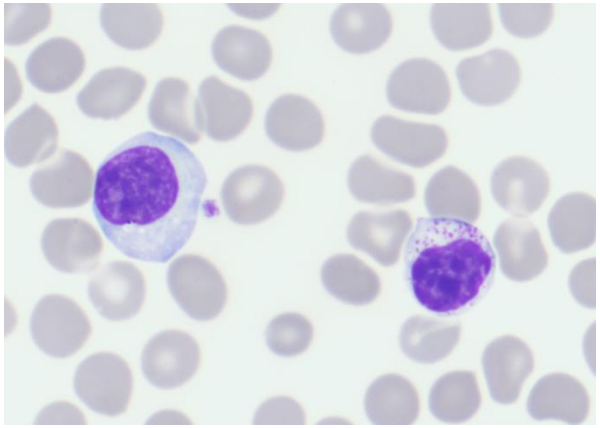
Lymphocyte

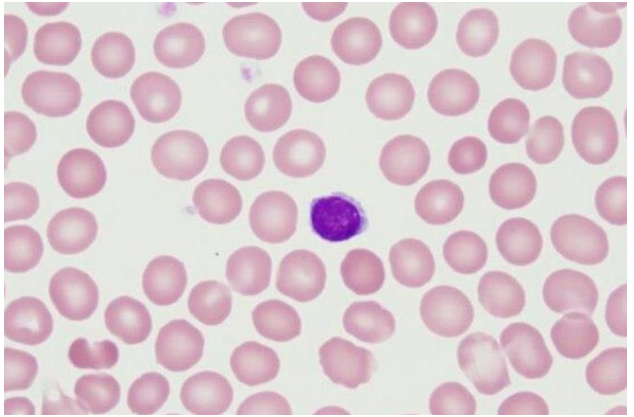
Lymphocytes seen in the peripheral blood are predominantly small (10–12 µm), or, less frequently large (12–16 µm).

Small lymphocytes are usually round in outline, and the nucleus is round with coarse, densely staining chromatin. Cytoplasm is scanty.

Large lymphocytes are usually irregular in outline, and the nuclear chromatin is not as coarse as in small lymphocytes. Cytoplasm is abundant and tends to be light sky blue in colour.

Large granular lymphocytes (LGLs) are of the same appearance as large lymphocytes but the cytoplasm contains prominent small red-violet granules. These cells can comprise up to 10–20% of the peripheral blood lymphocytes in normal subjects. LGLs are not routinely counted as a separate lymphocyte population.





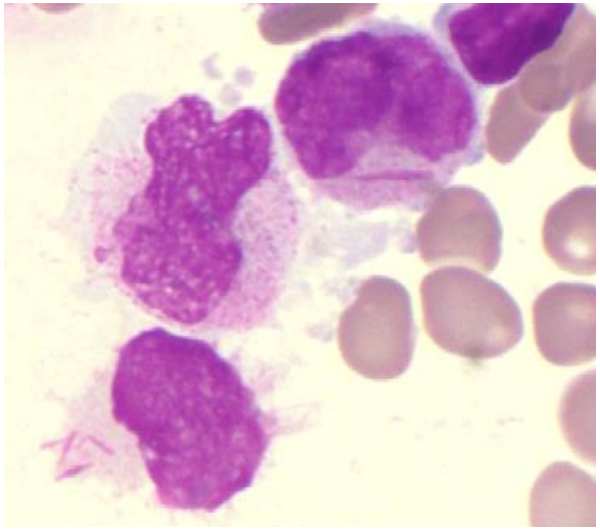
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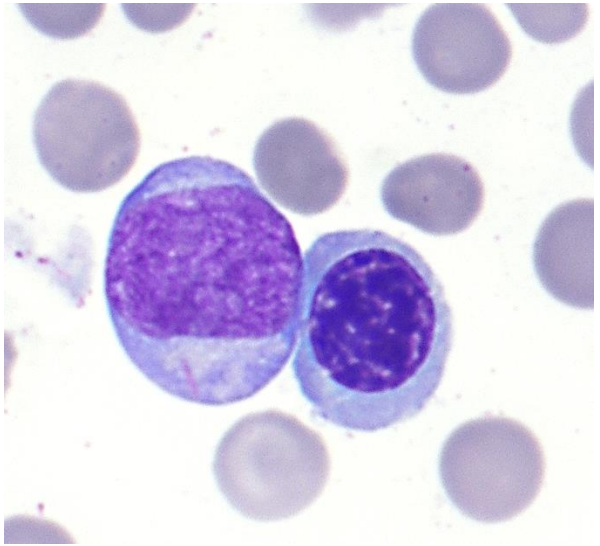
QUALITATIVE ABNORMALITIES IN MYELOID CELLS:

CYTOPLASMIC ABNORMALITIES

Auer rod

A sharply defined red rod or needle-like cytoplasmic inclusion formed by abnormal primary granule development. Found mainly in leukaemic myeloblasts or abnormal promyelocytes, they stain positively for myeloperoxidase and are a specific marker for myeloid lineage neoplasms. There may be several in a cell and may be arranged in bundles (faggots).





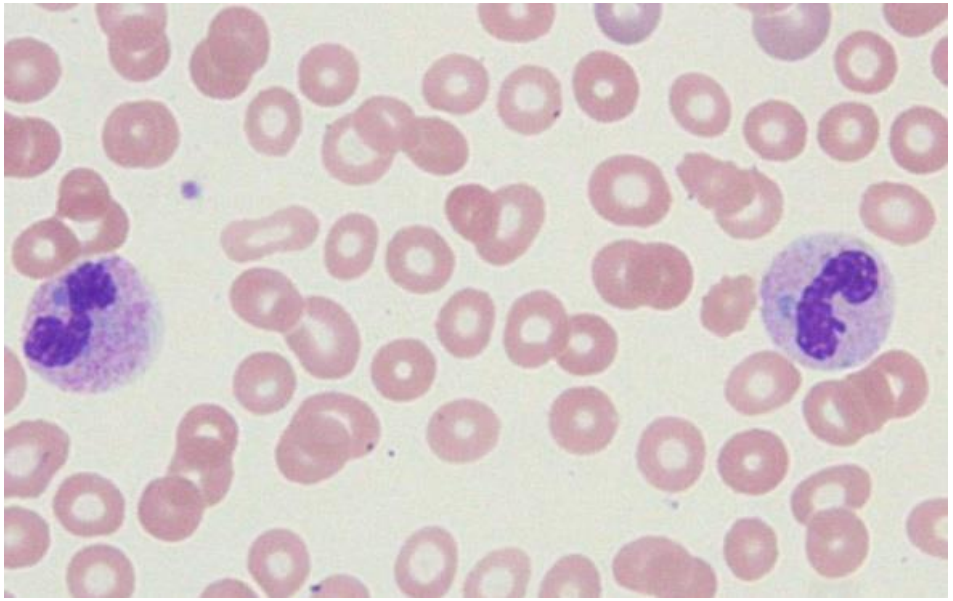
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

QUALITATIVE ABNORMALITIES IN MYELOID CELLS:

CYTOPLASMIC ABNORMALITIES

Döhle body

Pale light blue or grey, single or multiple, cytoplasmic inclusions found near the periphery of the neutrophil. Döhle bodies are a **non-specific reactive change** but may also indicate May-Hegglin anomaly if associated with thrombocytopenia and giant platelets. Döhle bodies may also be seen in patients on growth factor therapy such as granulocyte colony-stimulating factor (G-CSF).



ASH Image Bank

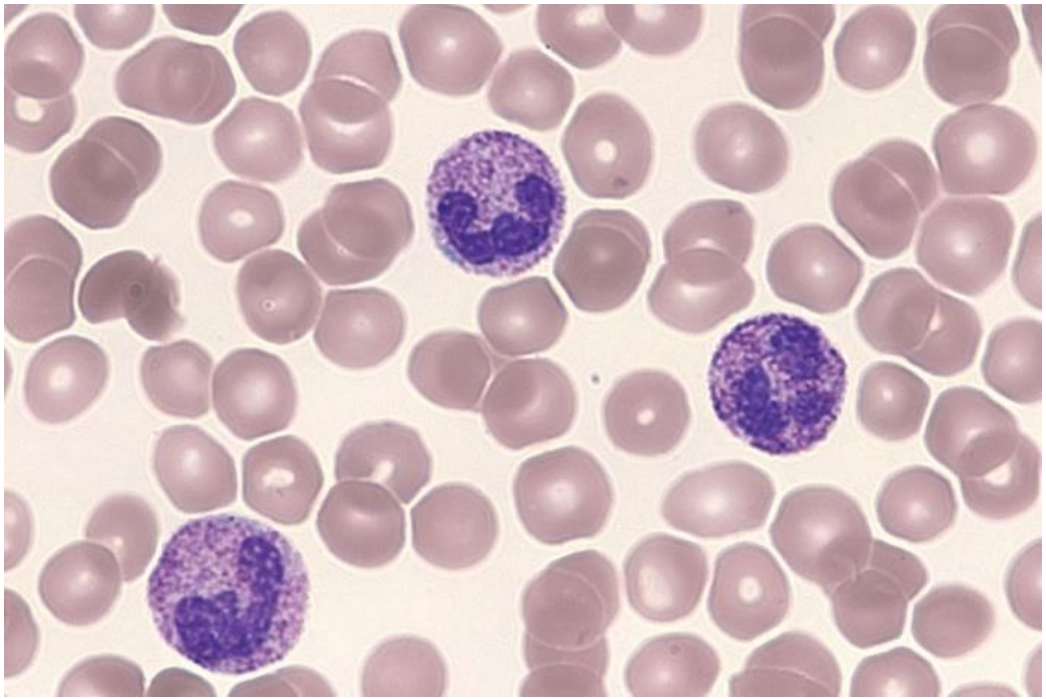
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

QUALITATIVE ABNORMALITIES IN MYELOID CELLS:

CYTOPLASMIC ABNORMALITIES

Hypergranulation – neutrophil (toxic granulation)

Coarse, purple staining primary (azurophilic) neutrophil cytoplasmic granules which occur as a **response to infection and inflammation**. A **non-specific reactive change**, it is a result of abnormal primary granule maturation with retention of their azurophilic staining properties.



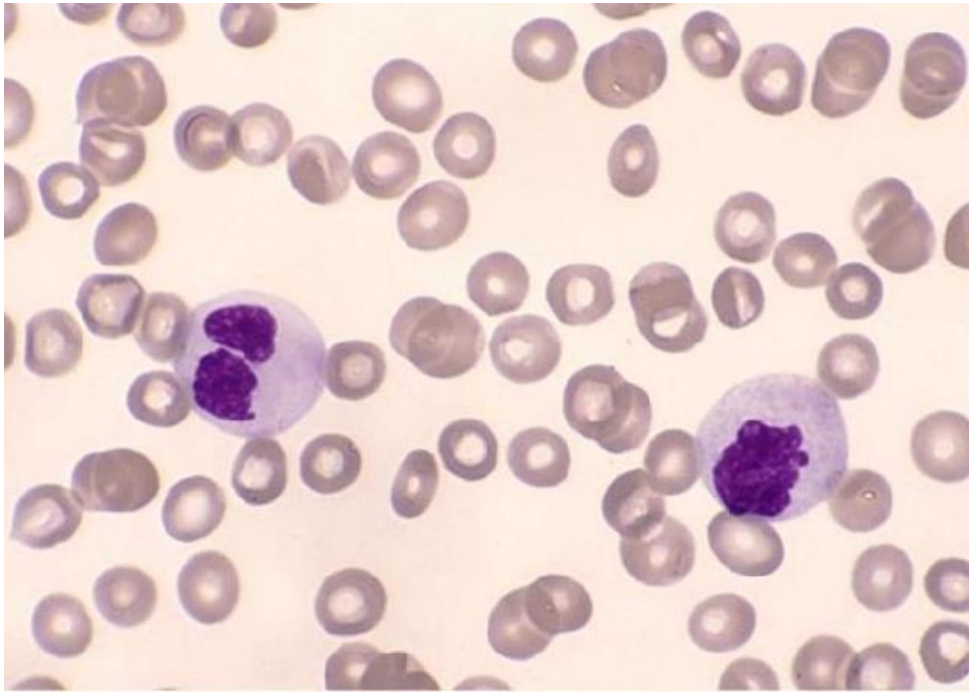
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

QUALITATIVE ABNORMALITIES IN MYELOID CELLS:

CYTOPLASMIC ABNORMALITIES

Hypogranulation – neutrophil

Reduced or absent neutrophil granulation causing the cytoplasm of mature neutrophils to appear blue grey. Frequently seen in Myelodysplastic Neoplasms (MDS) and other myeloid neoplasms. Often co-exists with nuclear abnormalities like the Pseudo-Pelger anomaly (hyposegmentation).



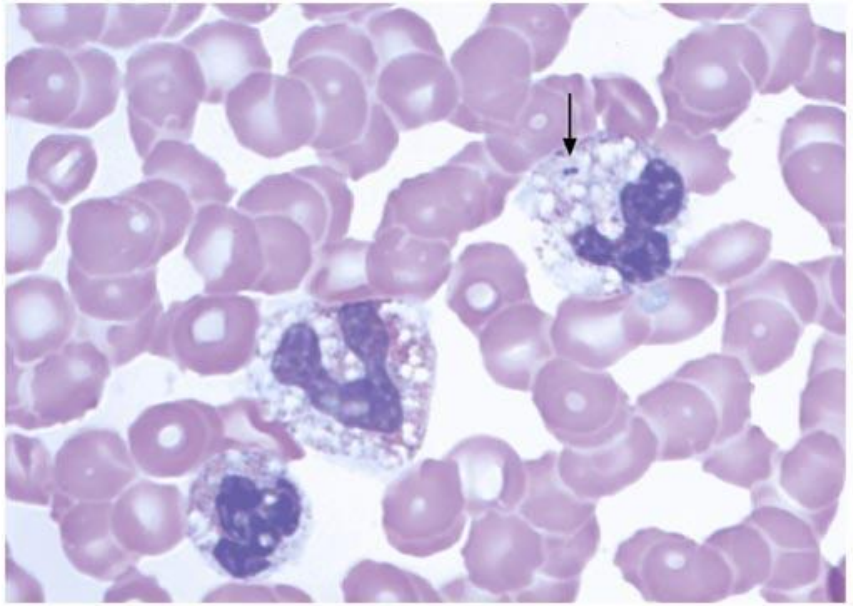
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

QUALITATIVE ABNORMALITIES IN MYELOID CELLS:

CYTOPLASMIC ABNORMALITIES

Vacuolation – neutrophil

Neutrophil cytoplasmic vacuolation in infection is due to granule fusion with a phagocytic vacuole and release of lysosomal contents to kill bacteria. This vacuolation may appear as 'pin-hole' vacuolation. Other causes of neutrophil vacuolation include alcohol toxicity and prolonged exposure to EDTA anticoagulant (storage artefact).



Hann CL et al. N Engl J Med 2004;351:2636

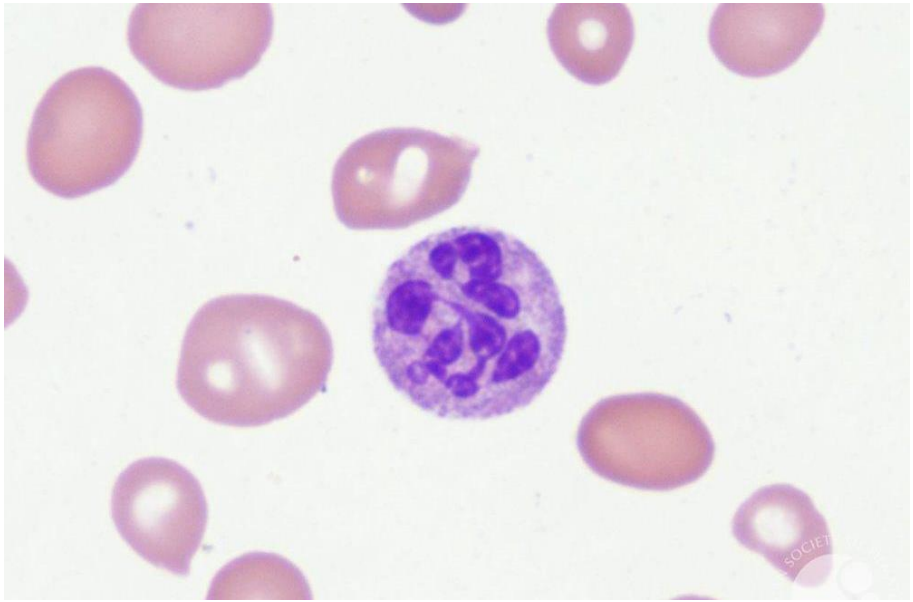
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

QUALITATIVE ABNORMALITIES IN MYELOID CELLS:

NUCLEAR ABNORMALITIES

Hypersegmented neutrophils

Normal neutrophils usually have 3–4 lobes (occasionally 2 and 5 lobes). Hypersegmented neutrophils have an increased number of distinct nuclear lobes with increased numbers of neutrophils having 5 or more nuclear segments. Neutrophil hypersegmentation is defined as any neutrophil having **6 or more lobes** or more than 3% of neutrophils having 5 lobes, when 100 neutrophils are examined.



Frequently associated with megaloblastic anemia (Vitamin B12 or Folate deficiency).

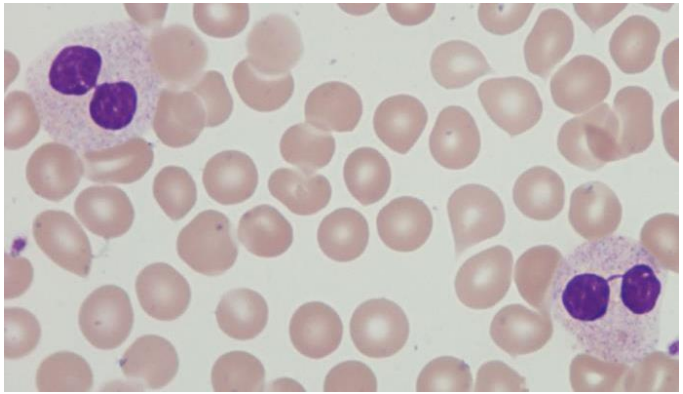
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QUALITATIVE ABNORMALITIES IN MYELOID CELLS:

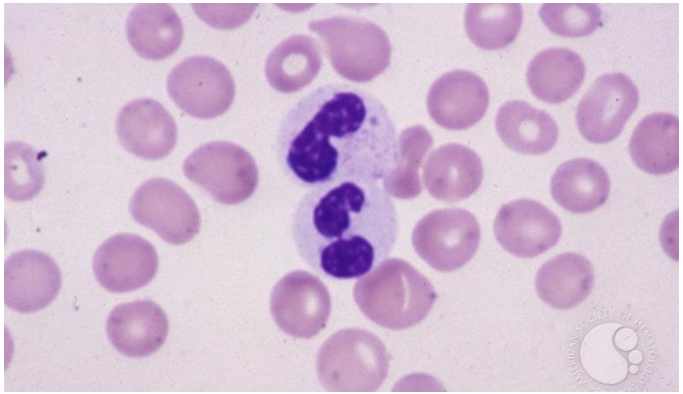
NUCLEAR ABNORMALITIES

Hyposegmented/ hypolobated neutrophils
(Pelger-Huët neutrophils e Pseudo-Pelger cells)

The Pelger-Huët anomaly is a benign inherited condition in which neutrophil nuclei fail to segment properly. The majority of circulating neutrophils have only two discrete equal-sized lobes connected by a thin chromatin bridge. The chromatin is coarsely clumped, and granule content is normal.



A similar acquired morphologic anomaly, known as **pseudo-Pelger cells or the acquired Pelger-Huët anomaly**, may be seen in myelodysplastic neoplasms, acute myeloid leukaemia with dysplastic maturation, and occasionally in chronic myelogenous leukaemia. The neutrophils are often hypogranular and they tend to have a markedly irregular nuclear pattern.



Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

QUALITATIVE ABNORMALITIES IN LYMPHOID CELLS

Lymphocyte morphology is subject to **wide variability** due to various immunological stimuli both in inflammatory and infectious diseases (particularly viral) as well as in neoplastic disorders (leukaemias and lymphomas), resulting in circulating lymphocytes with morphological abnormalities in various quantities.

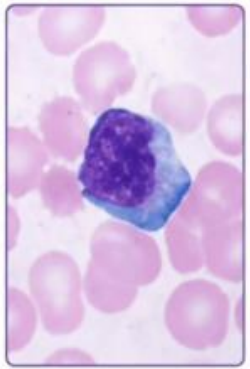
Terminology for these lymphocytes has been varied and confused with many different terms being used to describe the same thing including *variant, reactive, abnormal, activated and atypical lymphocytes, Downey cells Type1–3, Turk cells, immunoblasts*.

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

DOWNEY CLASSIFICATION OF REACTIVE LYMPHOCYTES

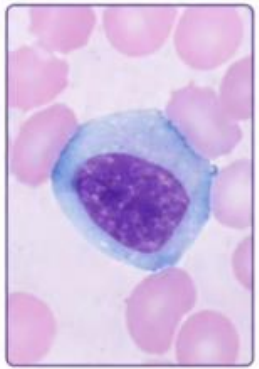
TYPE I

- Smaller size
- Indented to lobulated nucleus
- Cytoplasmic granules



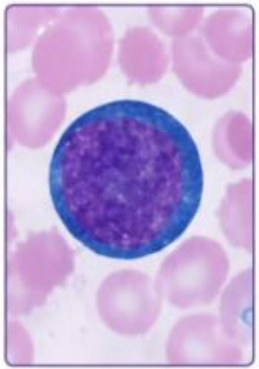
TYPE II

- Most common type
- Abundant agranular cytoplasm, darker at the periphery, molds around RBCs
- Radiating basophilia



TYPE III

- "Immunoblasts"
- Fine to coarse chromatin
- Nucleoli
- Deeply basophilic cytoplasm

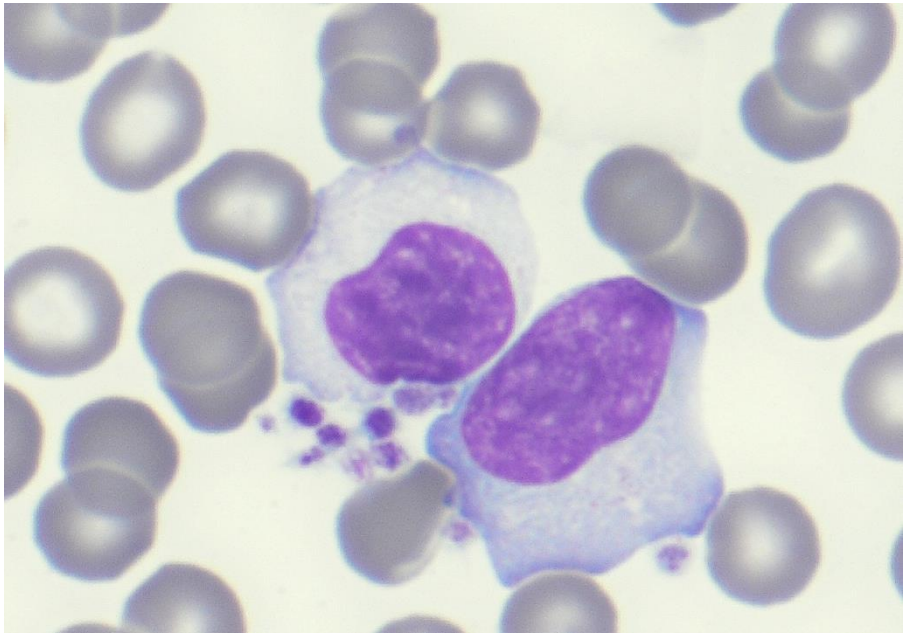


QUALITATIVE ABNORMALITIES IN LYMPHOID CELLS

Reactive lymphocytes (atypical lymphocyte, suspect reactive – European LeukemiaNet classification)

Abnormalities include increased cell size, immaturity of the nucleus including a visible nucleolus and lack of chromatin condensation, irregular nuclear outline or lobulation, cytoplasmic basophilia and vacuolation and irregular cell outline. The cytoplasm may be abundant with staining varying from pale blue to markedly basophilic especially at points of contact with adjacent cells.

The recommendation is to comment on the presence of reactive lymphocytes. They may be counted as a separate population in the differential if they are present in significant numbers.

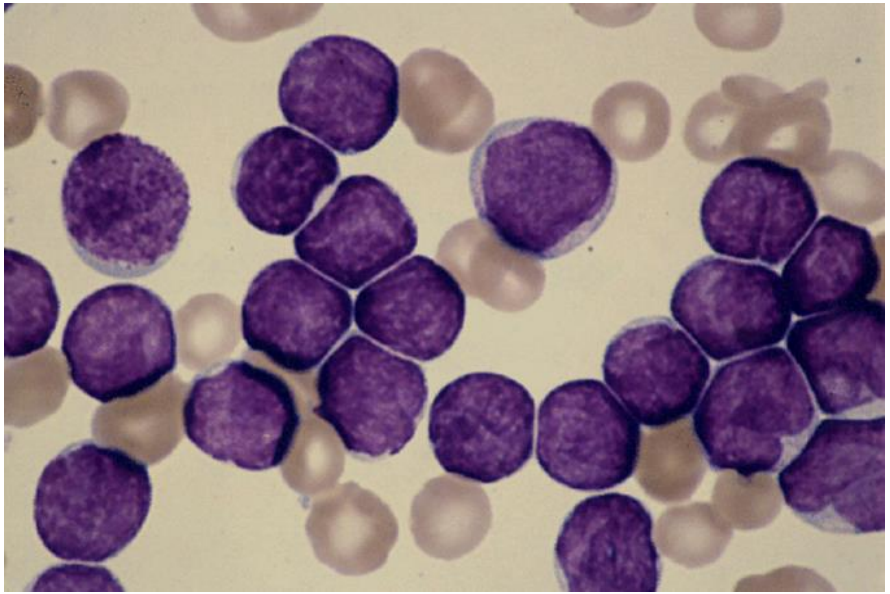


Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

QUALITATIVE ABNORMALITIES IN LYMPHOID CELLS

Abnormal lymphocytes (atypical lymphocyte, suspect neoplastic– European LeukemiaNet classification)

It is recommended that abnormal lymphoid cells that can be identified as a particular neoplastic cell type.
Limited diagnostic value of morphology in the lymphoproliferative neoplasms where the final diagnosis is determined by immunophenotyping by flow cytometry.



Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

QUALITATIVE ABNORMALITIES IN LYMPHOID CELLS



It is recommended that ***reactive lymphocyte*** is used to describe lymphocytes with a benign aetiology and ***abnormal lymphocyte,*** with an accompanying description of the cells, is used to describe lymphocytes with a suspected malignant or clonal aetiology.

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

WHEN SHOULD LEUKEMIA BE SUSPECTED?

Peripheral blood findings

- Circulating blasts—sometimes only a small proportion
- Anemia and/or thrombocytopenia, often involving more than one cell line
- Leukocytosis, leukopenia, or a normal total white-cell count
- Abnormal differential, immature cells, or an unexpected analyzer flag

Clinical clues that raise concern

- Persistent pallor, fatigue, fever, or recurrent infections
- Unexplained bruising, petechiae, or bleeding
- Bone or joint pain, limp, or refusal to walk
- Lymphadenopathy, hepatosplenomegaly, or unexplained deterioration

Absence of circulating blasts does NOT exclude acute leukemia

PERIPHERAL BLOOD MORPHOLOGY: LYMPHOBLASTS *vs* MYELOBLASTS

Feature	Lymphoblasts (ALL)	Myeloblasts (AML)
Cell size	Often small to intermediate; variable	Often intermediate to large; variable
Nucleus / cytoplasm	Usually high ratio	Variable
Chromatin & nucleoli	Chromatin often relatively condensed; nucleoli variable	Chromatin may be fine; nucleoli often prominent
Cytoplasmic granules	Usually absent	May be present
Auer rods	Absent	If present, strongly support myeloid differentiation

Lineage assignment relies primarily on immunophenotyping, integrated with cytogenetic and molecular studies

PERIPHERAL BLOOD MORPHOLOGY: LEUKAEMIA SUSPECT ALERTS

Morphological red flags

Blasts population (alert for Burkitt cells)

Auer rods or abnormal promyelocytes

Cytopenia

Cytosis with myeloyd and/or erithroid precursors

Next steps

Urgent clinical evalutation

Bone marrow evaluation and flow cytometry

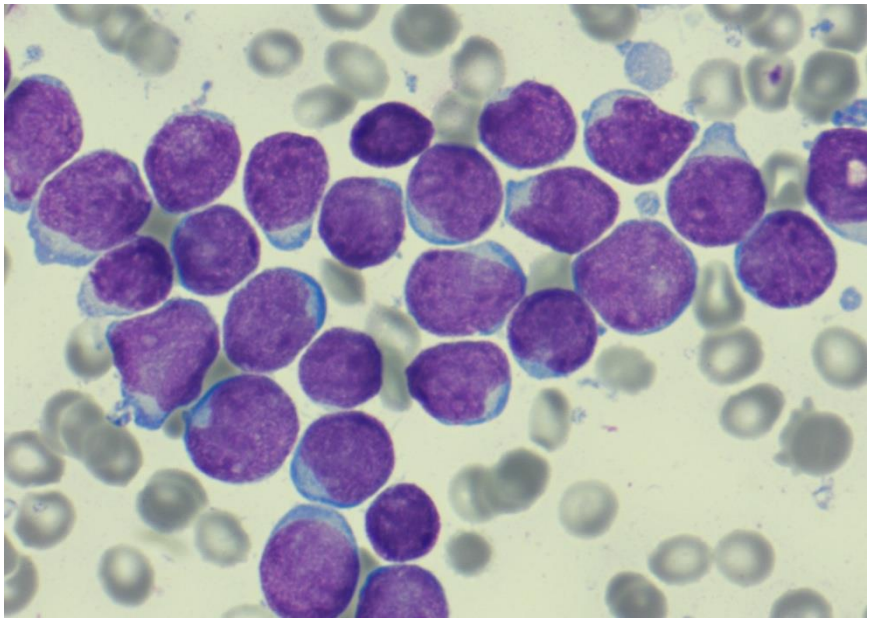
Cytogenetic and molecular testing for classification and risk stratification

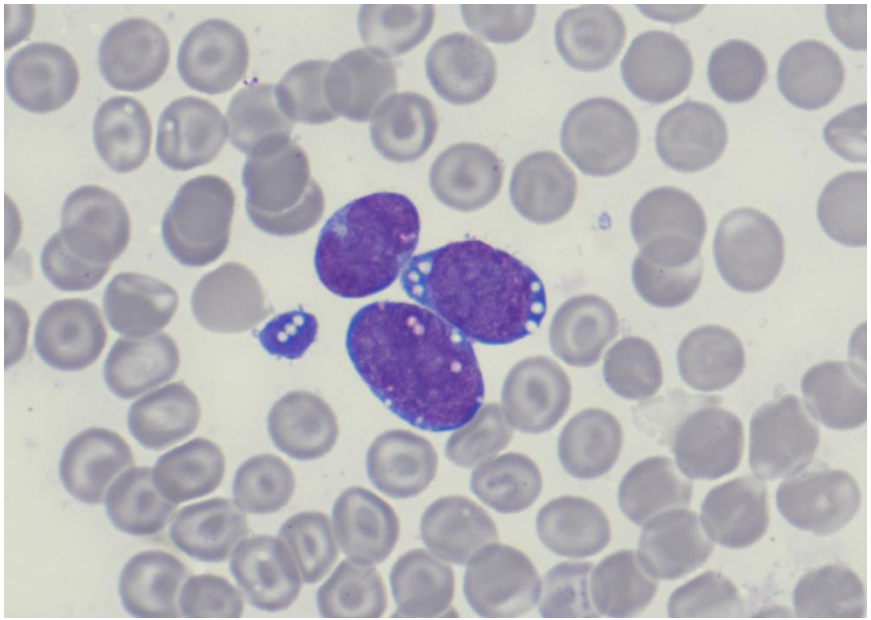


Suspected acute promyelocytic leukemia is a medical emergency because of potentially life-threatening coagulopathy

PERIPHERAL BLOOD MORPHOLOGY: SPOT THE CLUES

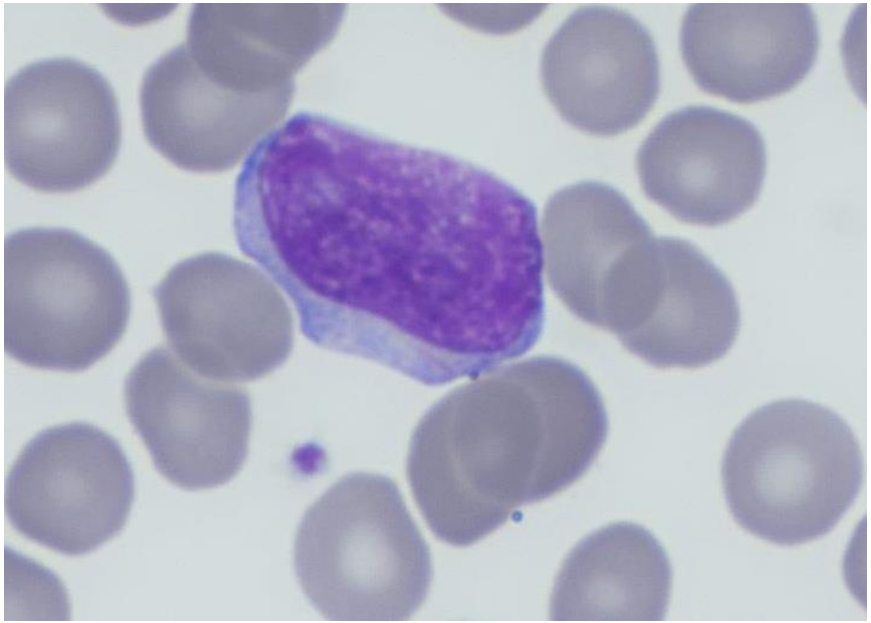
What is the most likely diagnosis?

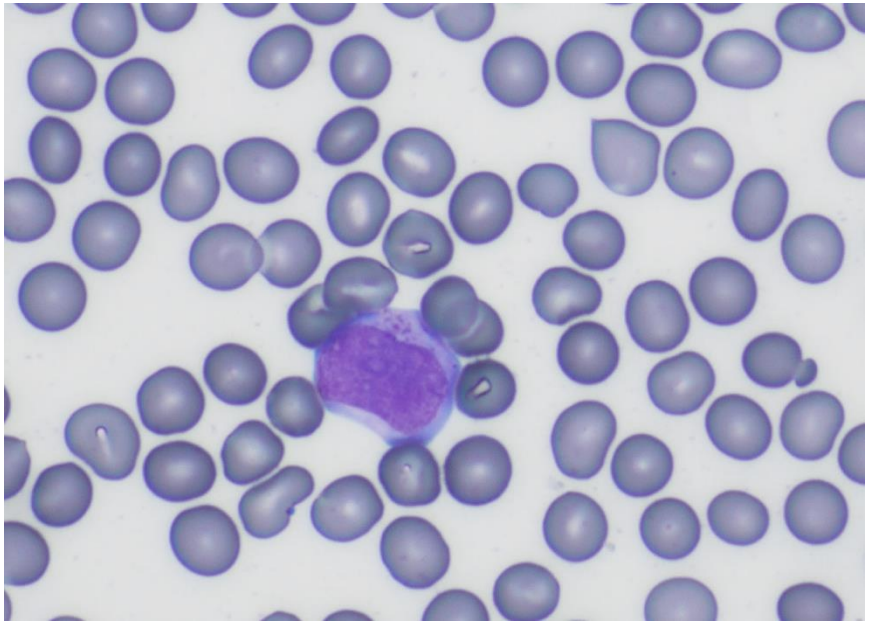




PERIPHERAL BLOOD MORPHOLOGY: SPOT THE CLUES

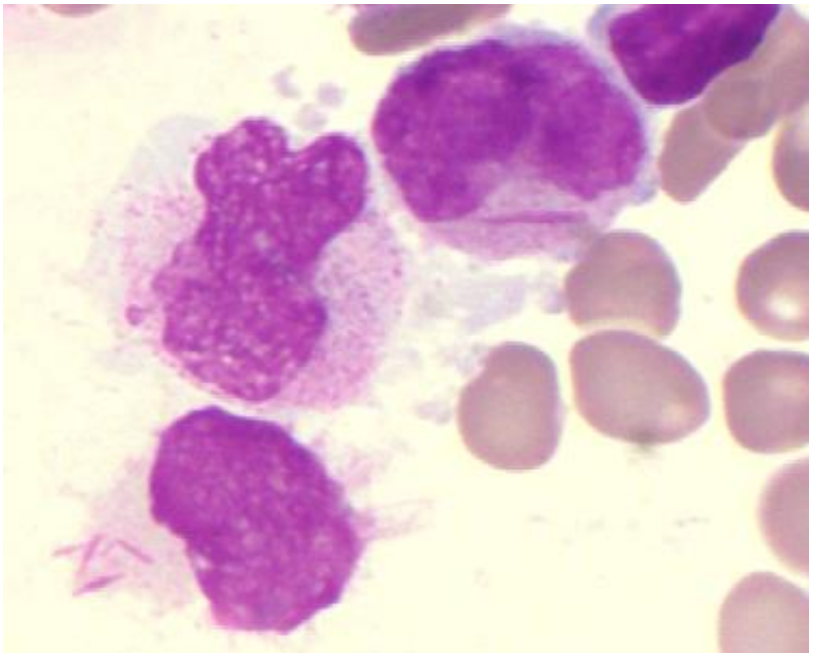
What is the most likely diagnosis?

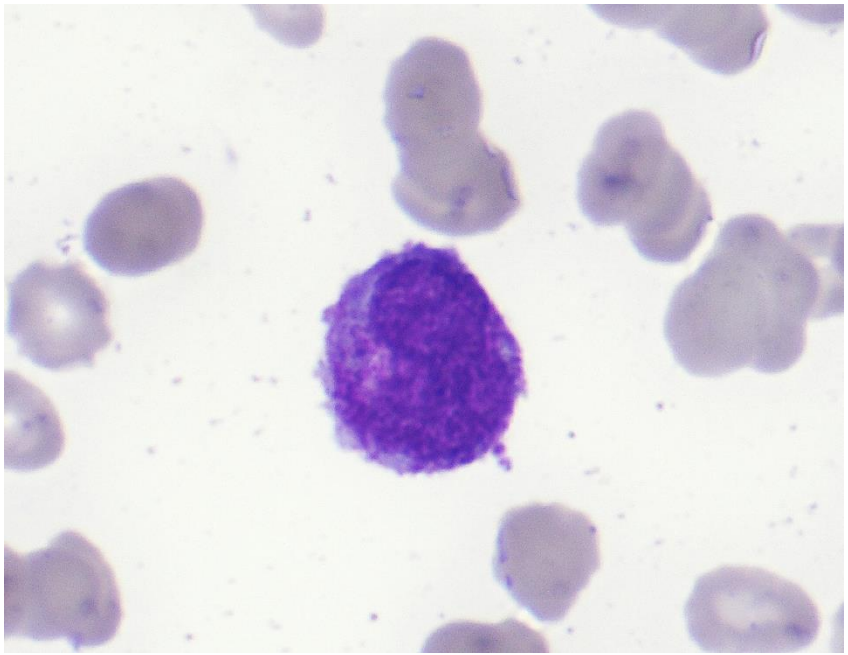




PERIPHERAL BLOOD MORPHOLOGY: SPOT THE CLUES

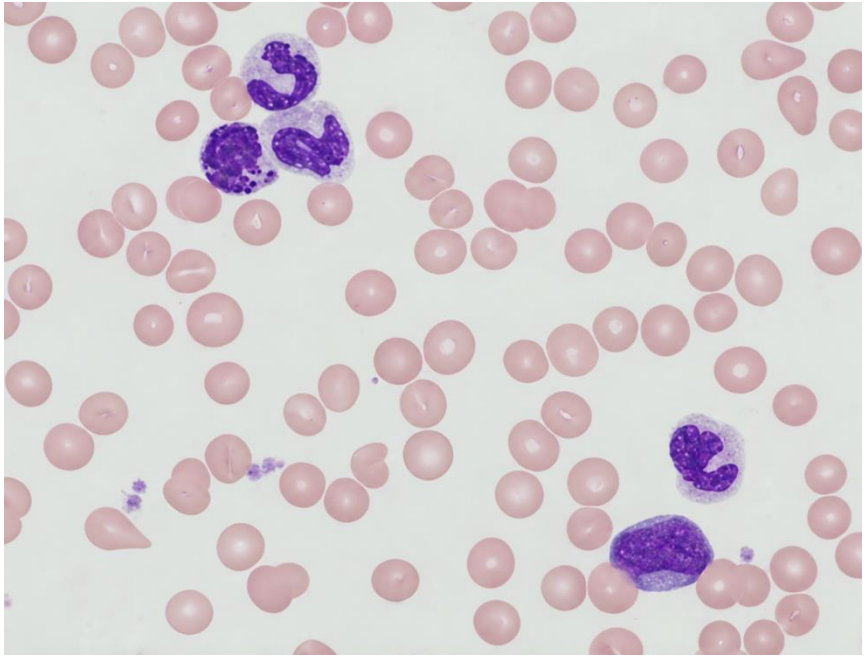
What is the most likely diagnosis?

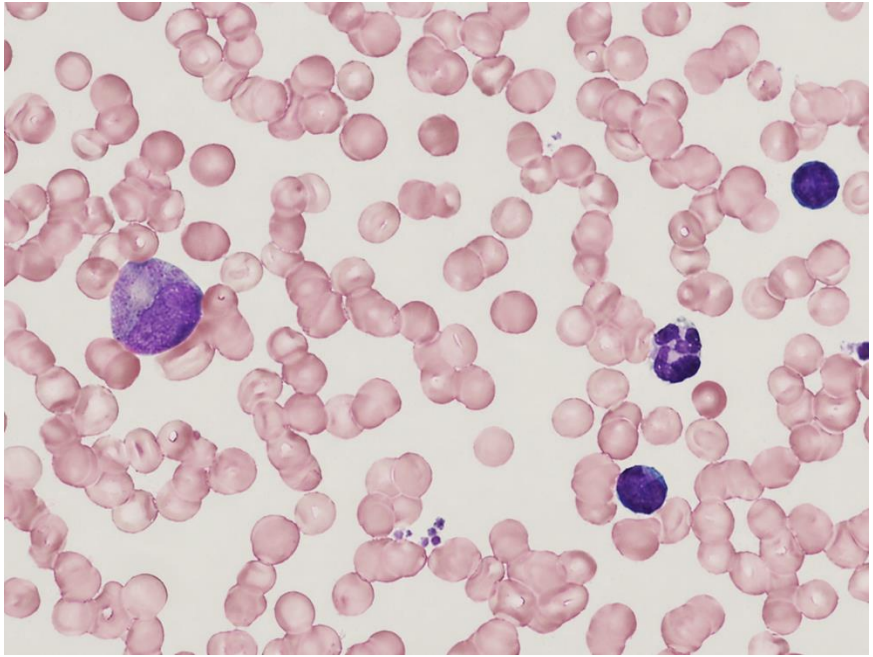




PERIPHERAL BLOOD MORPHOLOGY: SPOT THE CLUES

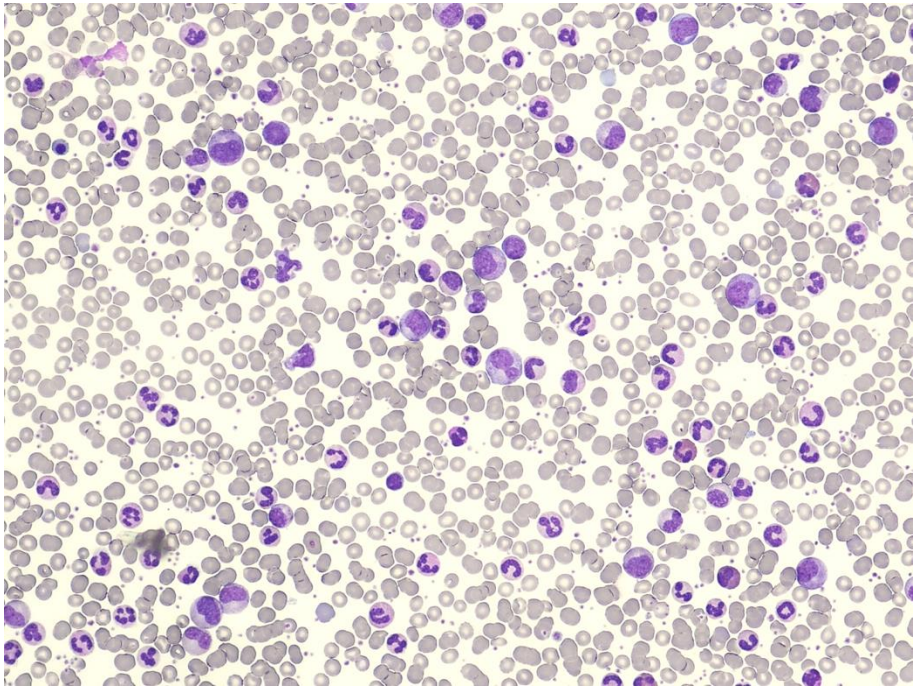
What is the most likely diagnosis?

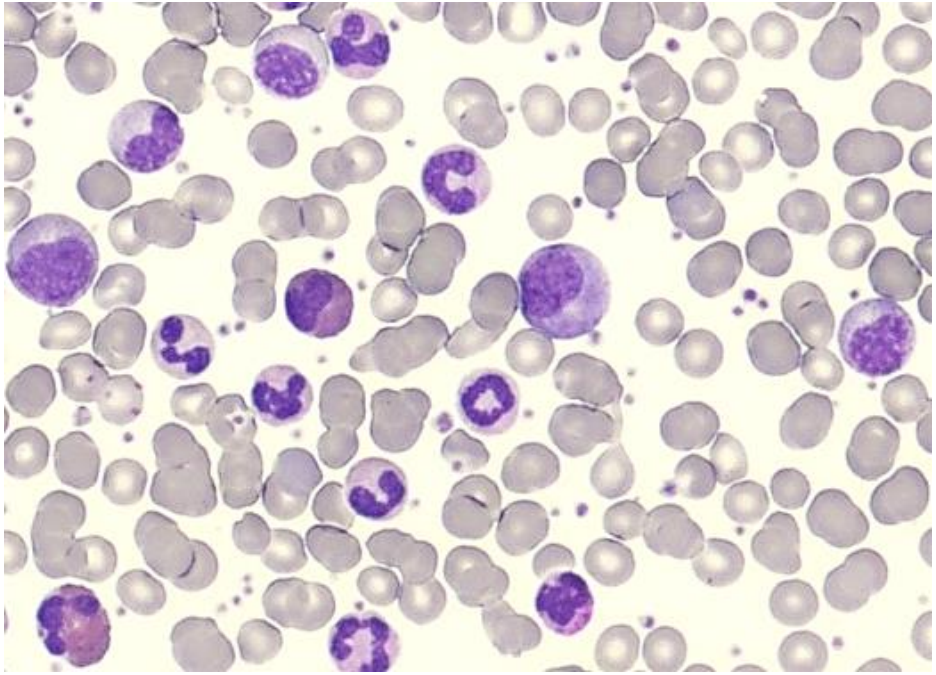


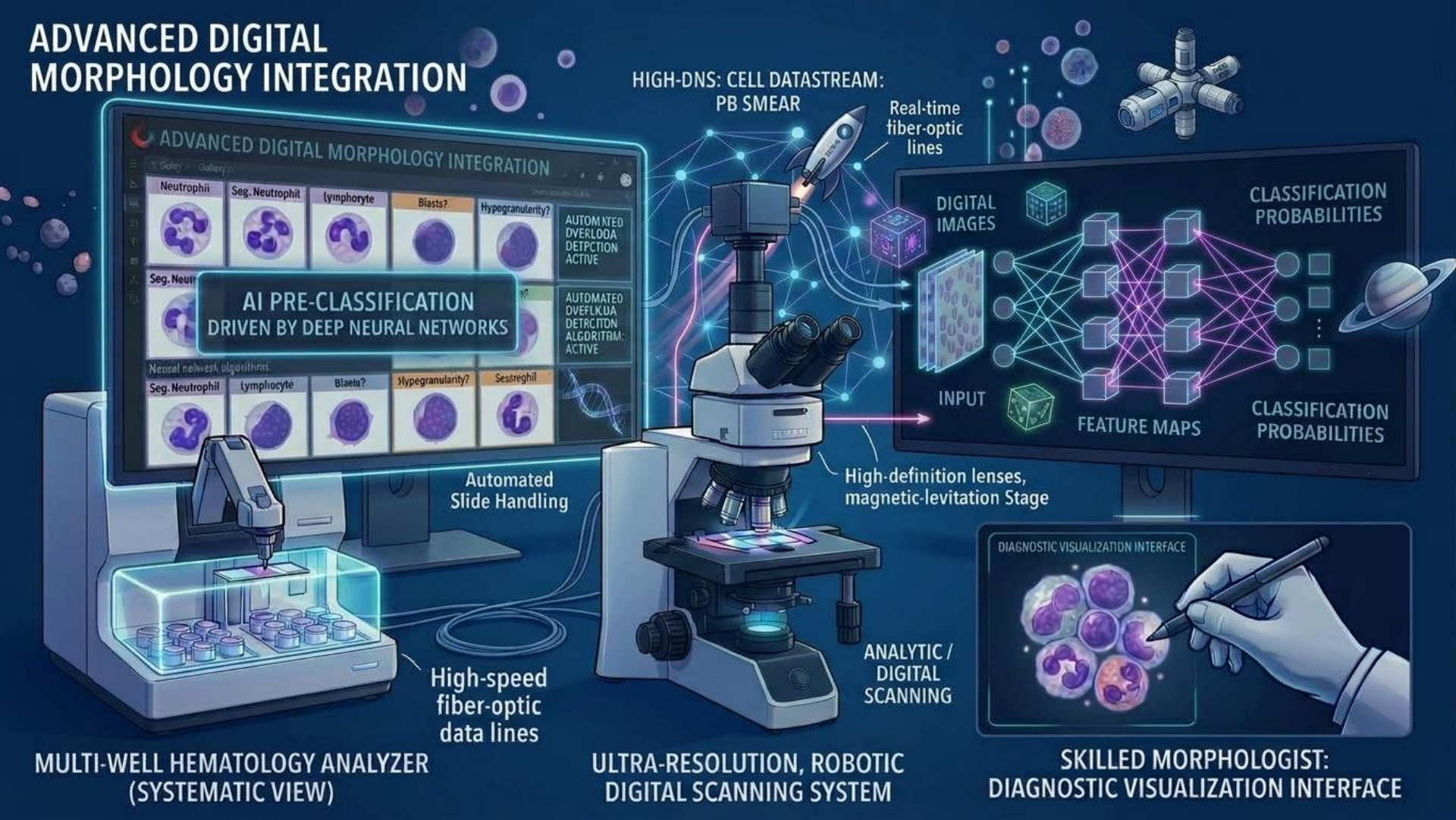


PERIPHERAL BLOOD MORPHOLOGY: SPOT THE CLUES

What is the most likely diagnosis?






ADVANCED DIGITAL MORPHOLOGY INTEGRATION
HIGH-DNS: CELL DATASTREAM: PB SMEAR
Real-time fiber-optic lines
ADVANCED DIGITAL MORPHOLOGY INTEGRATION
Neutrophil
Seg. Neutrophil
Lymphocyte
Blasts?
Hypogranularity?
AUTOMATED DVERLOAD DETECTION ACTIVE
AI PRE-CLASSIFICATION
DRIVEN BY DEEP NEURAL NETWORKS
AUTOMATED DVERLOAD DETECTION ALGORITHM: ACTIVE
Seg. Neutrophil
Lymphocyte
Blasts?
Hypogranularity?
Segmented
DIGITAL IMAGES
CLASSIFICATION PROBABILITIES
INPUT
FEATURE MAPS
CLASSIFICATION PROBABILITIES
Automated Slide Handling
High-definition lenses, magnetic-levitation Stage
DIAGNOSTIC VISUALIZATION INTERFACE
High-speed fiber-optic data lines
ANALYTIC / DIGITAL SCANNING
MULTI-WELL HEMATOLOGY ANALYZER (SYSTEMATIC VIEW)
ULTRA-RESOLUTION, ROBOTIC DIGITAL SCANNING SYSTEM
SKILLED MORPHOLOGIST: DIAGNOSTIC VISUALIZATION INTERFACE

DIGITAL MORPHOLOGY ANALYZERS IN HEMATOLOGY: ICSH REVIEW AND RECOMMENDATIONS

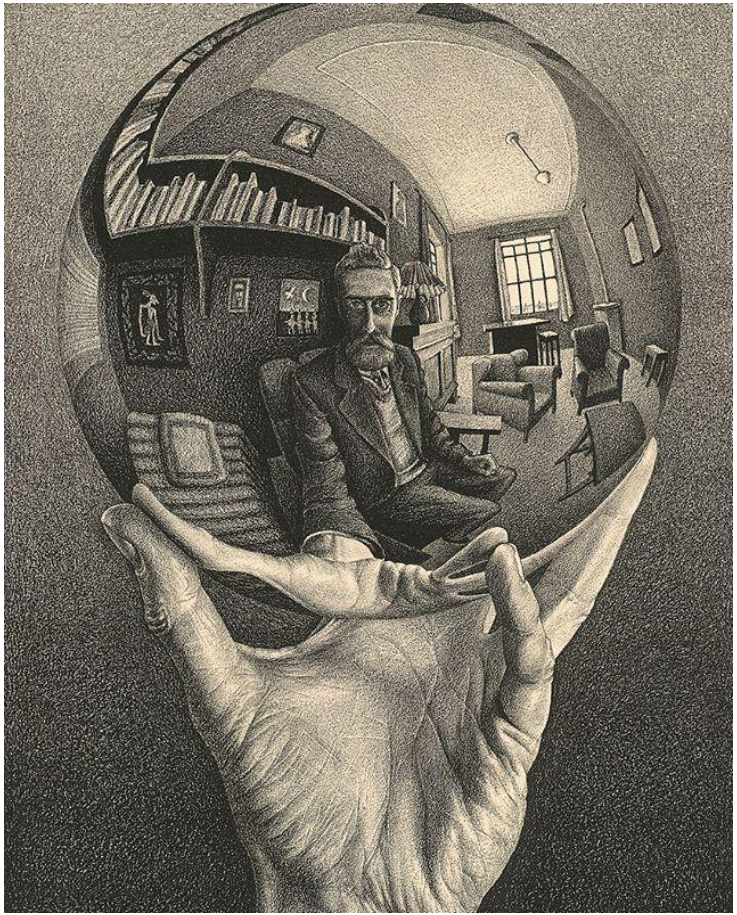
Not all pathological cells are reliably detected, and manual review remains necessary for certain cases (e.g., neonates, leukemia, suspected dysplastic cells, schistocytes, intracellular parasites, RBC agglutinates, and platelet clumps).

- Manufacturers of new instruments should focus on improving the accuracy of cell preclassifications, and the automated recognition and classification of pathological cell types.

- Cut-offs for grading morphological abnormalities should depend on clinical significance.

With all current devices, a skilled morphologist remains essential for cell reclassification and diagnostic interpretation of the blood smear.

Kratz A et al. Int J Lab Hematol. 2019;41:437–447.



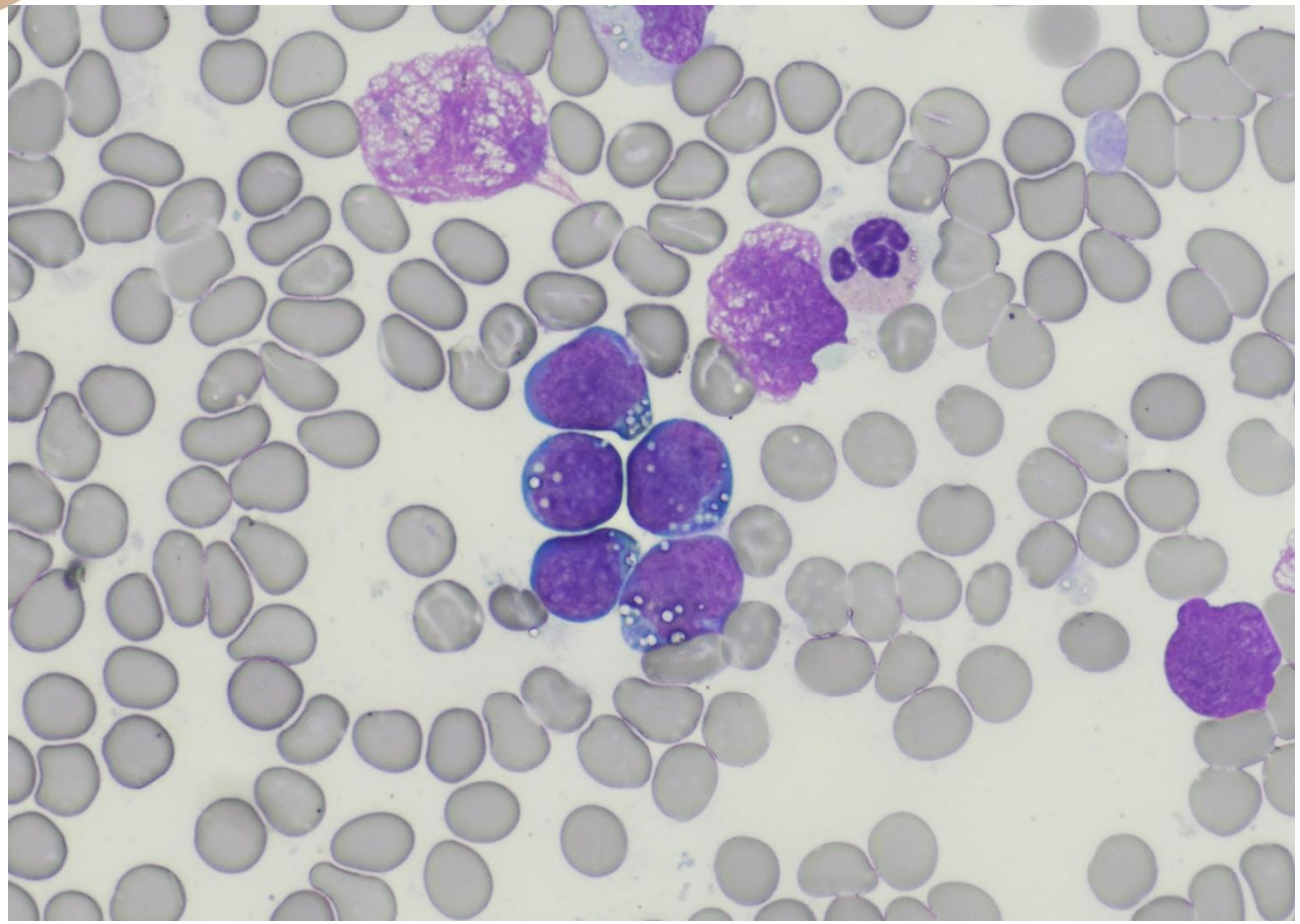
Grazie!

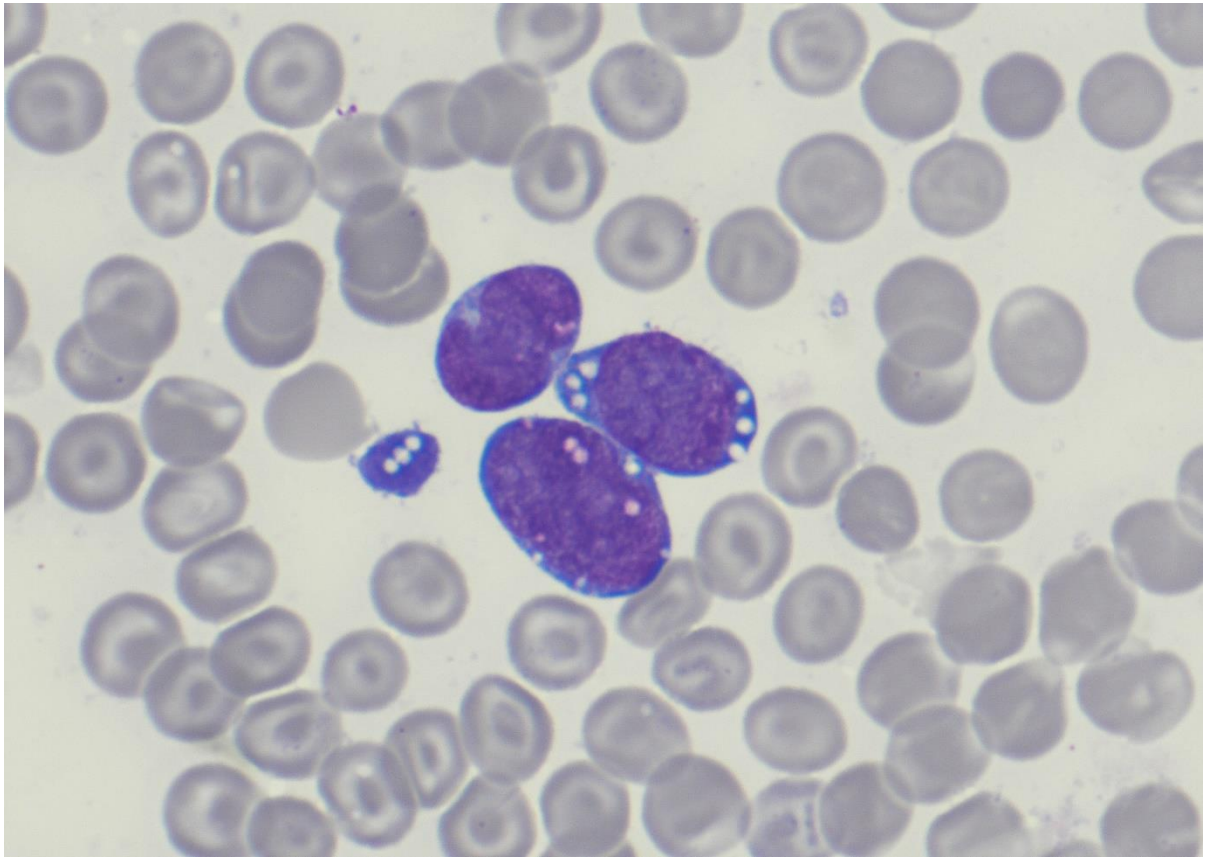
Look again, the first interpretation is not always the final one

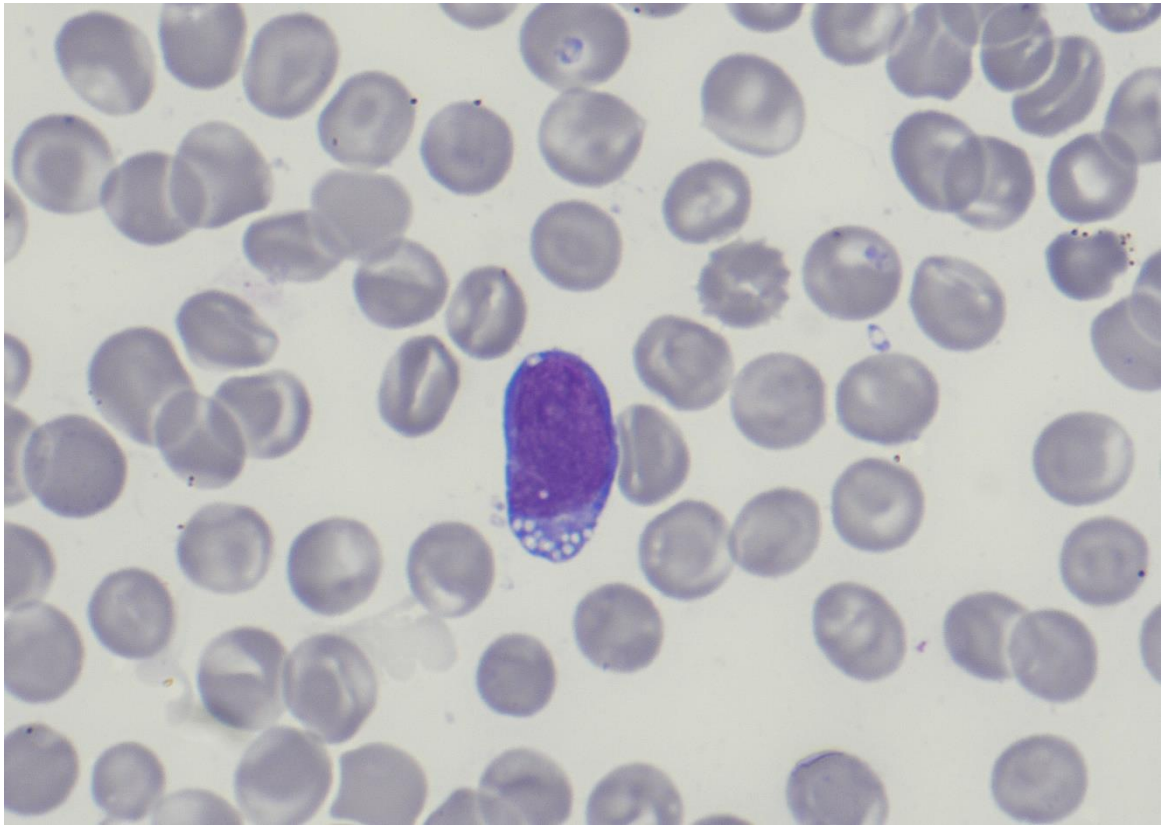
DIGITAL MORPHOLOGY ANALYZERS IN HEMATOLOGY: ICSH REVIEW AND RECOMMENDATIONS

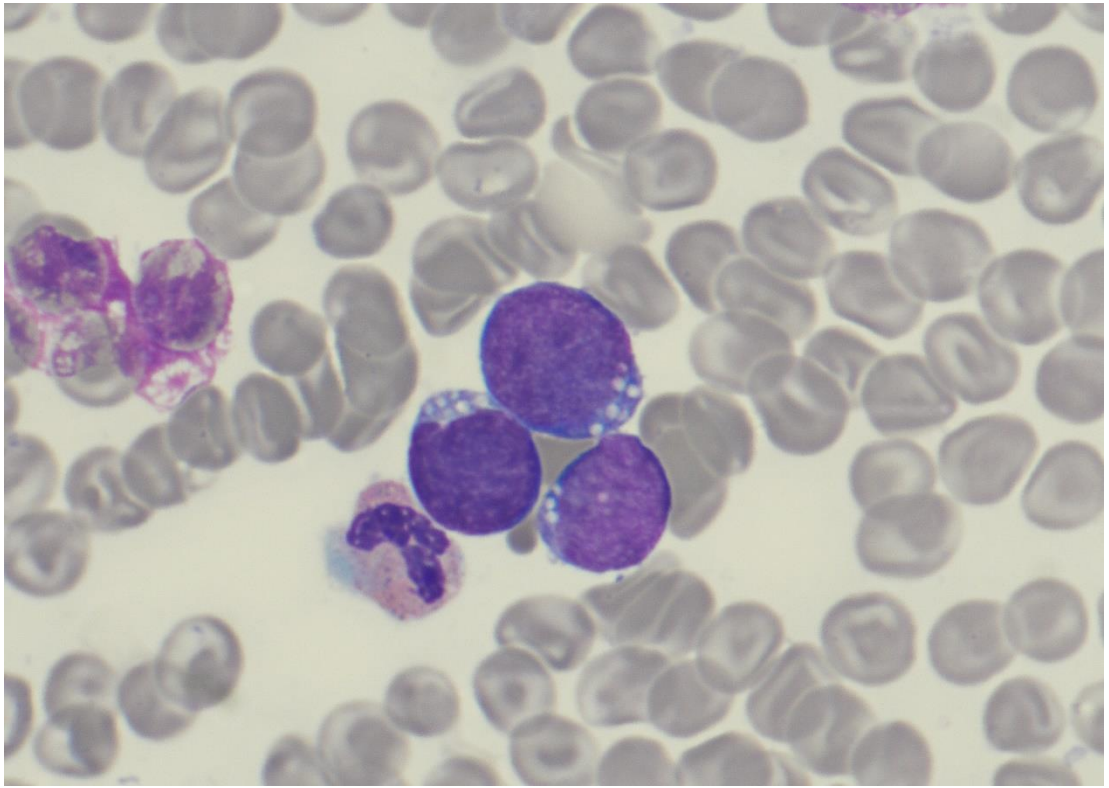
- Morphological assessment of blood smears has traditionally relied on manual microscopy. Recent advances in digital imaging and information technology have enabled the development of automated digital morphology analyzers.
- A panel of laboratory hematology experts reviewed the literature on digital imaging for blood smear analysis, evaluating strengths and weaknesses and proposing improvements. Digital analyzers use artificial intelligence to preclassify cells, speeding up slide reviews and enabling remote consultations, digital archiving, education, and quality assurance.
- Different instruments from several manufacturers are available, but *there is a lack of standardization of staining methods, optical magnifications, color and display characteristics, hardware, software, and file formats.*
- In order to realize the full potential of Digital Morphology Hematology Analyzers, *pre-analytic, analytic, and post analytic parameters should be standardized*.

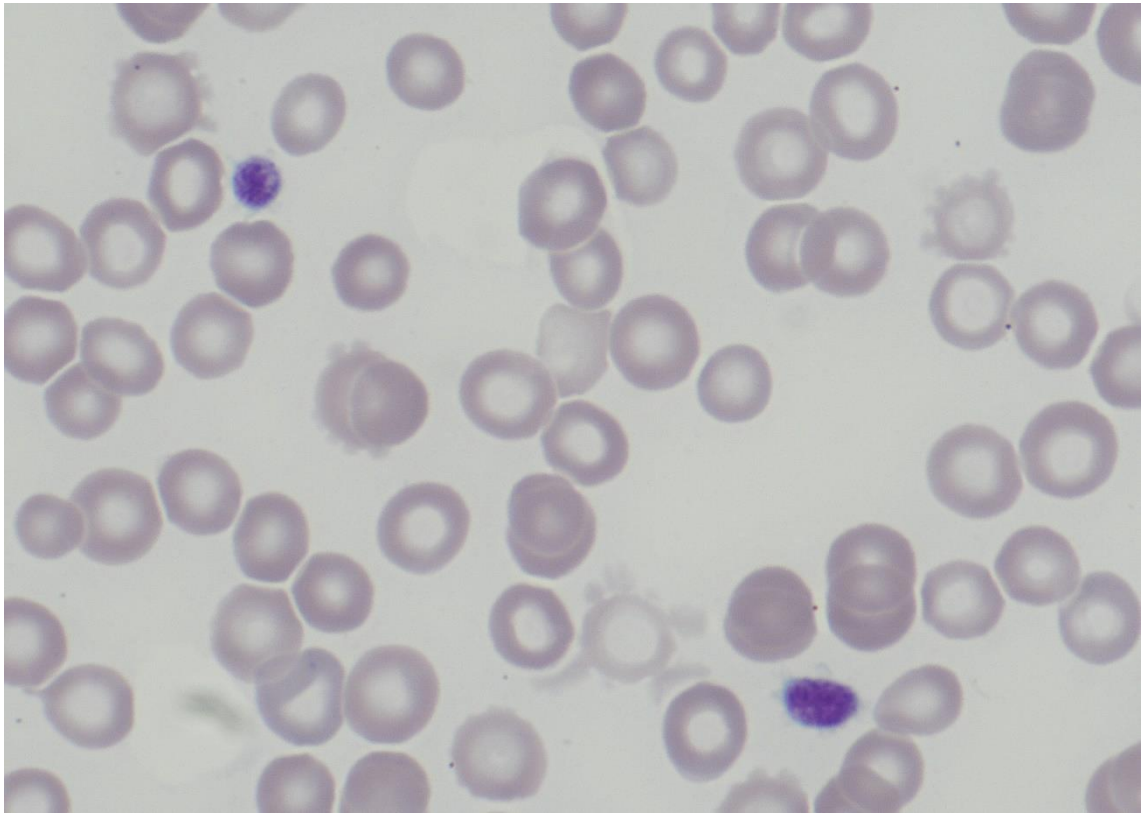
Kratz A et al. Int J Lab Hematol. 2019;41:437–447.

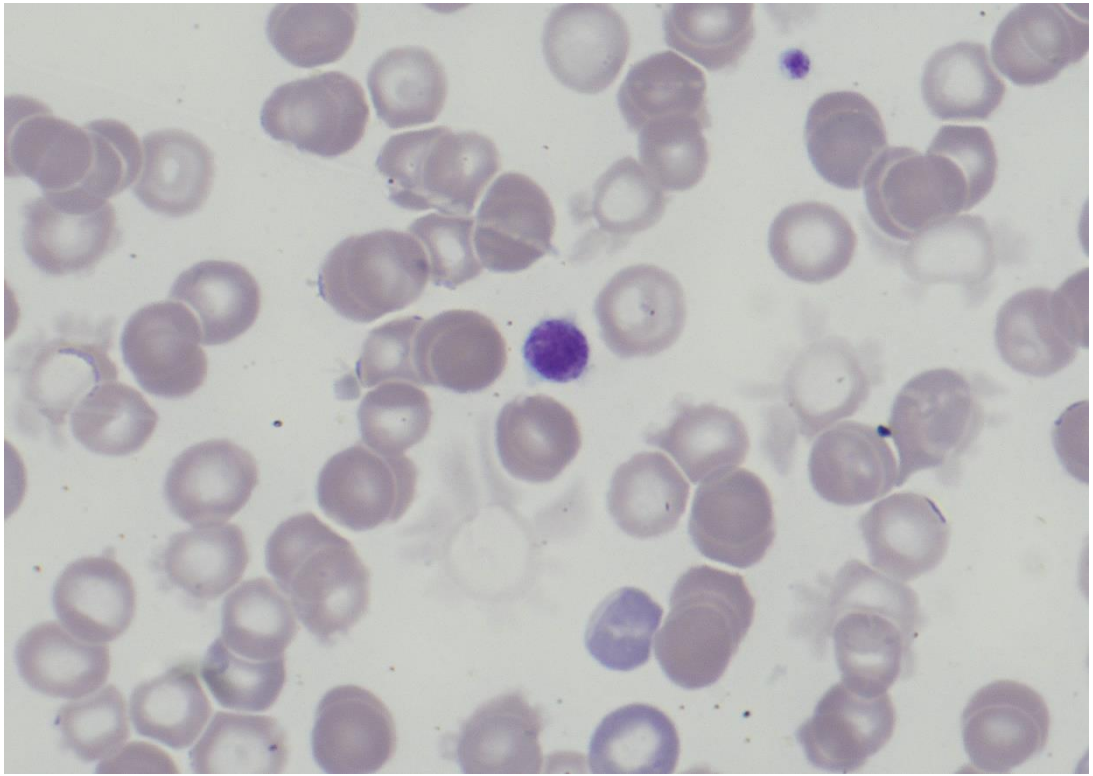


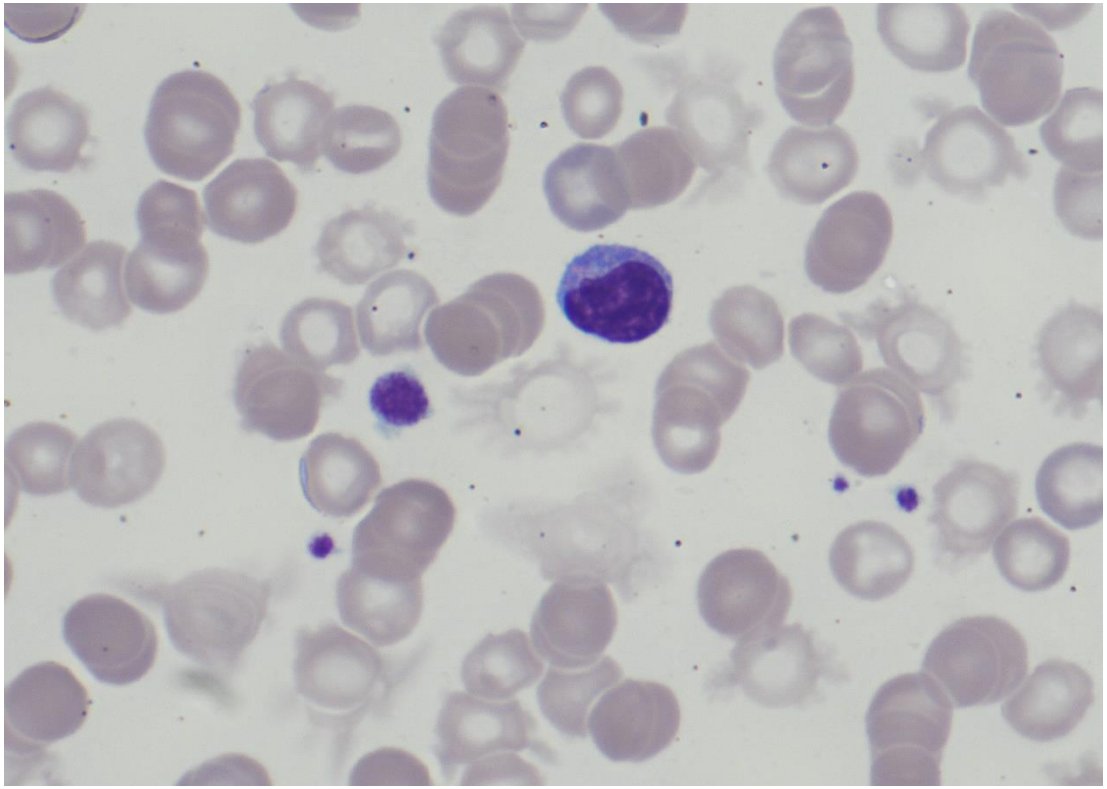


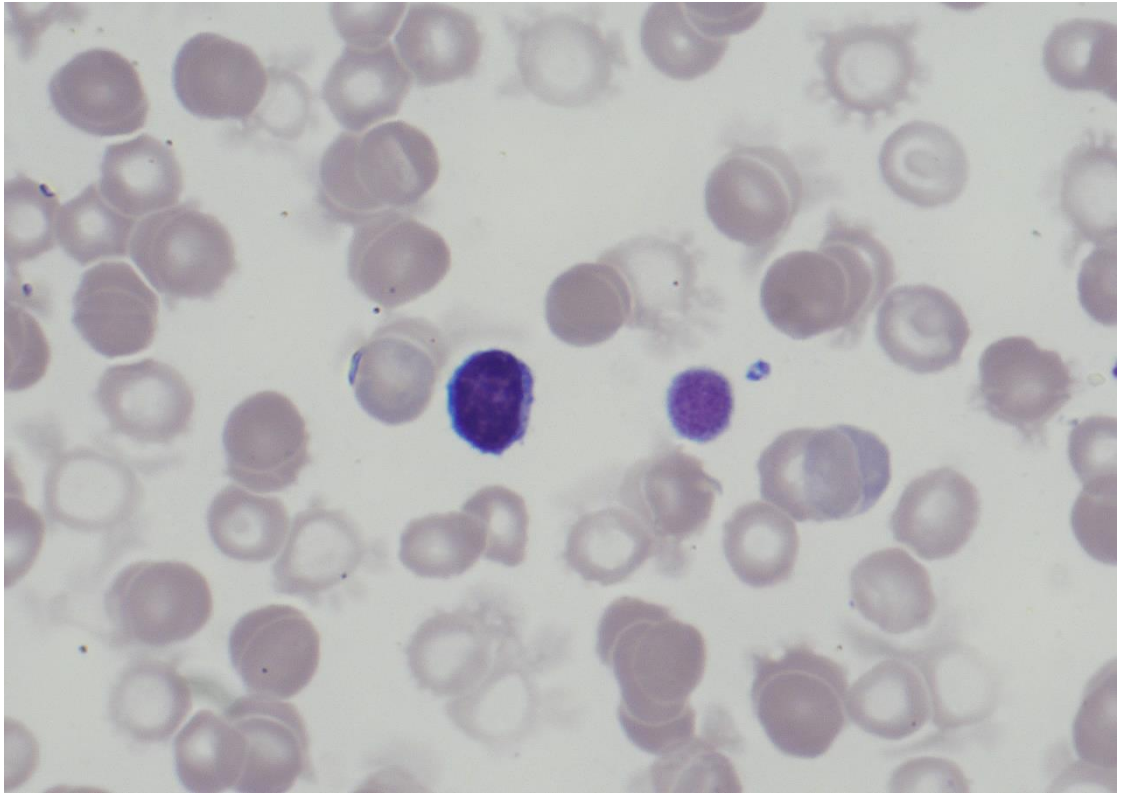




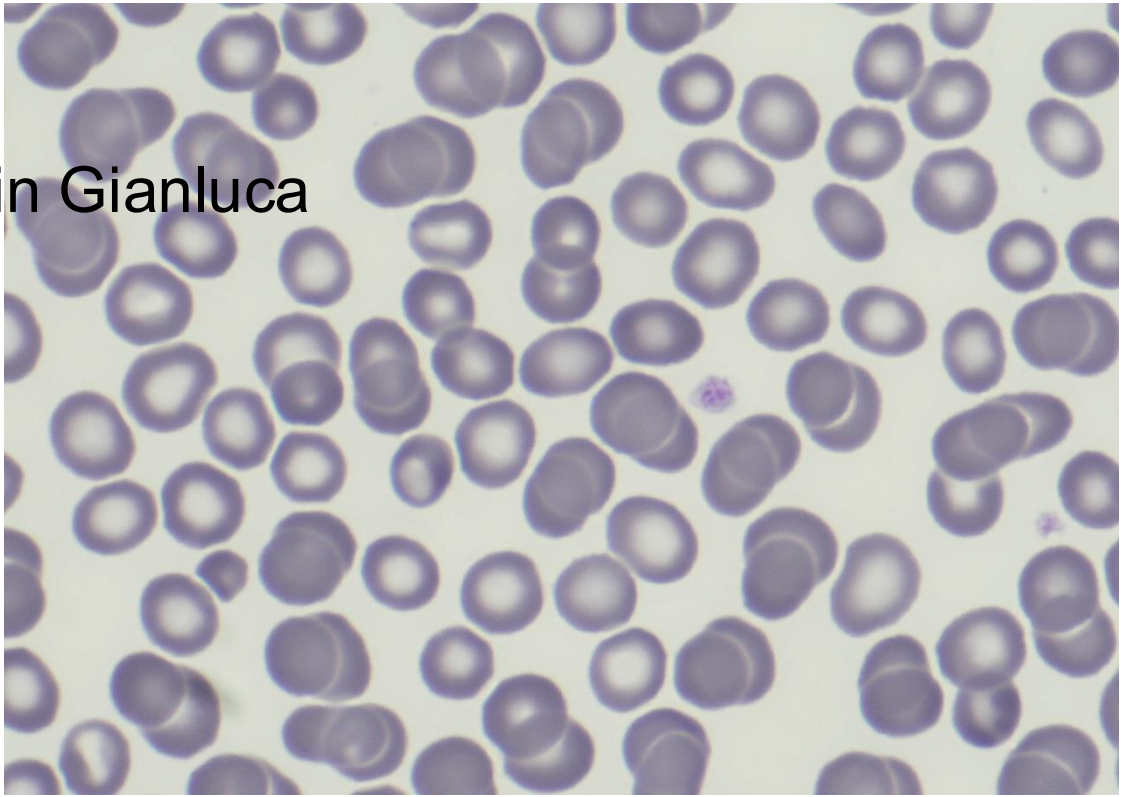


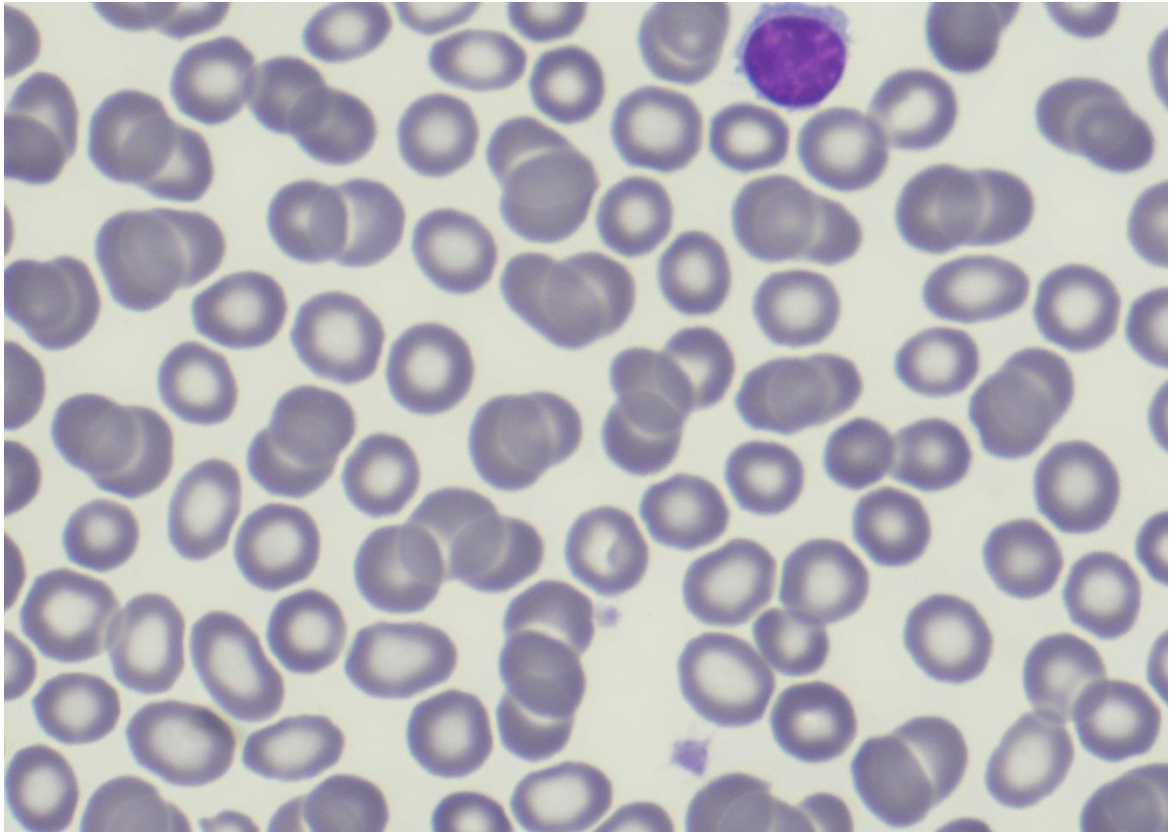


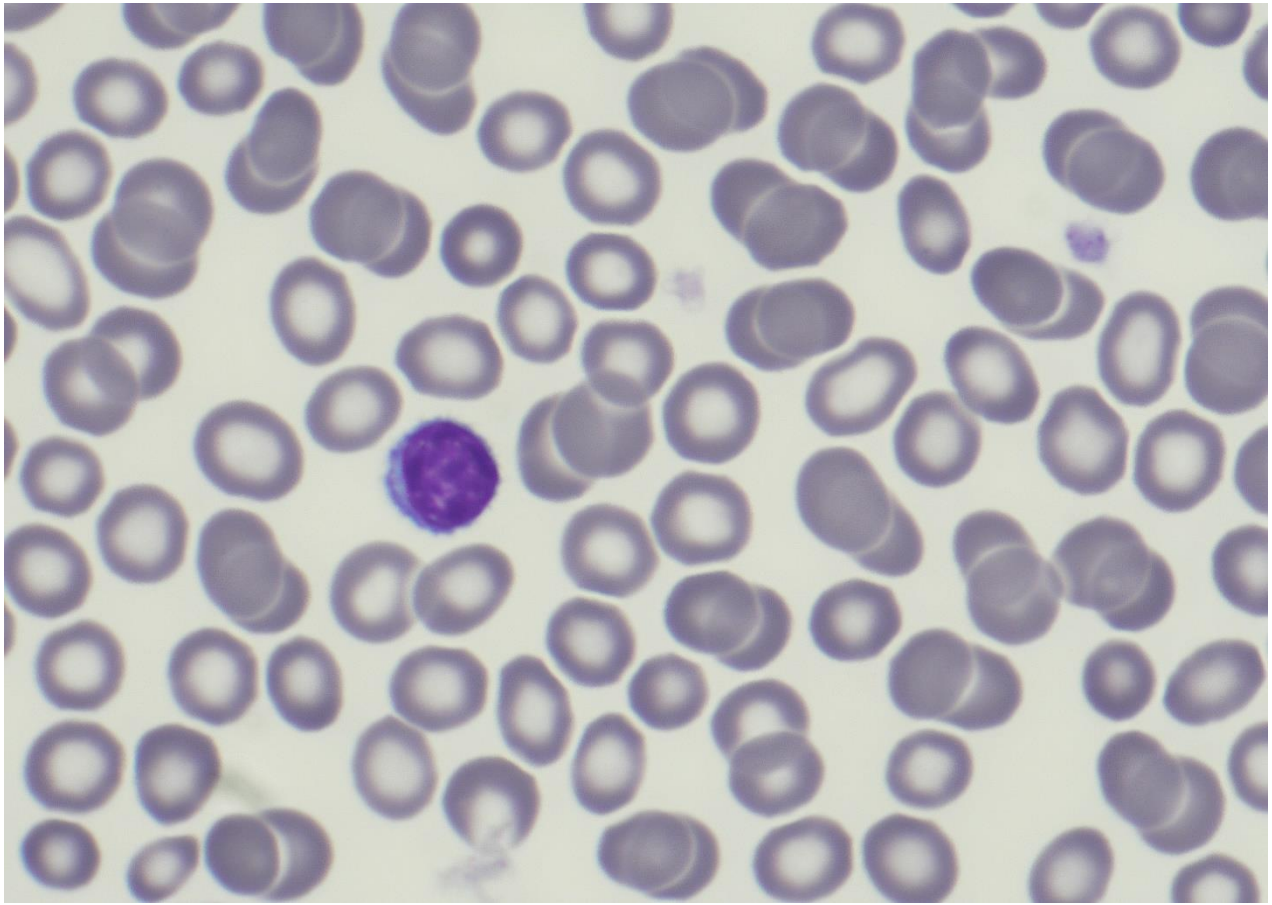


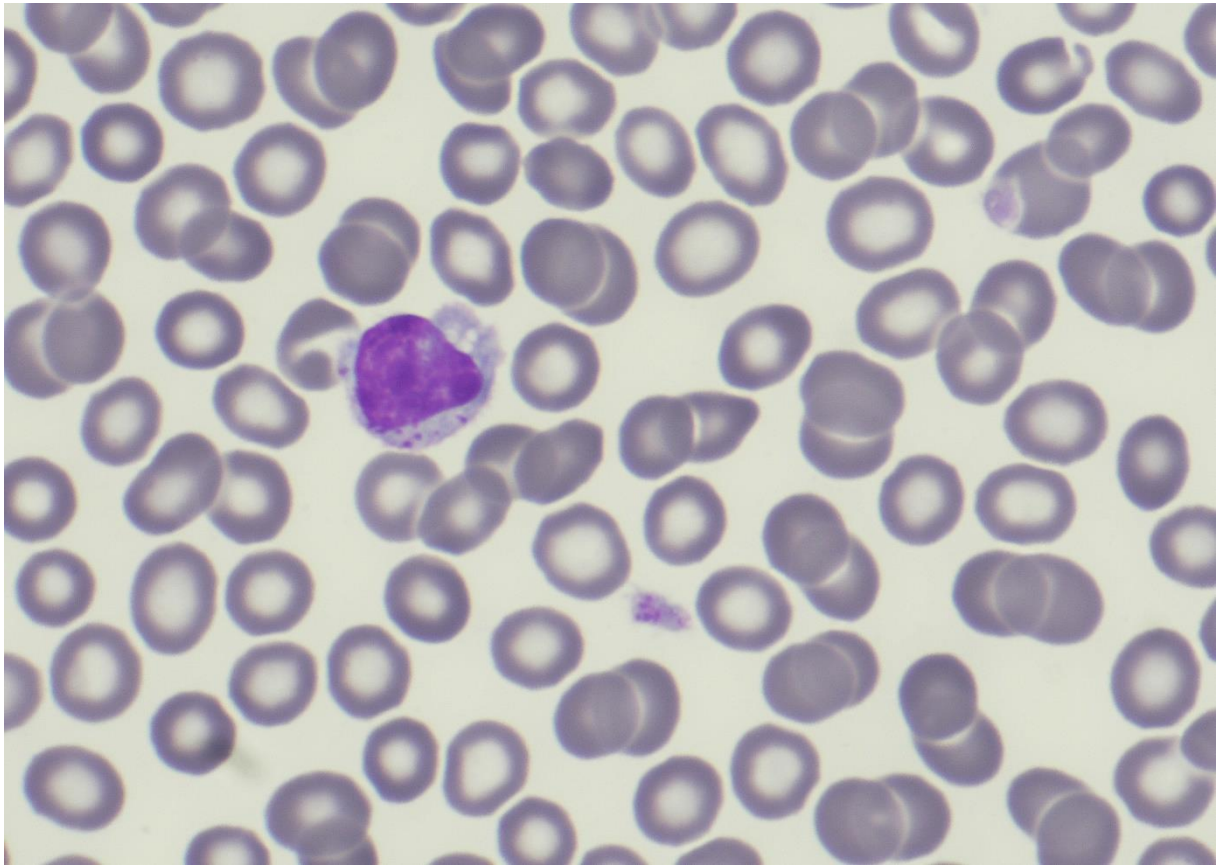


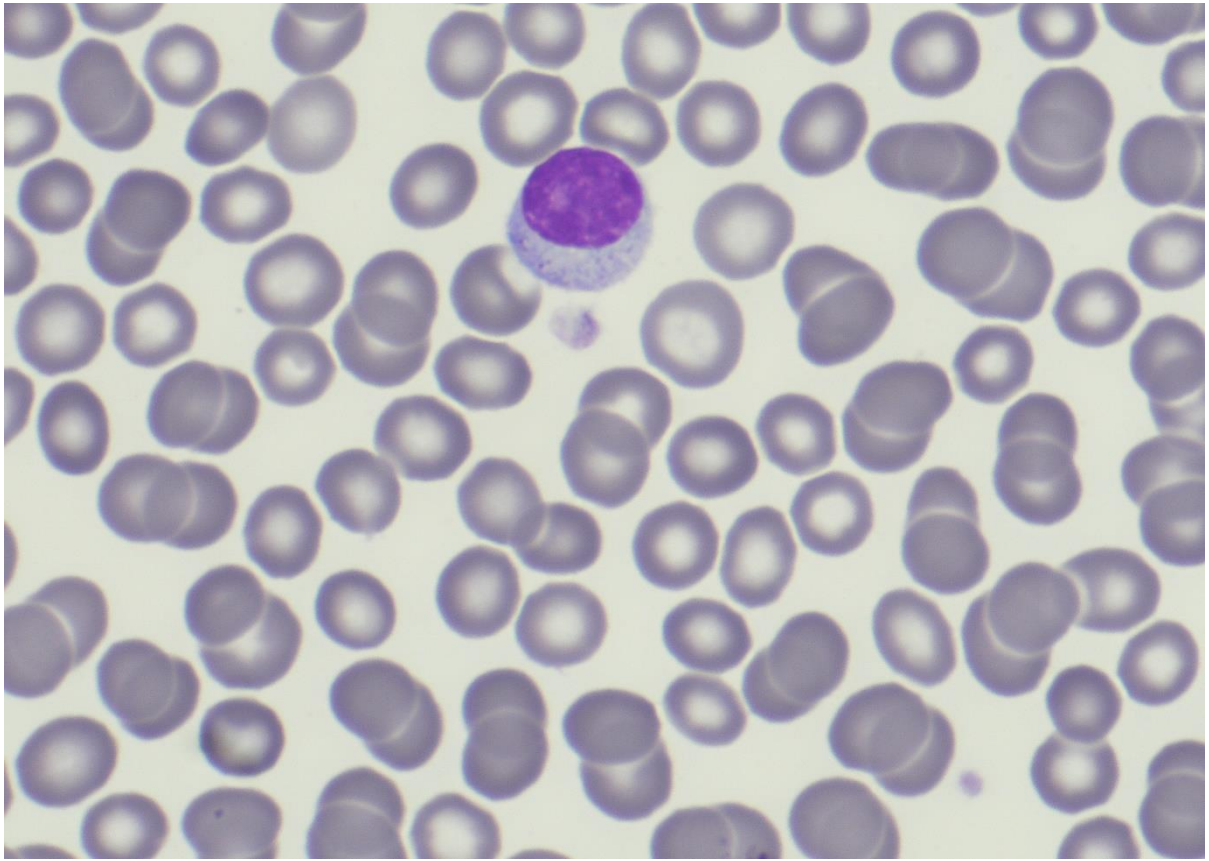
iscontro in Gianluca



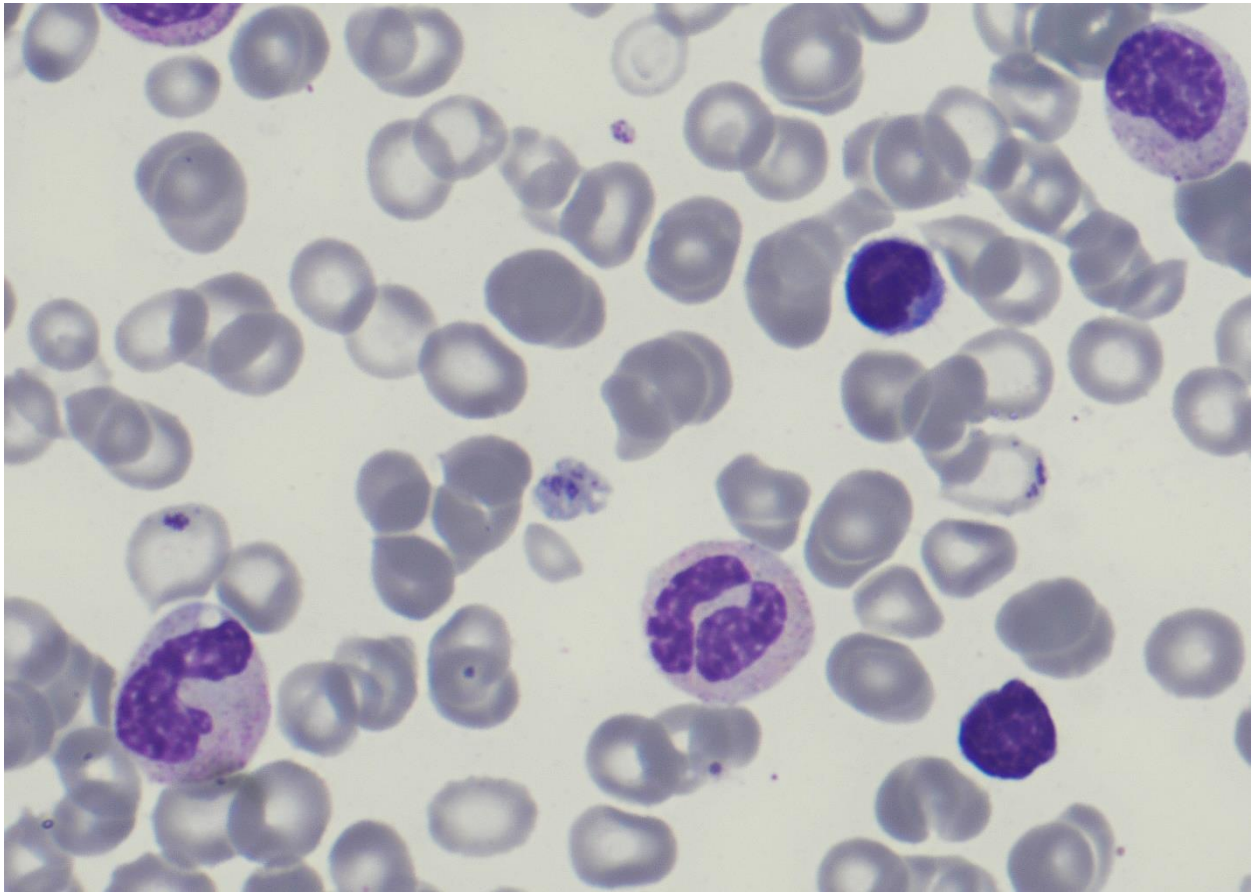








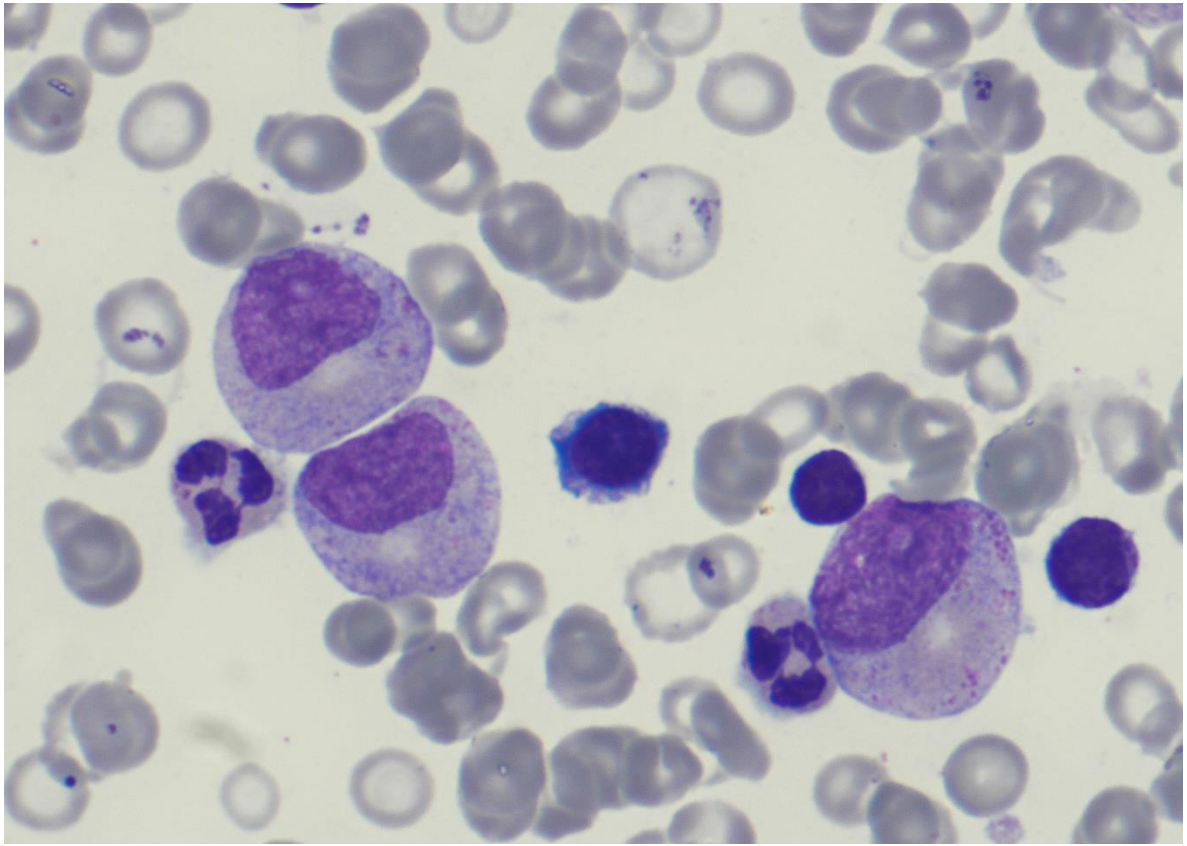
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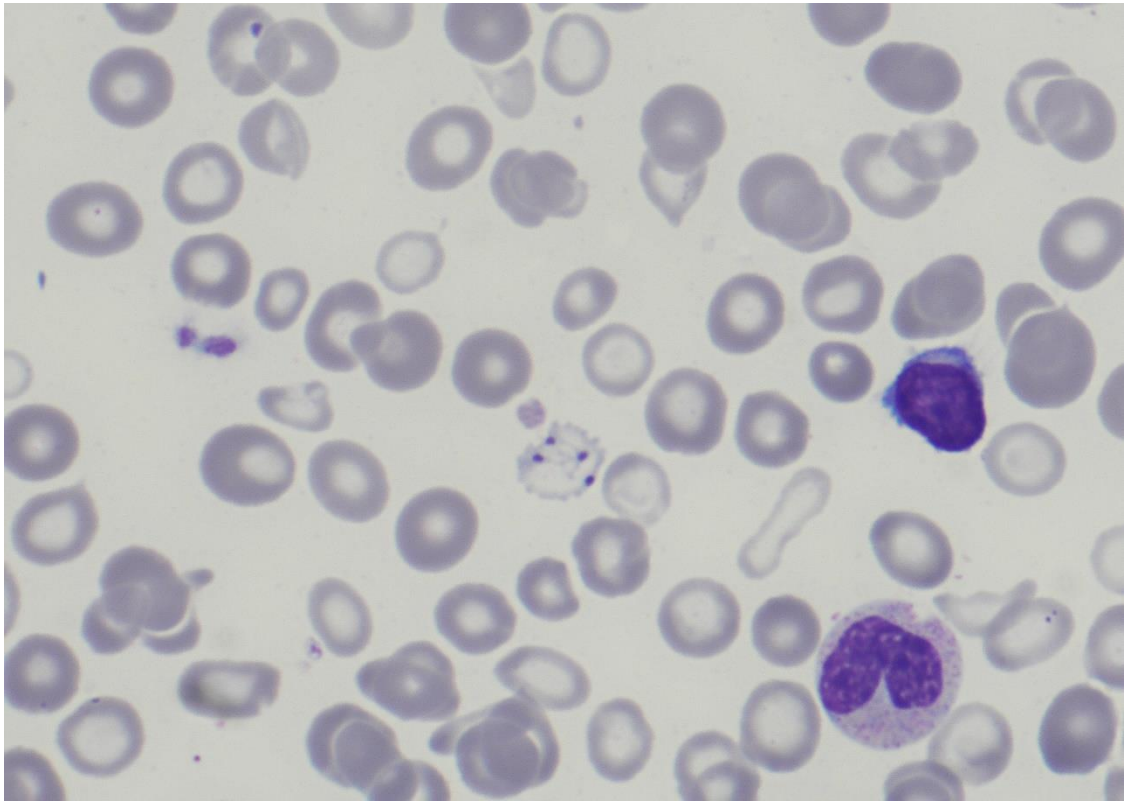


•**Eritrociti con inclusioni periferiche:** Si notano distintamente alcuni globuli rossi che ospitano alla loro periferia piccoli ammassi o grappoli di granuli di colore blu-viola scuro. Ad esempio, guardando il globulo rosso situato in basso a sinistra (subito sopra il neutrofili segmentato), è visibile un netto cluster focale di granuli. Un'altra cellula con un'inclusione simile e ben definita si trova nella parte centrale destra del vetrino.

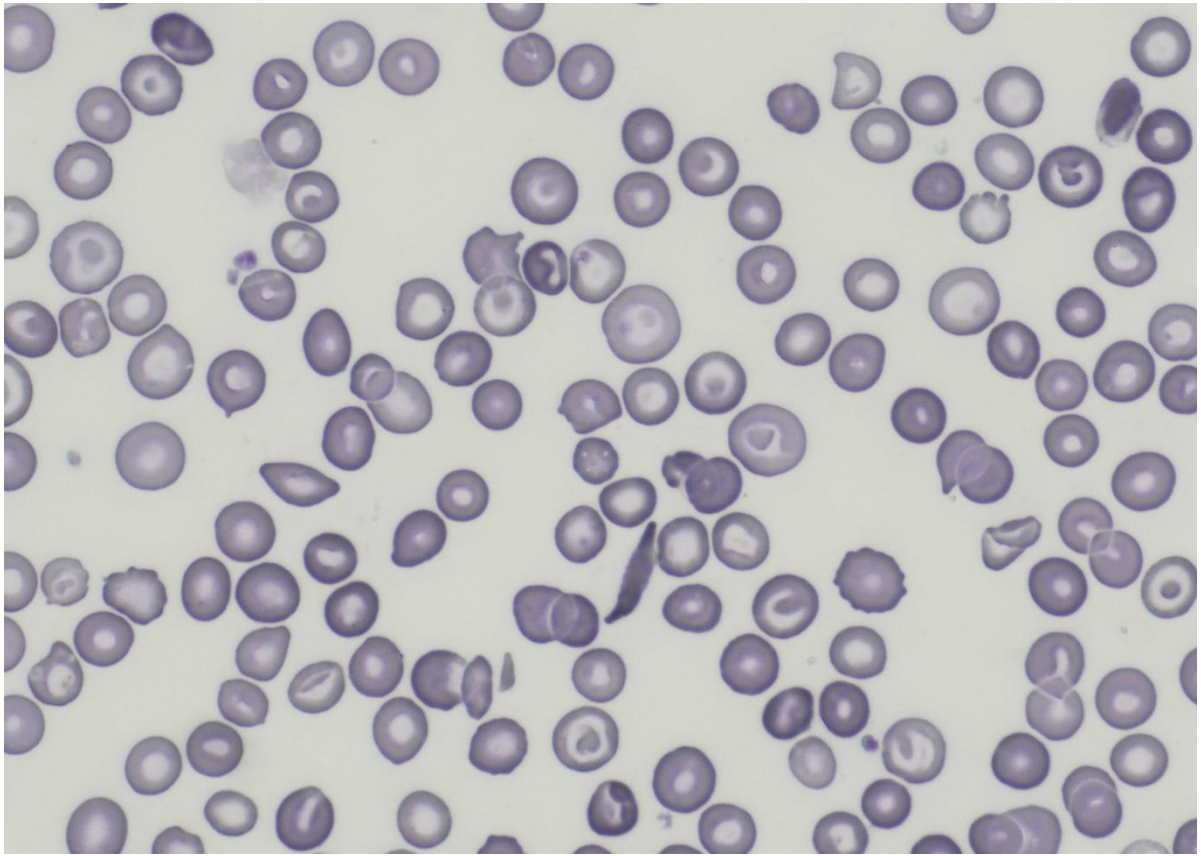
•**Caratteristiche tipiche:** La disposizione geometrica e la morfologia di questi elementi corrispondono perfettamente alla definizione ematologica dei corpi di Pappenheimer (siderociti nel sangue periferico), escludendo artefatti da colorazione o altre inclusioni strutturate.

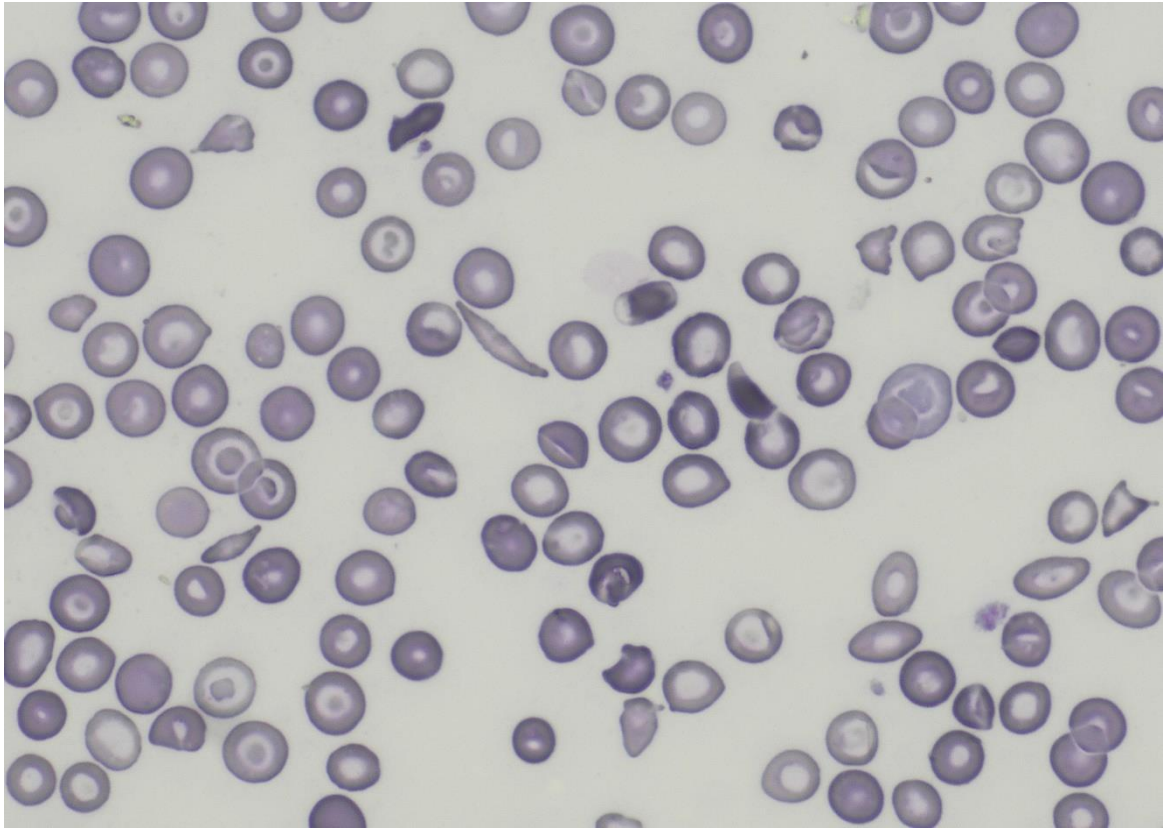
•**Morfologia di fondo:** Si osserva una spiccata eterogeneità nella forma e nella dimensione dei globuli rossi (anisopoichilocitosi), con la compresenza di cellule ipocromiche e cellule normocromiche, un reperto strettamente associato a difetti congeniti o acquisiti della sintesi dell'eme, come l'**anemia sideroblastica**.

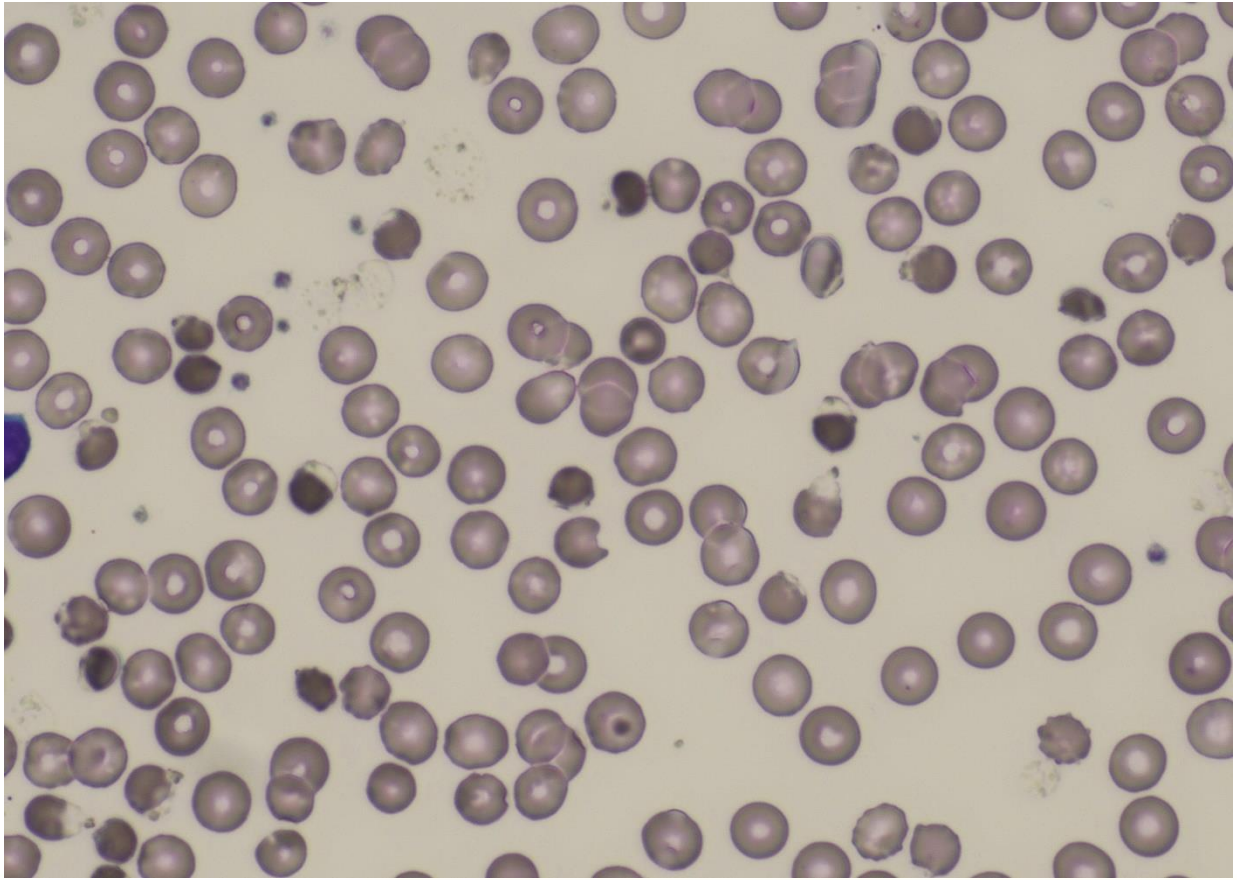


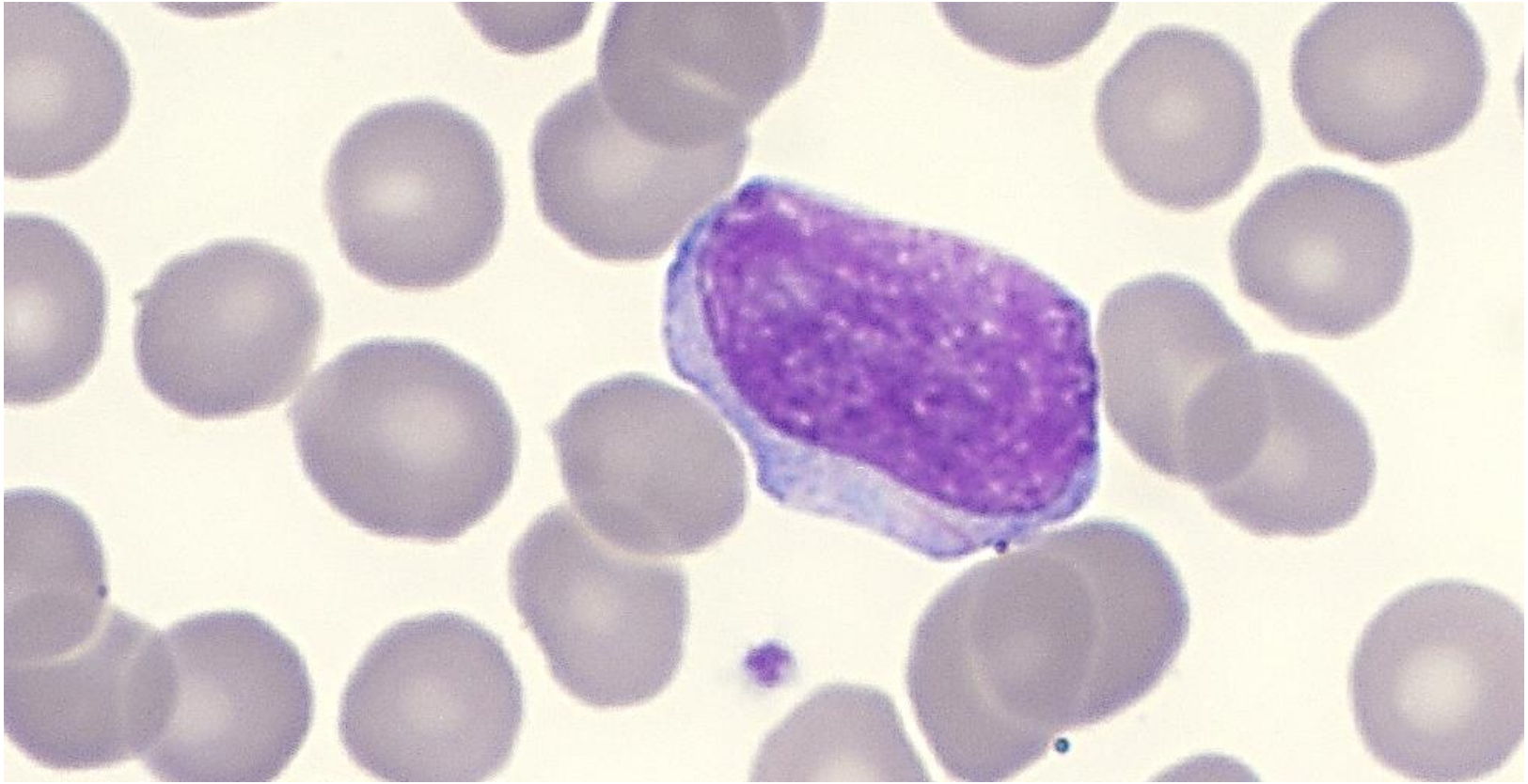


Conferma della diagnosi ottenuta con colorazione di Perls su striscio di sangue midollare, che mostra un quantitativo di sideroblasti ad anello >15%, e conferma di displasia eritroide (taccuino).
Si contattano i genitori e si informano, proponendo alcune date per appuntamento di controllo (in attesa di conferma)

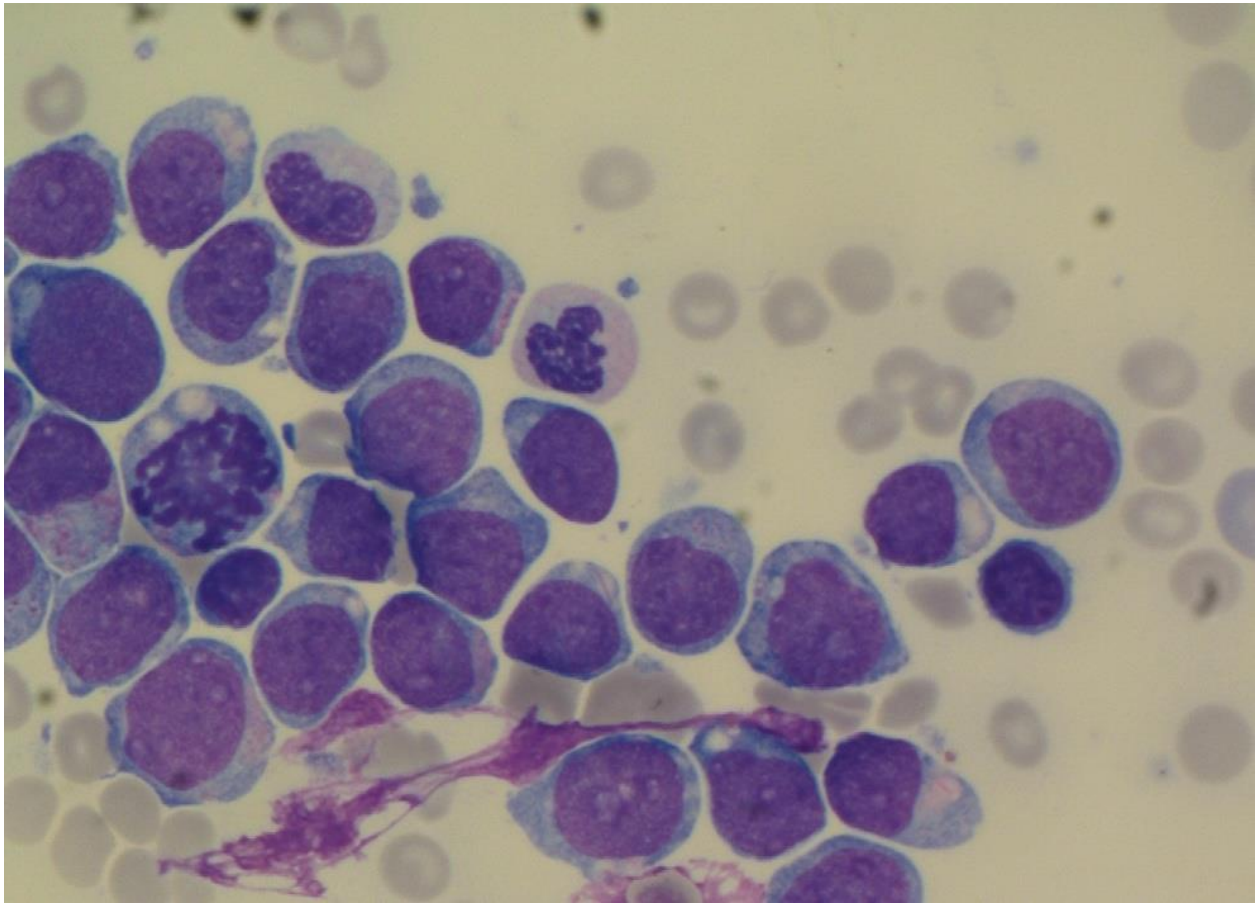




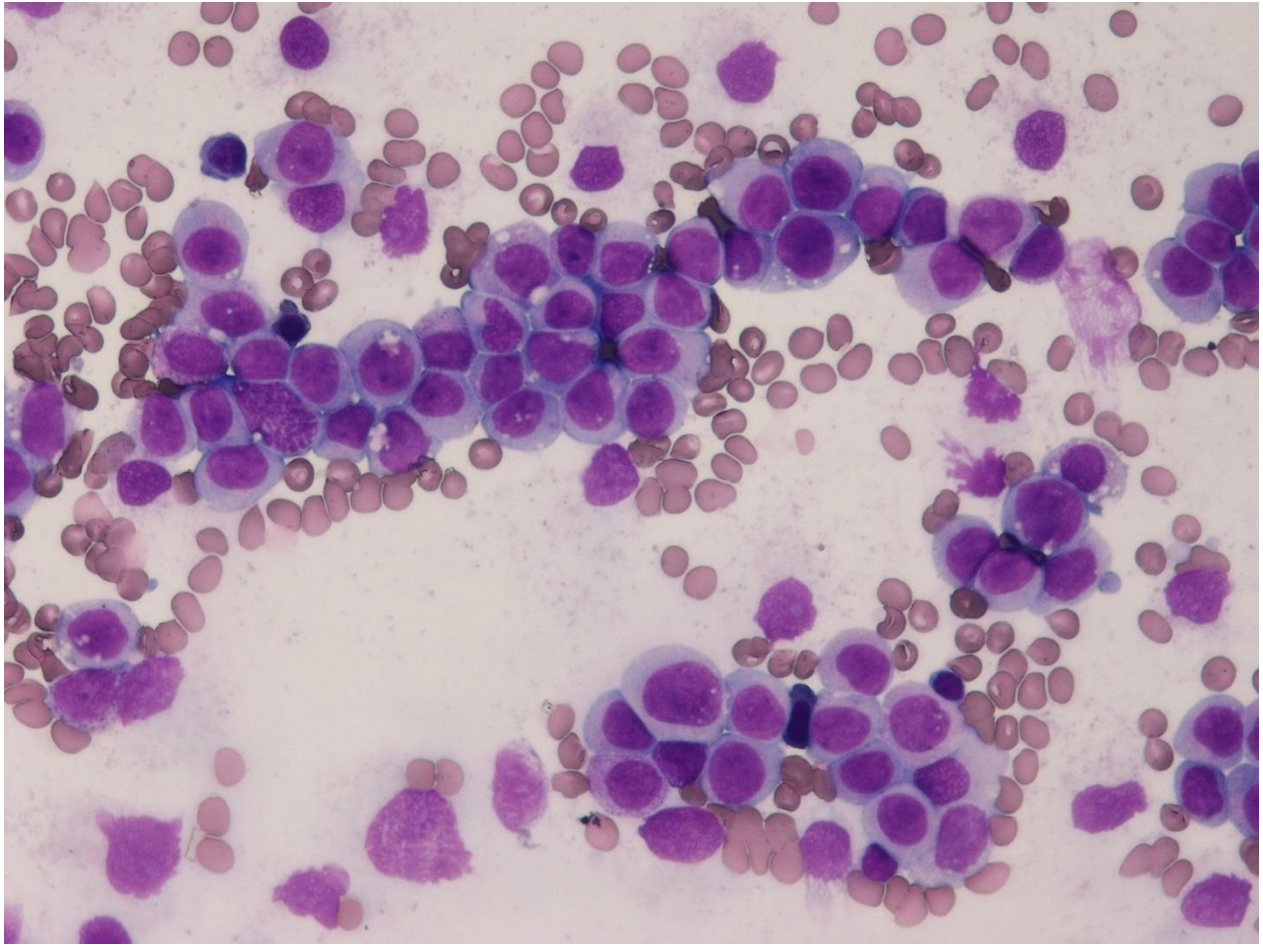




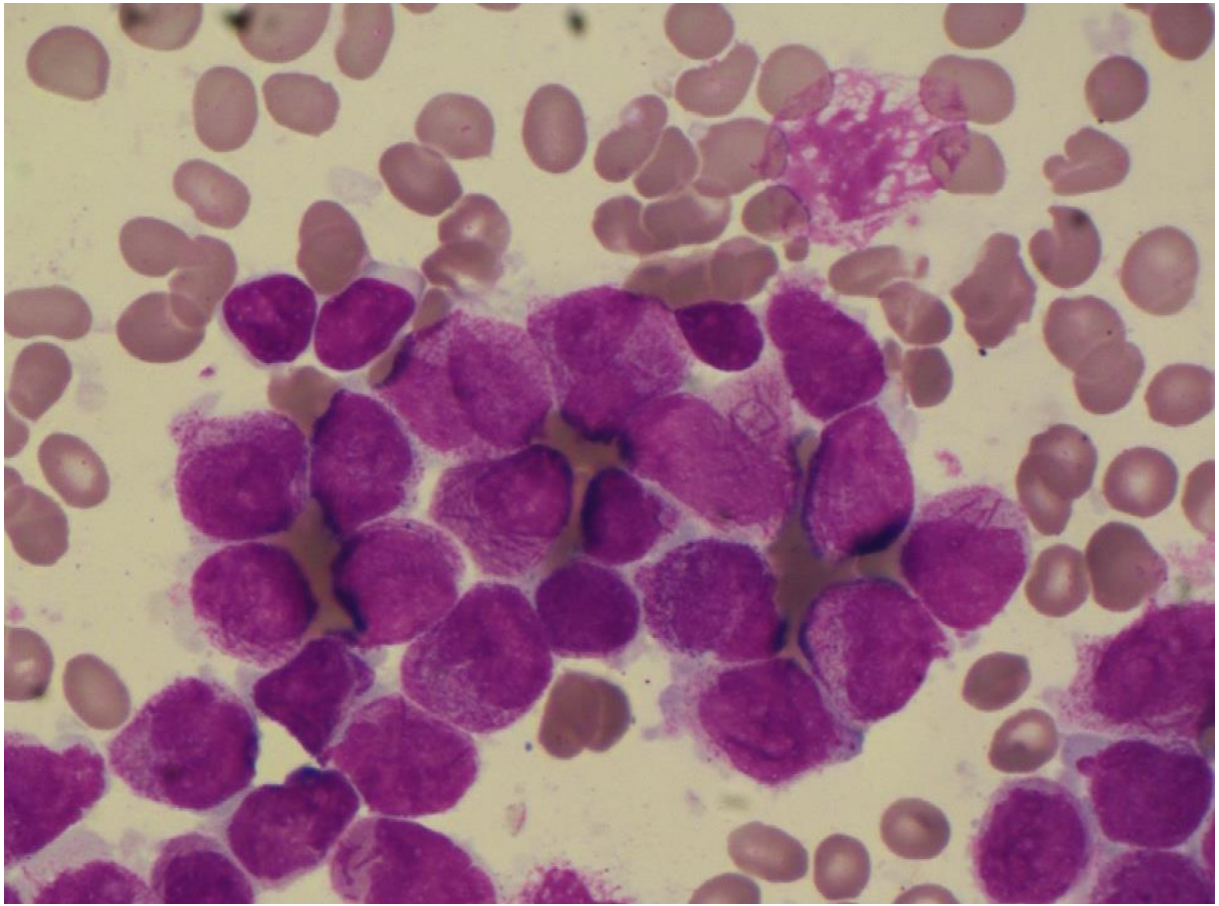
LEUCEMIA MIELOIDE ACUTA



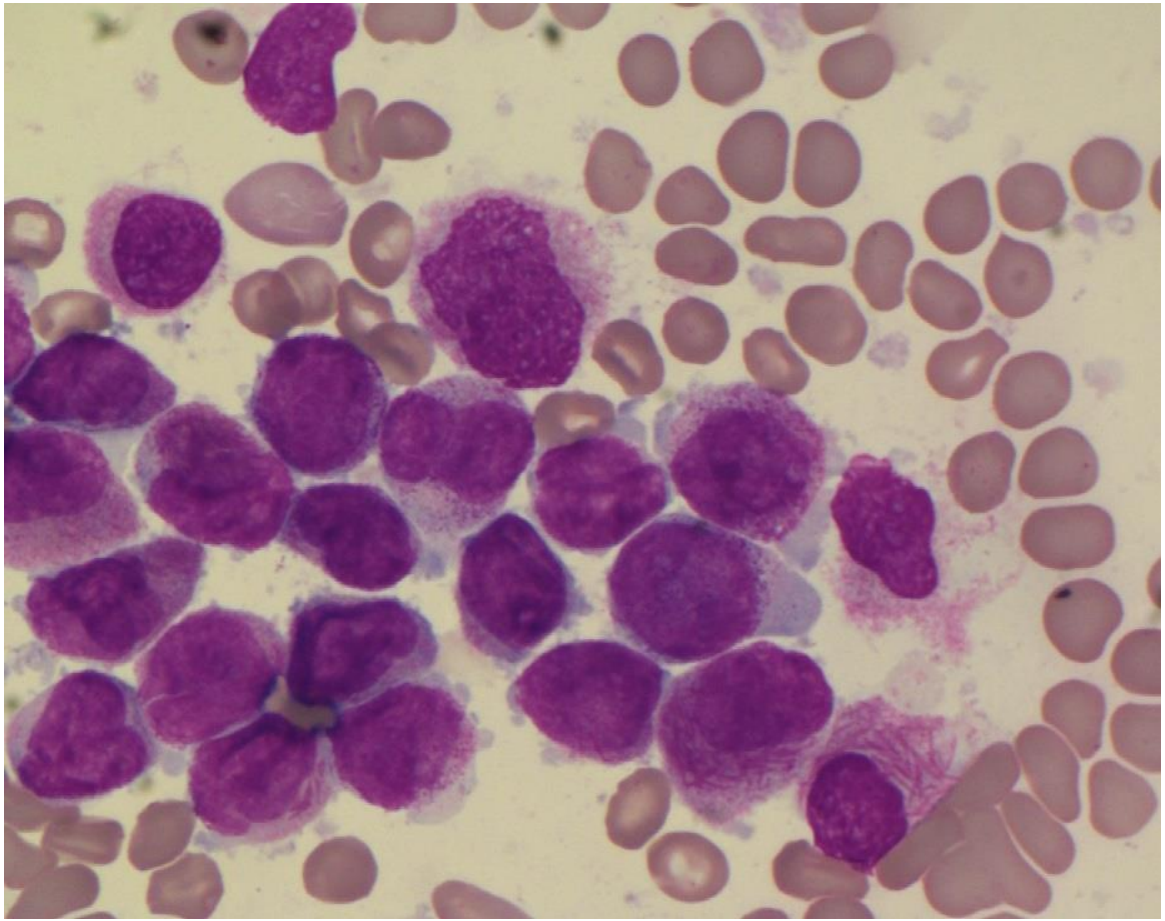
LEUCEMIA MIELOIDE ACUTA



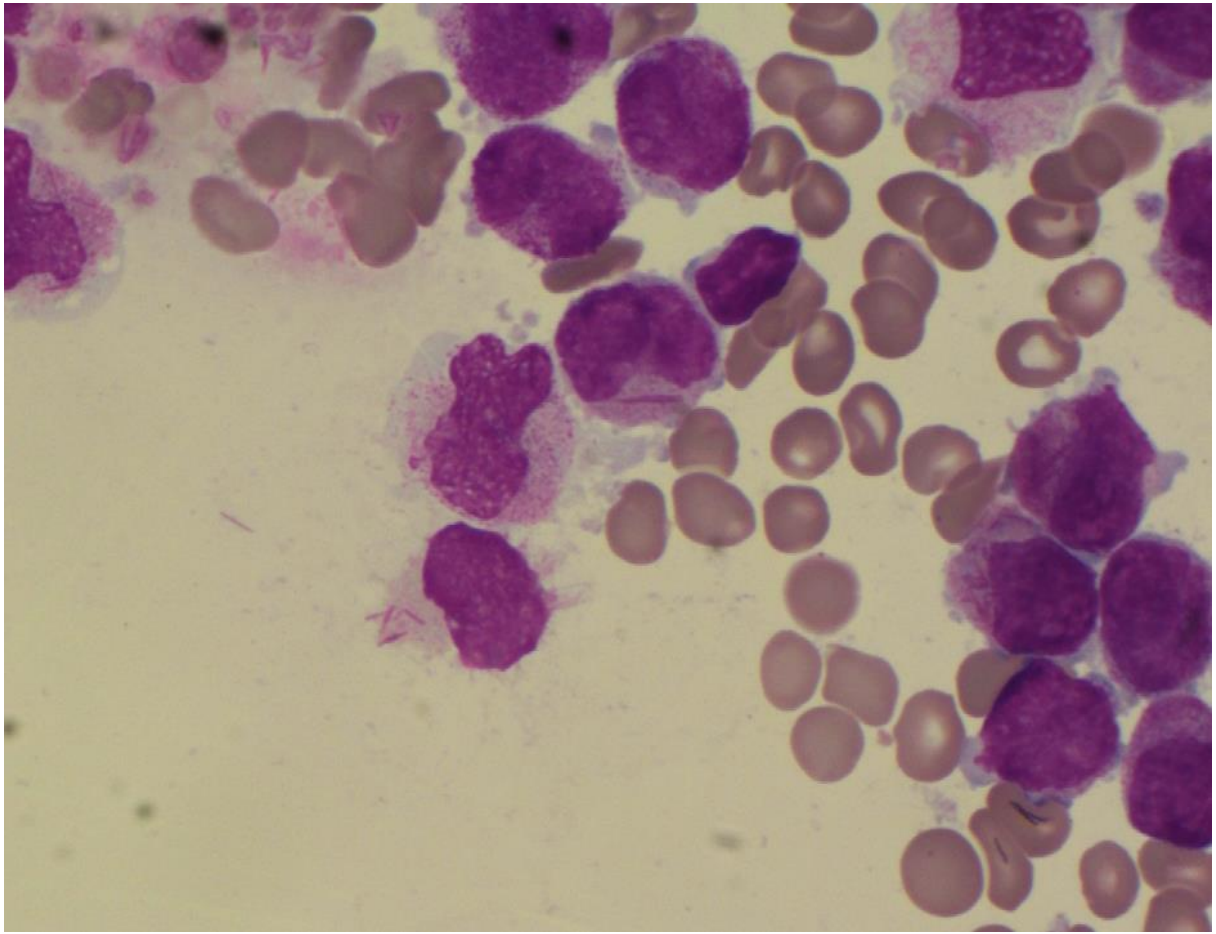
LEUCEMIA MIELOIDE ACUTA PROMIELOCITICA



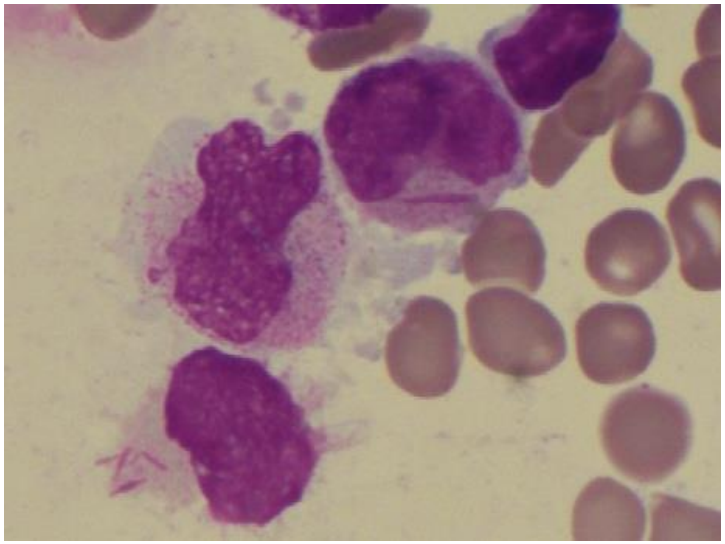
LEUCEMIA MIELOIDE ACUTA PROMIELOCITICA

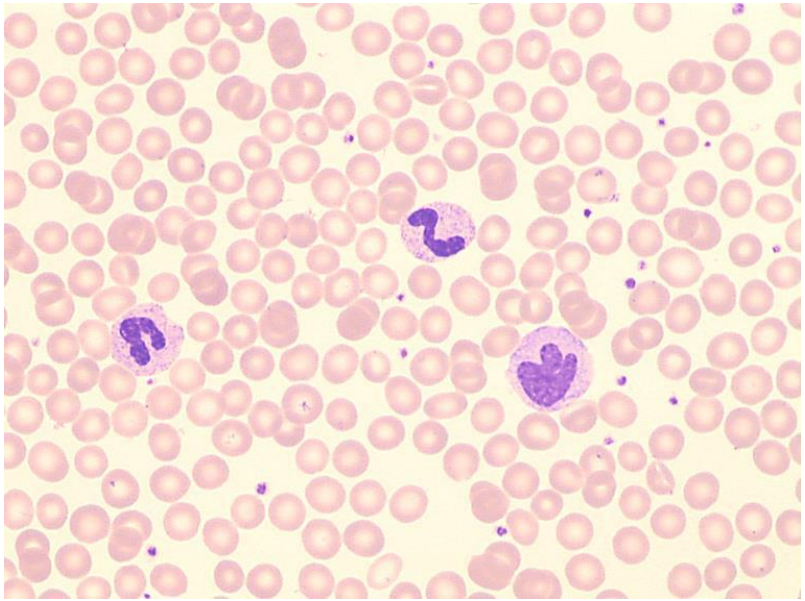


LEUCEMIA MIELOIDE ACUTA PROMIELOCITICA



LEUCEMIA MIELOIDE ACUTA PROMIELOCITICA





MORFOLOGIA

HA ANCORA UN RUOLO NELLA DIAGNOSTICA EMATOLOGICA?





The identification of staining and preparation artifact is necessary to allow for p

- **Titolo:** L'Esame Morfologico Oggi

  - Il primo step fondamentale nella diagnostica ematologica.
  - Integrazione sinergica con l'emocromo automatizzato
  - Obiettivo: identificare anomalie cellulari qualitative non rilevabili dalle macchine.

CITOMETRIA AUTOMATIZZATA
vs
MORFOLOGIA

EMOCROMO

- Alterazioni valori WBC, Hb, PLTS
- Allarmi
- LUC
- BLASTI

MORFOLOGIA

- striscio sp
- ASPIRATO MIDOLLARE

susi

LA VALUTAZIONE DEL VETRINO AL MICROSCOPIO OTTICO È UN PROCESSO MULTISTEP:

1. **obiettivo 10x (total magnification 100 x): scelta del miglior campo per il midollo osseo, valutazione cellularità MK, istiociti, ecc VALUTAZIONE DELLA QUALITA'**

2. **obiettivo medio ingrandimento 40-60x (total magnification 400-600 x): distribuzione delle filiere emopoietiche, scelta della migliore zona**

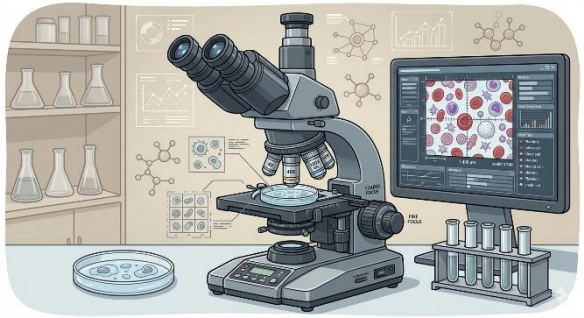
3. **obiettivo 100x (oil high magnification 1000x): definizione delle caratteristiche morfologiche/ conta differenziale (sangue periferico –midollo osseo)**



scelta dell'area più

Si definisce **INGRANDIMENTO** il rapporto tra le dimensioni dell'oggetto originale, e quelle dell'immagine ottenuta. E' dato dal prodotto tra l'ingrandimento dovuto all'oculare (solitamente 10x) moltiplicato l'ingrandimento dovuto agli obiettivi (solitamente 10x, 20x, 40x, 60x,100x)

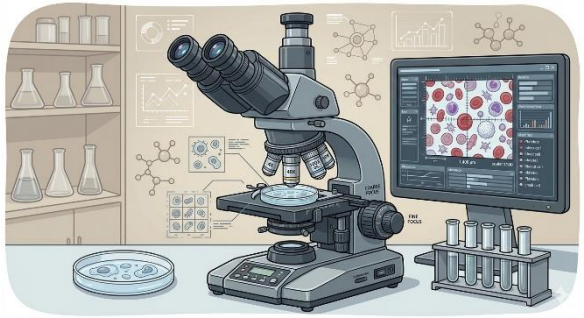
AIEOP GIOVANI | LECCE, 12 ottobre 2026



- **Manual review of the PBS via light microscopy** or digital whole slide imaging is strongly preferred over single high-power field images.

- **Independent use of the compound light microscope** is a mandatory skill that should be attained by graduation.

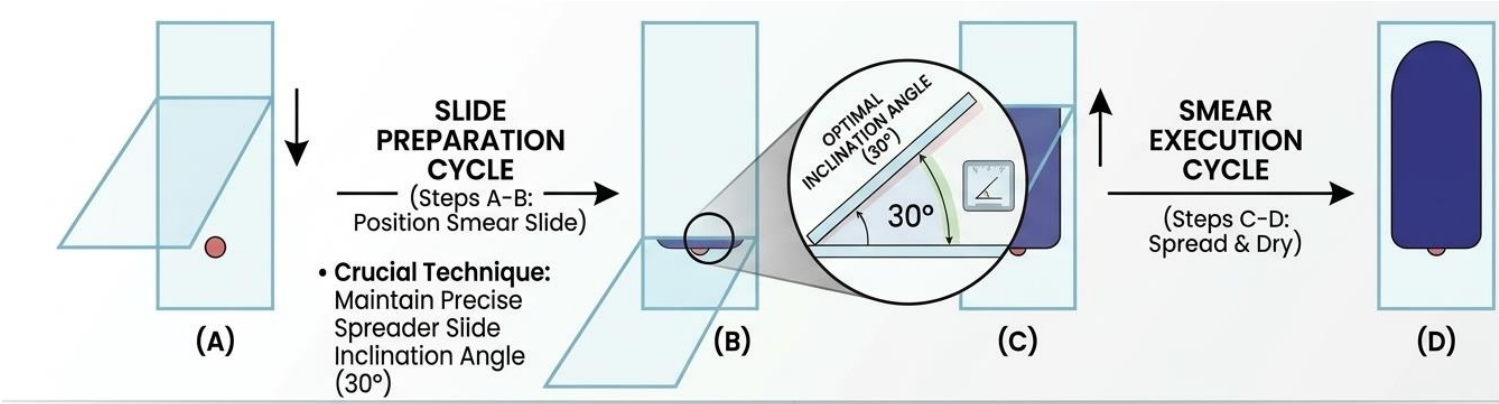
Chase ML et al, Consensus recommendations on peripheral blood smear review. Blood Advances 2023, 7; 3244-3252



- Identification of both **normal and pathologic cytoplasmic and nuclear characteristics.**

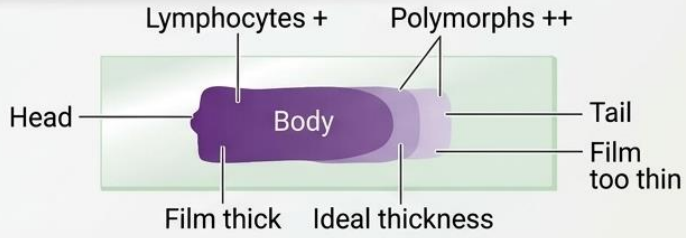
Chase ML et al, Consensus recommendations on peripheral blood smear review. Blood Advances 2023, 7; 3244-3252

HOW TO PREPARE A PERIPHERAL BLOOD SMEAR





Peripheral Blood Smear on a Microscope Slide



Peripheral Blood Film Zone Analysis & White Cell Distribution (Schematic View)

Figure 3.2 Schematic drawing of a blood film. Spread from left to right. An indication is given of the white blood cells are distributed. Cells are identified and quantified based on their characteristic locations: lymphocytes are concentrated in the 'Body' and intermediate zones, while polymorphs are found at the 'Head' and 'Tail' where the film thickness varies.

Dacie and Lewis, Practical Haematology 2011, 11th Edition. Hematology Lab Protocol – Staining & Distribution Guide.

DIFFERENTIAL DIAGNOSIS: ARTIFACT VS. APOPTOSIS

Morphological features of apoptosis:

Cell shrinkage and cytoplasmic condensation around the nuclear membrane.
Nuclear indentations followed by fragmentation.
Cell remnants form dense, basophilic masses (**apoptotic bodies**).

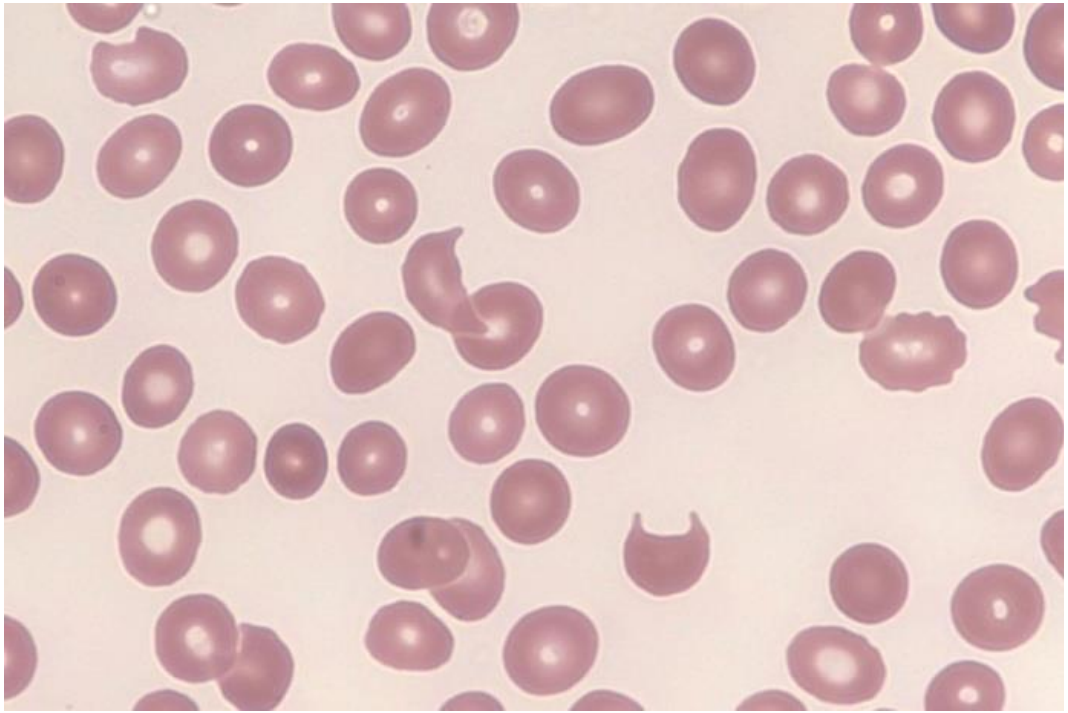
Diagnostic pitfall: Apoptotic neutrophils with a single body can be easily misidentified as **nucleated red cells** if cytoplasmic details are overlooked.
Clinical context: Rarely seen in normal films due to rapid phagocytosis, but more frequent in **leukaemia**.

Dacie and Lewis, Practical Haematology 2011, 11th Edition

MORPHOLOGICAL ANOMALIES OF RBC SHAPE

Bite cells/keratocytes

Bite cells are RBC with peripheral single or multiple arcuate defects (bites), usually one pair or two pairs of spicules. They may be formed either by removal of Heinz bodies by the spleen, or by mechanical damage. (oxidant haemolysis, microangiopathic haemolytic anaemias anaemia).



Palmer L et al. ICSH recommendations. Int. Jnl. Lab. Hem. 2015, 37, 287–303

DIAGNOSI DIFFERENZIALE MORFO-QUANTITATIVA DEI BLASTI NELLE LEUCEMIE ACUTE (WHO)

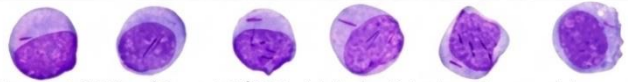
variante "CUP-LIKE BLAST"



Dimensioni: 10-20 µm | **Rapporto N/C:** 7:1 a 4:1.
Nucleo: Profonda indentazione (bocca di pesce). **Cromatina:** Fine, lassa.
Associazione WHO: Mutazioni NPM1, FLT3-ITD.

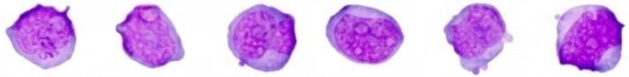
CRIAZIONE HD BASATA SU:
MTCLS Bilal Masood
Tecnologo Medico,
Scienziato di Laboratorio Clinico

MIELOBLASTI CON CORPI DI AUER (AML)



Dimensioni: 15-20 µm | **Rapporto N/C:** 7:1 a 4:1. **Nucleo:** Rotondo, ovale, o convoluto.
Nucleo: Rotondo, ovale, o convoluto. **Cromatina:** Fine, lassa. Citamamente meniulato.
Citoplasma: Contiene 1 o più Corpi di Auer patognomonici (inclusioni aghiformi). **Marcatore WHO:** MPO+.

MIELOBLASTI AGRANULARI / COMUNI (AML)



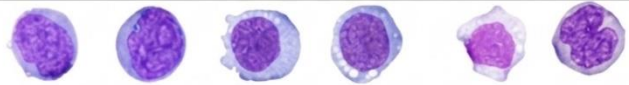
Dimensioni: 10-20 µm | **Rapporto N/C:** 7:1 a 5:1.
Nucleo: Rotondo/ovale, talvolta indentato. **Cromatina:** Fine, lassa.
Citoplasma: Tipicamente privo di granulazioni macroscopiche. **Marcatore WHO:** MPO+.

LINFOBLASTI (ALL)



Dimensioni: 10-20 µm (variabile) | **Rapporto N/C:** 7:1 a 4:1 (Elevato). **Nucleo:** Rotondo, indentato o fessurato. **Cromatina:** Densa, condensata ("chiusa"). **Citoplasma:** Scarso, rigidamente agranulato.
Variante: Aspetto 'a specchio a mano'. **Marcatore WHO:** CD19+ (B) / cCD3+ (T).

MONOBLASTI (AML M5)



Dimensioni: 15-25 µm (Molto Grande) | **Rapporto N/C:** 7:1 a 3:1 (Basso).
Nucleo: Irregolare, lobato, cerebriforme. **Cromatina:** Lassa, reticolata.
Citoplasma: Abbondante, grigio-blu (vetro smerigliato). **Marcatore WHO:** NSE+ (Esterasi Aspecifiche).

MORPHOLOGICAL ANOMALIES OF RBC SHAPE

Poikilocyte

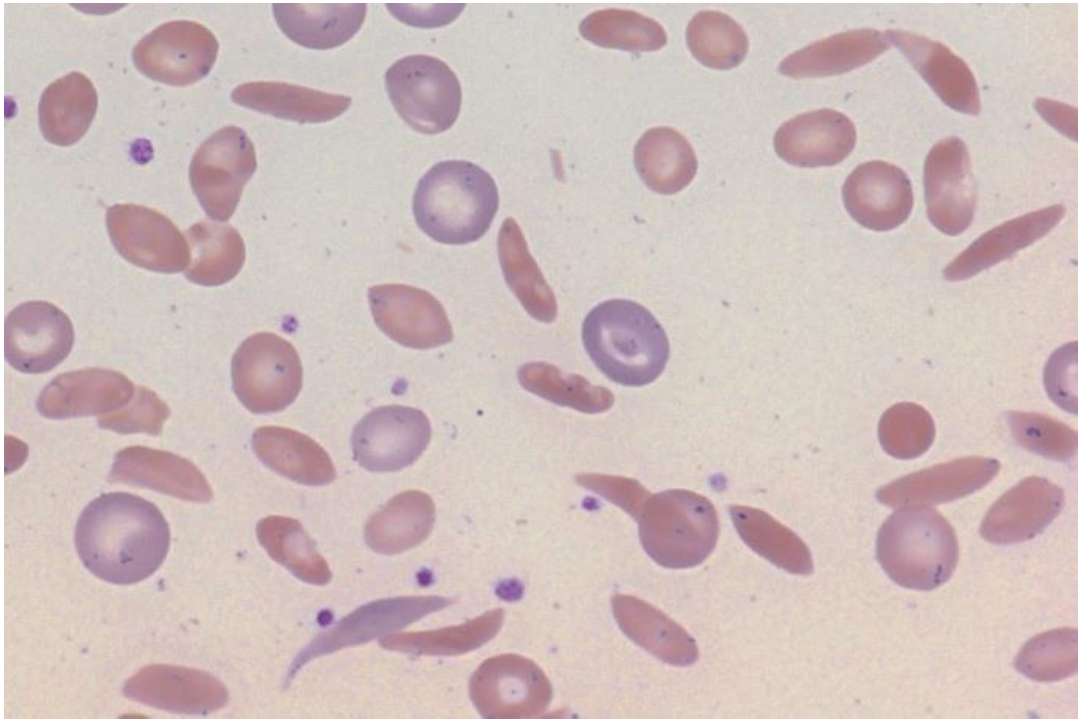
A poikilocyte is a red cell of abnormal shape. Poikilocytosis is a non-specific abnormality but poikilocytes of specific shapes may be associated with a particular disorder e.g. elliptocytes in hereditary elliptocytosis.

Palmer L et al. ICSH recommendations. Int. Jnl. Lab. Hem. 2015, 37, 287–303

MORPHOLOGICAL ANOMALIES OF RBC SHAPE

Sickle cells

Sickle cells are red cells that become crescent or sickle-shaped with pointed ends as a result of polymerization of HbS.
A sickle screen or haemoglobinopathy screen is recommended.



Sickle cell disease typical forms together with other features, notably polychromasia, target cells, and a spherocytic cell.

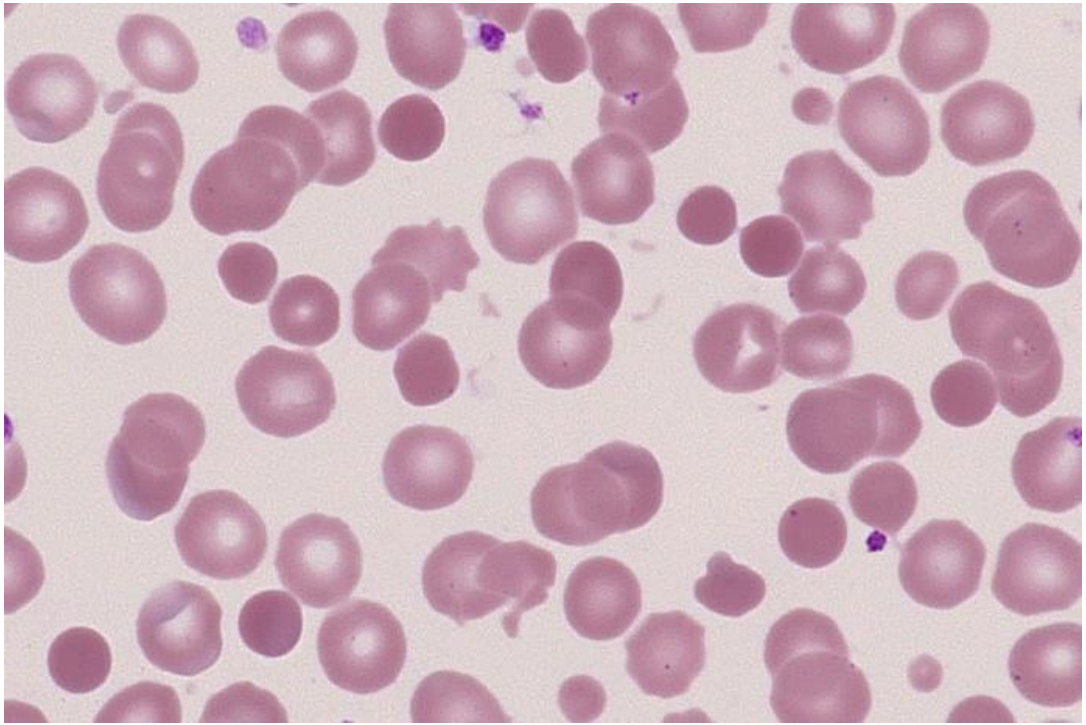
Palmer L et al. ICSH recommendations. Int. Jnl. Lab. Hem. 2015, 37, 287–303

MORPHOLOGICAL ANOMALIES OF RBC SHAPE

Spherocytes

Spherocytes are of small diameter (<6.5 lm) and are dense spheroidal RBC with a normal or decreased MCV and an absence of central pallor. They may be formed as a consequence of an abnormality of the RBC cytoskeleton and membrane, immune and microangiopathic haemolysis and direct damage to the red cell membrane.

Hereditary spherocytosis, ABO and warm AIHA, Clostridium perfringens sepsis, burns.



Autoimmune haemolytic anaemia – typical round, dense red cells

Palmer L et al. ICSH recommendations. Int. Jnl. Lab. Hem. 2015, 37, 287–303

MORPHOLOGICAL ANOMALIES OF RBC SHAPE

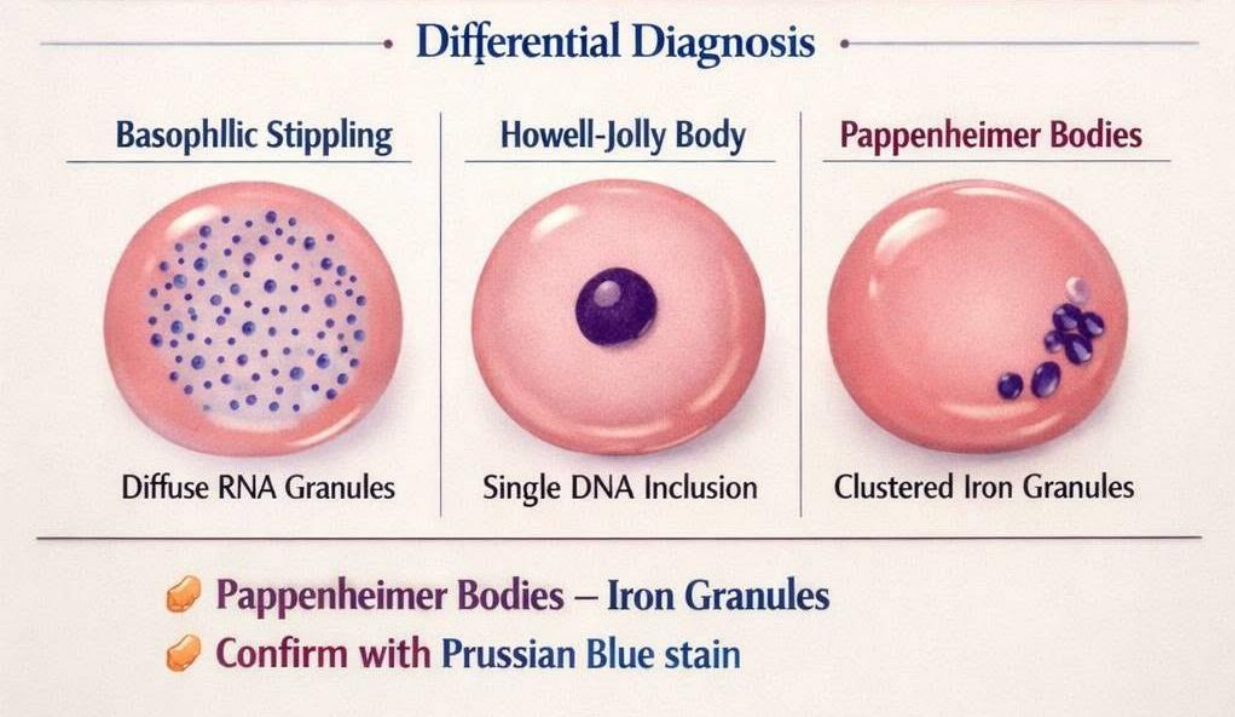
The term target cell refers to a cell in which there is a central round stained area and a peripheral rim of haemoglobinized cytoplasm separated by non-staining or more lightly staining cytoplasm. Target cells result from cells having a surface that is disproportionately large compared with their volume.
They may be normal in size, microcytic or macrocytic. They are seen in films in chronic liver diseases in which the cell
membrane may be loaded with cholesterol, in hereditary hypo-betalipoproteinaemia,16 and in varying numbers in iron deficiency anaemia and in thalassaemia. They are often conspicuous in certain haemoglobinopathies, e.g. haemoglobin C disease, haemoglobin
H disease, sickle cell anaemia, sickle cell/haemoglobin
C disease, sickle cell/b thalassaemia, and
haemoglobin E disease. Smaller numbers are usual in haemoglobin
C trait, haemoglobin E trait and postsplenectomy.
Splenectomy in thalassaemia may result in an extreme
degree of leptocytosis and target cell formatio

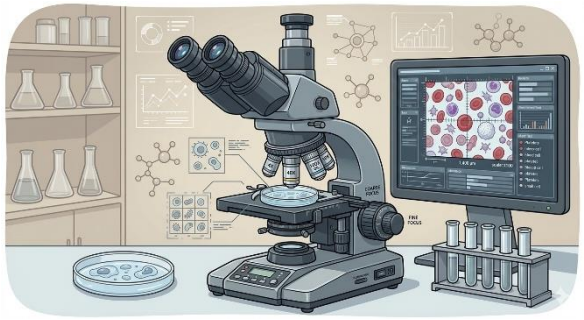
Haemoglobin C disease

Liver disease, haemoglobinopathies, thalassaemia.

Palmer L et al. ICSH recommendations. Int. Jnl. Lab. Hem. 2015, 37, 287–303

INCLUSION IN RBC





- Manual review of the PBS via light microscopy or digital whole slide imaging is strongly preferred over single high-power field images. **Independent use of the light microscope** is a mandatory skill that should be attained by graduation.

- Identification of both **normal and pathologic cytoplasmic and nuclear characteristics.**

- Identification of **staining and preparation artifact.**

Chase ML et al, Consensus recommendations on peripheral blood smear review. Blood Advances 2023, 7; 3244-3252

EFFECTS OF STORAGE ON BLOOD CELL MORPHOLOGY:

Lymphocytes and Red Blood Cells

Lymphocytes:

Appearance of a few cytoplasmic vacuoles.
Nuclei stain more homogeneously than usual.
Nuclear budding (resulting in nuclei with 2 or 3 lobes).

Red Blood Cells:

Largely unaffected for up to 6 hours at room temperature.
Longer delays lead to progressive crenation and sphering.
Critical Note: An excess of EDTA causes marked crenation within just a few hours

Dacie and Lewis, Practical Haematology 2011, 11th Edition

EFFECTS OF STORAGE ON BLOOD CELL MORPHOLOGY:

White Blood Cells (Neutrophils & Monocytes)

Alterations After 3–18 Hours

Neutrophils (partially affected):

- Nuclei stain more homogeneously.
- Nuclear lobes may separate.
- Cytoplasmic margins appear ragged or poorly defined.
- Appearance of small cytoplasmic vacuoles.

Monocytes (marked changes):

- Development of small cytoplasmic vacuoles.
- Irregular nuclear lobulation (potentially leading to near disintegration).

Dacie and Lewis, Practical Haematology 2011, 11th Edition

TECHNIQUE OF EXAMINATION OF BLOOD FILMS

- **Leukocyte Profiling**: Semi-quantitative estimation of leukocyte density, spatial distribution, and tinctorial characteristics.
- **Monolayer Identification**: Localization of the optimal examination field where erythrocytes exhibit a homogenous distribution without artifactual distortion.
- **Atypical Cell Surveillance**: Extensive screening to detect low-frequency pathogenic entities, including immature granulocytic precursors or nucleated erythrocytes.

Dacie and Lewis, Practical Haematology 2011, 11th Edition

TECHNIQUE OF EXAMINATION OF BLOOD FILMS

- **Primary Morphological Evaluation (x40 / x50 / x60)**: Serves as the principal modality for erythrocyte analysis, providing superior field-of-view architecture compared to maximum magnification.
- **Erythrocytic Pathomorphology**: Detailed assessment of anisocytosis, poikilocytosis, and chromasia variants.
- **Intracellular Inclusions**: Targeted detection of diagnostic cytological markers such as toxic granulation, Howell-Jolly bodies, and Pappenheimer bodies.

Dacie and Lewis, Practical Haematology 2011, 11th Edition

HOW TO PREPARE A PERIPHERAL BLOOD SMEAR

- the film should be made with a rapid movement using a smooth glass spreader.
- the film must not be too thin and the tail of the film should be smooth.
- If the film is too thin or if a rough-edged spreader is used, many of the white cells, perhaps even 50% of them, accumulate at the edges and in the tail.
- SOLO DETTA

Dacie and Lewis, Practical Haematology 2011, 11th Edition

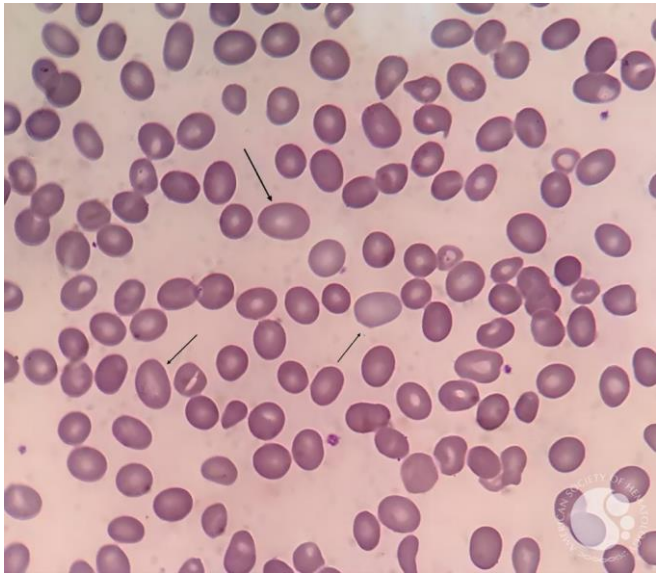
ABNORMALITIES OF RBC SIZE AND/OR COLOUR

- **Microcytes (<7 µm; MCV <80 fL)**: frequently linked to hypochromia. Target cells can falsely lower the MCV. Interpretation must be **age-contextualized** (physiologically smaller in healthy children, larger in neonates).

- **Macrocytes (>8.5 µm; MCV >100 fL)**: Enlarged cells (round or oval); elevated MCV can be triggered by reticulocytosis or neonate physiology. **Oval macrocytes** require compulsory visual grading due to diagnostic relevance.

- *Methodological Directive*: The automated **MCV** is the definitive gold standard for gauging micro-/macrocytosis; manual microscopic grading is reserved for specific histogram anomalies (normal MCV with abnormal RDW) or non-advanced laboratories.

Palmer L et al. ICSH recommendations Int. Jnl. Lab. Hem. 2015, 37, 287–303

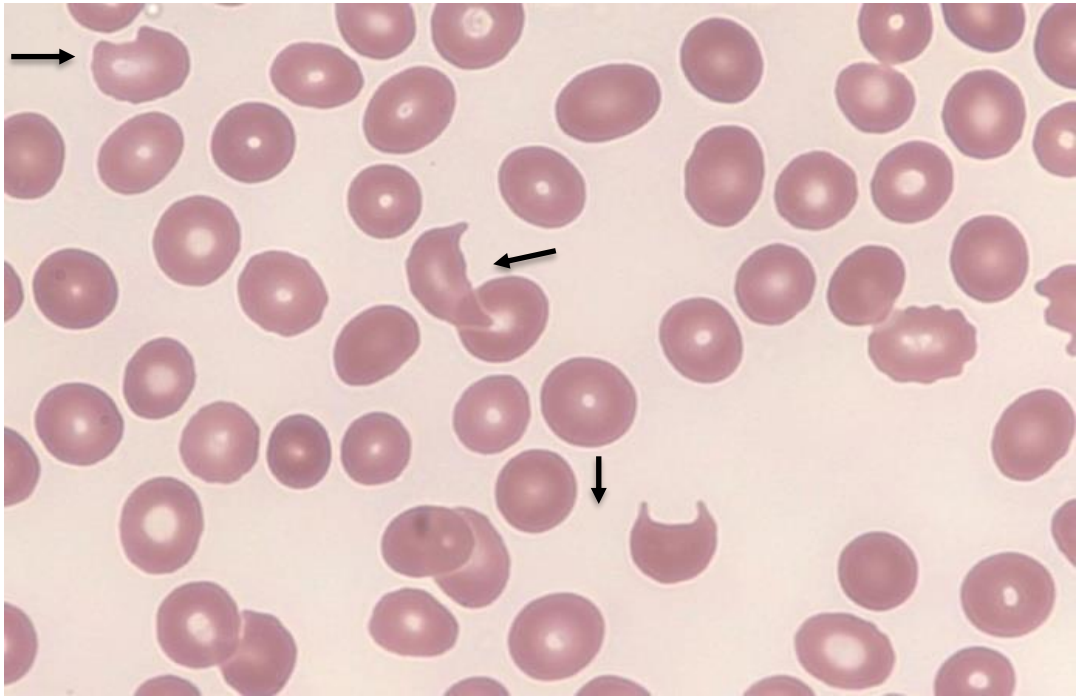


Peripheral blood smear showing megaloblastic change despite a normal MCV. Scattered macro-ovalocytes, for example those indicated by arrows, demonstrate classic features of impaired DNA synthesis.

ABNORMALITIES OF RBC SHAPE

Bite cells/keratocytes

RBC characterized by single or multiple distinct peripheral, arcuate (semi-circular) defects resembling "bites," typically demarcated by one or two pairs of prominent spicules. They may be formed via the selective removal (pitting) of denatured hemoglobin precipitates (Heinz bodies) by splenic macrophages. Generated through direct microvascular shear stress and mechanical damage.



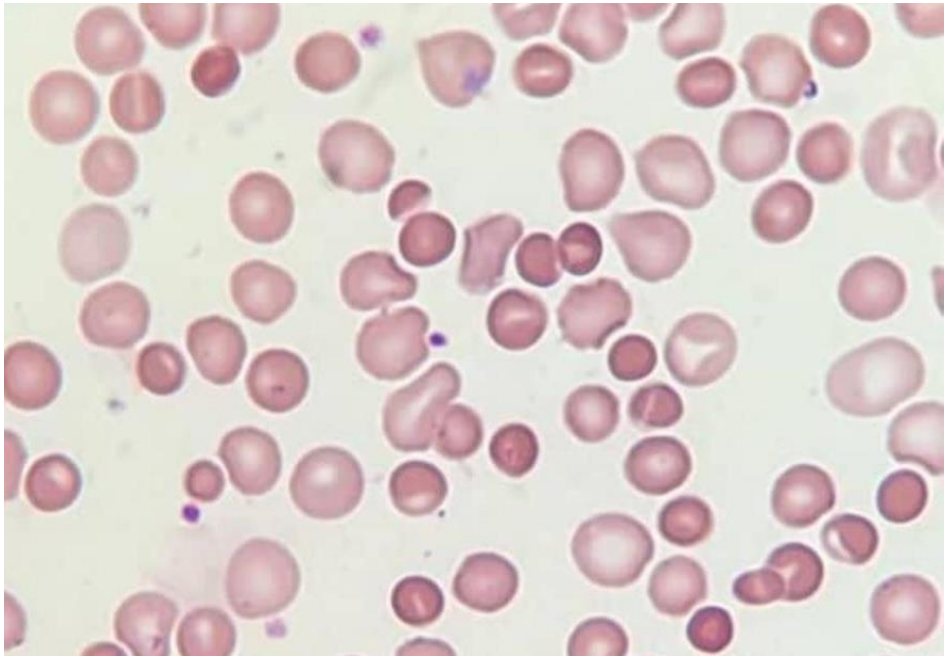
G6PD deficiency and microangiopathic hemolytic anemias.

Palmer L et al. ICSH recommendations. Int. Jnl. Lab. Hem. 2015, 37, 287–303

ABNORMALITIES OF RBC SHAPE

Spherocytes

Peripheral blood smear of a newborn diagnosed with ABO incompatibility and positive direct coombs test shows increased spherocytes and microspherocytes. Spherocyte formation occurs due to a partial loss of the red blood cell membrane leading to a decrease in the surface to volume ratio of the red blood cell. Conditions associated with spherocytosis include hereditary spherocytosis, autoimmune hemolytic anemia, drug -induced immune hemolytic anemia, allo-immune-mediated hemolysis (transfusion reactions, hemolytic disease of the fetus and newborn), glucose-6-phosphate (G6PD) deficiency, transfused cells or severe burns.



newborn diagnosed with ABO incompatibility

Palmer L et al. ICSH recommendations. Int. Jnl. Lab. Hem. 2015, 37, 287–303

Acantocita: stella marina deforme: le sue punte sono poche, lunghe, asimmetriche e nascono da punti casuali della cellula.

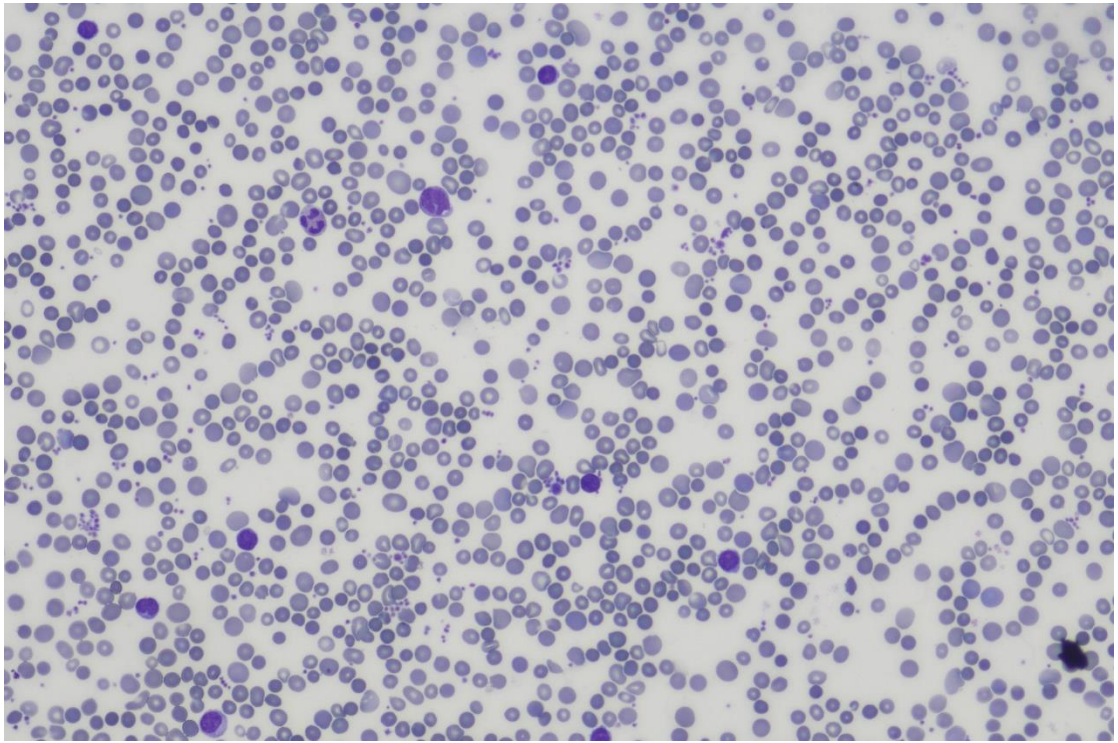
Echinocita: riccio di mare o ingranaggio: cellula tondeggiante che ha mantenuto la sua simmetria, ma presenta piccoli "denti" tutti uguali e distribuiti in modo impeccabile su tutta la circonferenza. Concetto importante della assoluta regolarità.

ABNORMALITIES OF RBC SHAPE

Spherocytes

Spherocytes are of small diameter (<6.5 lm) and are dense spheroidal RBC with a normal or decreased MCV and an absence of central pallor. They may be formed as a consequence of an abnormality of the RBC cytoskeleton and membrane, immune and microangiopathic haemolysis and direct damage to the red cell membrane.

Hereditary spherocytosis, ABO and warm AIHA, Clostridium perfringens sepsis, burns.



Hereditary spherocytosis

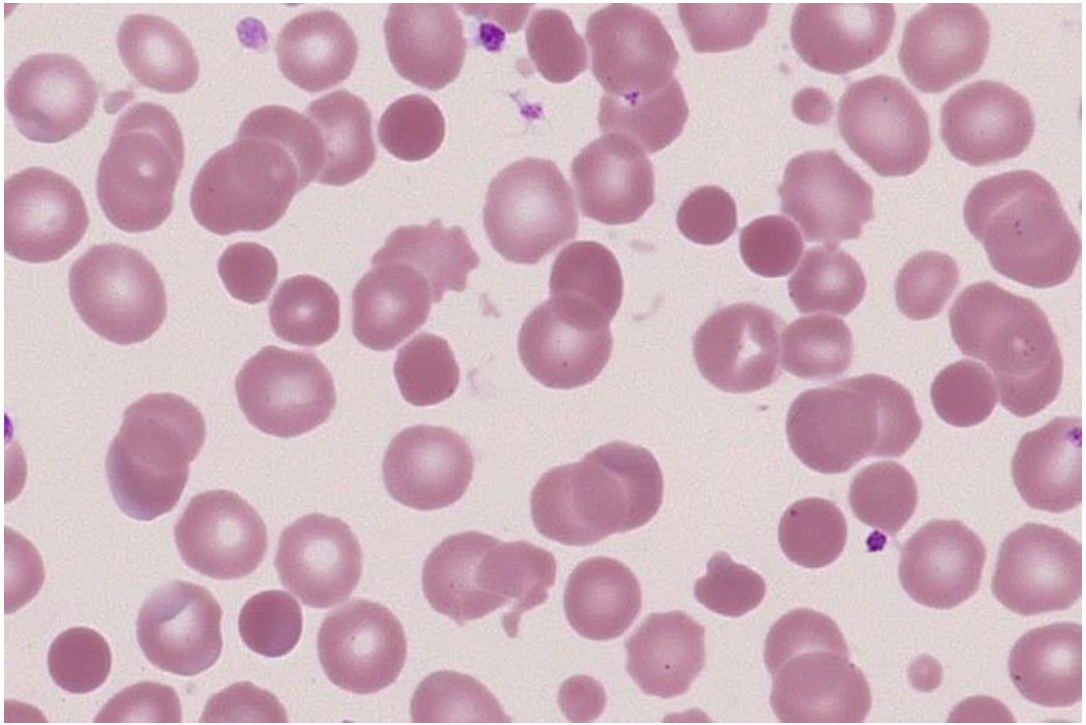
Palmer L et al. ICSH recommendations. Int. Jnl. Lab. Hem. 2015, 37, 287–303

MORPHOLOGICAL ANOMALIES OF RBC SHAPE

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Autoimmune haemolytic anaemia – typical round, dense red cells

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Teardrop cells

MORPHOLOGICAL ANOMALIES OF RBC SHAPE

Dacrocytes (Teardrop Cells)

Erythrocytes characterized by a distinct pear-shaped or teardrop-like elongation, featuring a single, blunt, or tapered elongated spicule at one pole of the cell.

Formed during the mechanical extrusion of erythrocytes containing rigid inclusions (such as Heinz bodies or Howell-Jolly bodies) through the endothelial slits of the spleen or bone marrow sinuses.

Myelofibrosis and Space-Occupying Lesions: Highly indicative of Primary Myelofibrosis (PMF) or secondary myelophthisic anemias, where the bone marrow architecture is severely distorted by fibrosis or tumor infiltration.

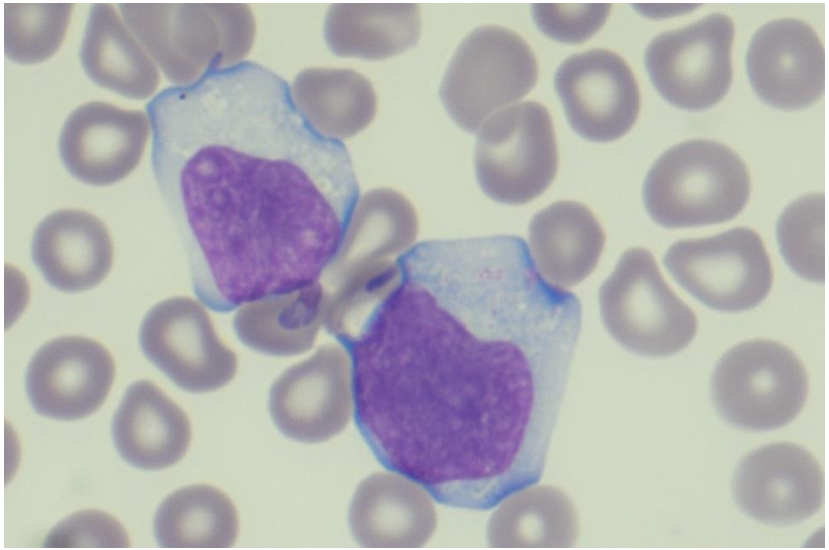
Severe Anemias: Frequently observed in severe Iron Deficiency Anemia (IDA), Thalassemias, and megaloblastic processes due to dyserythropoiesis.

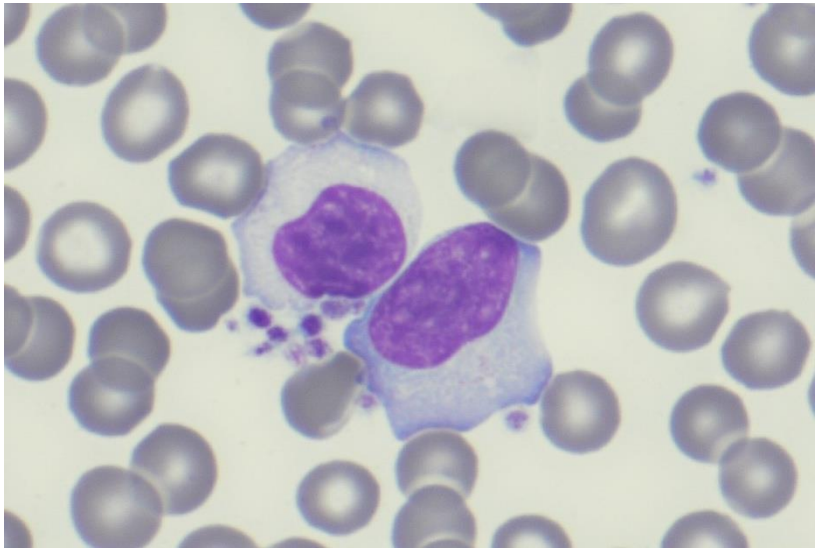
Artifactual Differentiation: True dacrocytes must be distinguished from preparation artifacts, which are easily identified because their tails point in the exact same direction across the film.

FARE MEGLIO

Myelofibrosis

Palmer L et al. ICSH recommendations. Int. Jnl. Lab. Hem. 2015, 37, 287–303





JMML



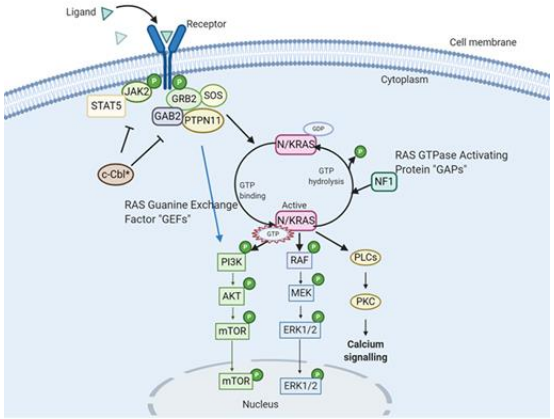


Table 1. Comparison between the WHO 5th edition and ICC criteria for JMML.

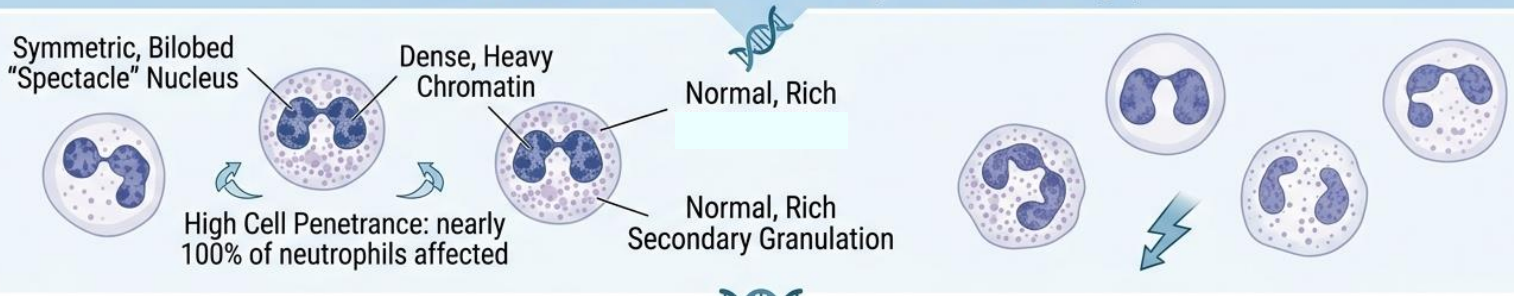
Criteria	WHO classification 5th edition (WHO 2022)	ICC
Nomenclature	Juvenile myelomonocytic leukemia	Juvenile myelomonocytic leukemia
Peripheral Blood	Peripheral blood monocyte count ≥1 $\times 10^9$/L	Peripheral blood monocyte count ≥1 $\times 10^9$/L
Peripheral blood and bone marrow blasts	<20%	<20%*
Organ infiltration	Evidence of organ infiltration, most commonly splenomegaly	Splenomegaly
Bone marrow findings	A combination of cytologic and proliferative features	Not clearly stated
Exclusions	*BCR::ABL1* fusion *KMT2A* rearrangements	*BCR::ABL1* fusions*
Genetic studies	Detection of a molecular pathogenic lesion in 1 of the 5 canonical RAS pathway driver genes in leukemic cells -biallelic pathogenic alterations in *NF1* -pathogenic *PTPN11* variant -pathogenic *NRAS* variant -pathogenic *KRAS* variant -biallelic pathogenic variants involving the *CBL* gene (in some cases heterozygous only) -alternatively, non-canonical genetic abnormalities such as gene fusions leading to upstream RAS pathway activation e.g. - *ALK, PDGFR, FLT3* -pathogenic variants in non-canonical RAS pathway genes such as *RRAS* or *RRAS2*	*Somatic mutations in *PTPN11, NRAS, KRAS* or *RRAS* Germline *NF1* mutation and LOH of *NF1*, or a clinical diagnosis of neurofibromatosis Germline *CBL* mutation and LOH of *CBL* (in some cases heterozygous only)
Other criteria	Cases not meeting genetic criteria (particularly in resource-restricted setting) must have the following criteria in addition to the clinical, hematological and laboratory criteria. ≥ 2 of the following -increased fetal hemoglobin for age -myeloid and erythroid precursors on the peripheral blood smear -thrombocytopenia in the context of a hypercellular marrow with decreased megakaryocytes -hypersensitivity of myeloid precursors to GM-CSF as tested in clonal genetic assays, or by measuring STAT5 phosphorylation in the absence, or with low-dose exogenous GM-CSF	None

ICC International Consensus Classification.
*Per ICC, these criteria must be met for the diagnosis of JMML.

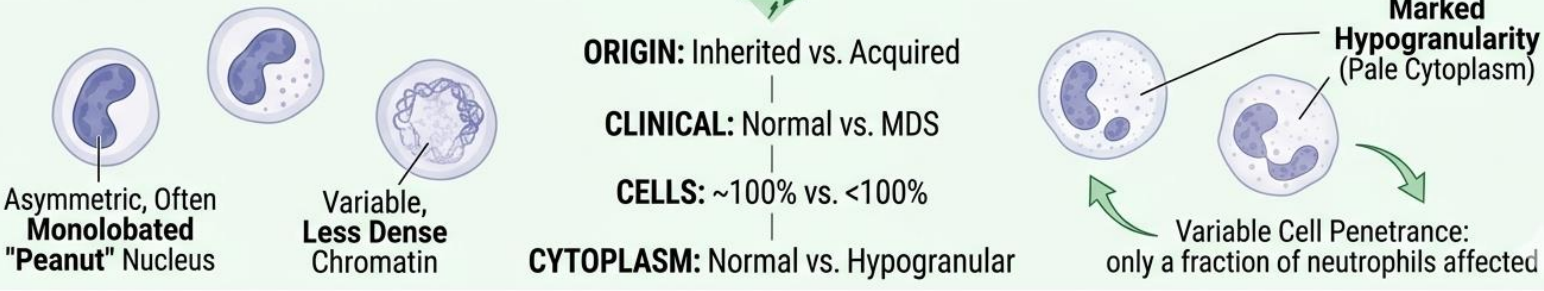
Elghetany, M.T., Cavé, H., De Vito, R. et al. Juvenile myelomonocytic leukemia; moving forward. Leukemia 2023, 720–722

DIFFERENTIAL DIAGNOSIS: PELGER-HUËT VS. PSEUDO-PELGER-HUËT ANOMALY

1. **TRUE PELGER-HUËT ANOMALY** (Inherited / Benign)



2. **PSEUDO-PELGER-HUËT ANOMALY** (Acquired / MDS-associated)

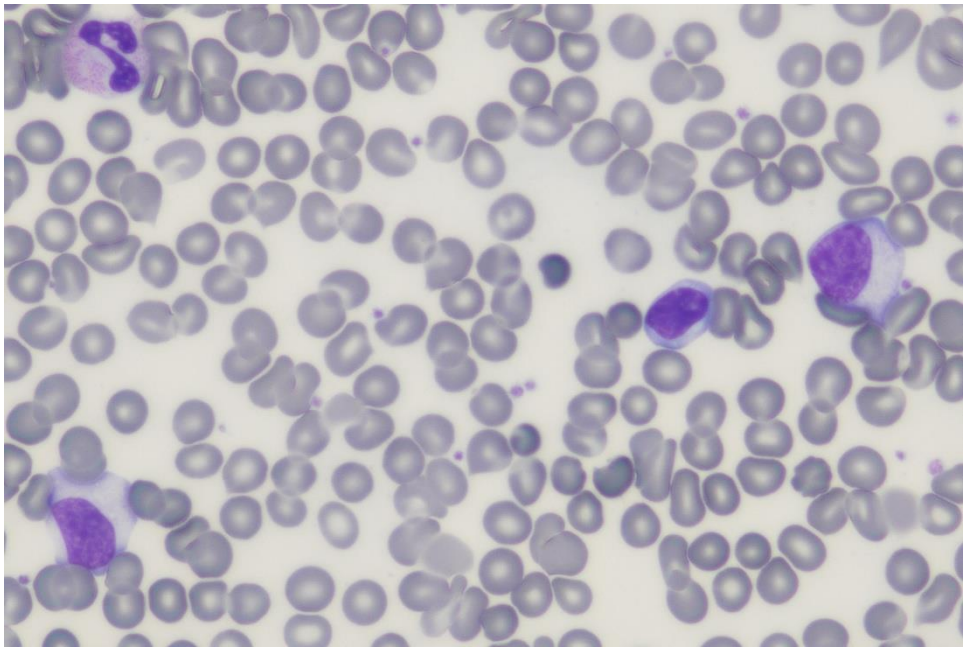


QUALITATIVE ABNORMALITIES IN LYMPHOID CELLS

Reactive lymphocytes (atypical lymphocyte, suspect reactive – European LeukemiaNet classification)

Abnormalities include increased cell size, immaturity of the nucleus including a visible nucleolus and lack of chromatin condensation, irregular nuclear outline or lobulation, cytoplasmic basophilia and vacuolation and irregular cell outline. The cytoplasm may be abundant with staining varying from pale blue to markedly basophilic especially at points of contact with adjacent cells.

The recommendation is to comment on the presence of reactive lymphocytes. They may be counted as a separate population in the differential if they are present in significant numbers.



Palmer L et al. ICSH recommendations. Int. Jnl. Lab. Hem. 2015, 37, 287–303

PERIPHERAL BLOOD FILM MORPHOLOGY: SUMMARY SCHEME

BLAST IDENTIFICATION
(WHO/FAB Concepts):



MYELOBLAST (Normal):
Rare in peripheral blood



LEUKEMIC BLAST:
>20% defines AML (WHO 2022)



MYELOBLAST
(MPO+)

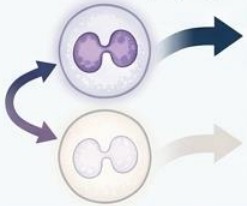
VS



MONOBLAST
(NSE+)

Cytochemical Distinction

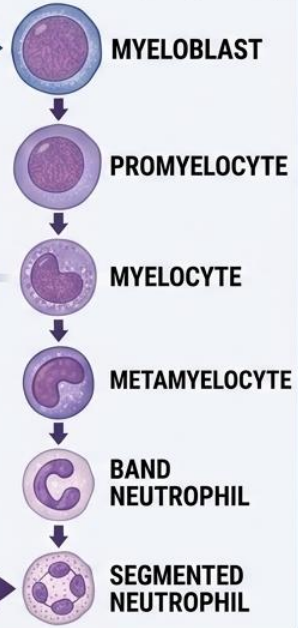
MYELODYSPLASIA HALLMARKS
(Dysgranulopoiesis):



PSEUDO-PELGER-HUËT NEUTROPHIL
Nuclear Hyposegmentation

HYPOGRANULAR NEUTROPHIL
Cytoplasmic Pallor

NORMAL MYELOID MATURATION (Simplified)



HYPERSEGMENTATION
(Megaloblastic):



Hypersegmented Neutrophil (≥6 lobes)

→ Hallmark of Vitamin B12 / Folate Deficiency

HYPOSEGMENTATION DIFFERENTIATION:



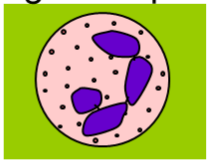
TRUE PELGER-HUËT
(Congenital):
Symmetric nucleus
Dense Chromatin
Normal Granulation



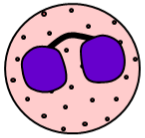
PSEUDO-PELGER-HUËT
(Acquired/MDS):
Asymmetric nucleus
Clumpy Chromatin
Hypogranularity

KEY ASSOCIATIONS: Hypogranularity/Pseudo-Pelger = MDS (frequent); **Hypersegmentation** = Megaloblastic (frequent)

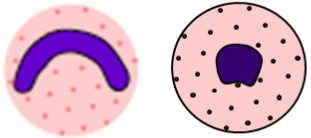
Dysgranulopoiesis



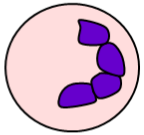
Normal segmented neutrophil



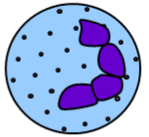
Pseudo-Pelger-Hüet anomaly



Bizarre nuclear shape

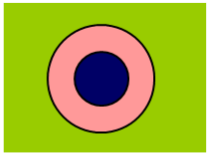


Hypo-, agranulation of cytoplasm

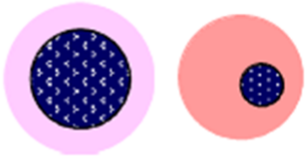


Asynchr. maturation nucleus - cytoplasm

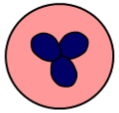
Dyserythropoiesis



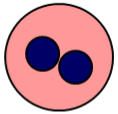
Normal erythroblast



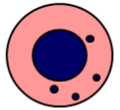
Macrocytic / megalo-blastic changes



Nuclear lobulation

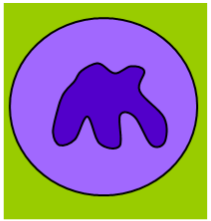


Multiple nuclei

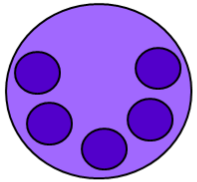


Cytoplasmic granules

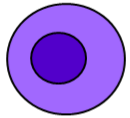
Dysmegakaryopoiesis



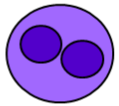
Normal megakaryocyte



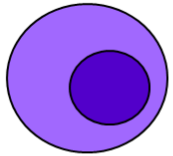
Separated single Nuclei



Mikromega-karyocyte



Small binucleated megakaryocyte



Rund, non-lobulated megakaryocyte

Rajnoldi Cantù A, Fenu S, et al. Evaluation of dysplastic features in myelodysplastic syndromes: experience from the morphology group of EWOG-MDS. Ann Hematol (2005) 84: 429–433

When Should Leukemia Be Suspected?

There is no single CBC pattern that rules leukemia in or out

Peripheral blood findings

Circulating blasts—sometimes only a small proportion

Anemia and/or thrombocytopenia, often involving more than one cell line

Leukocytosis, leukopenia, or a normal total white-cell count

Abnormal differential, immature cells, or an unexpected analyzer flag

Clinical clues that raise concern

Persistent pallor, fatigue, fever, or recurrent infections

Unexplained bruising, petechiae, or bleeding

Bone or joint pain, limp, or refusal to walk

Lymphadenopathy, hepatosplenomegaly, or unexplained deterioration

Important: absence of circulating blasts does NOT exclude acute leukemia.

If clinical suspicion remains high, urgent specialist assessment and further investigations are required.

Take-Home Messages & Selected References

For pediatric practice

Take-home messages

Review the smear when the CBC or clinical picture is concerning.

Leukemia may present with high, low, or normal WBC counts.

No circulating blasts does not rule out leukemia.

Morphology guides urgency and the next tests; it does not replace immunophenotyping or genetics.

Look at the smear. Think leukemia. Act early.

Educational content; follow local referral and laboratory pathways.

Selected references

Arber DA et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias. Blood. 2022;140:1200–1228. doi:10.1182/blood.2022015850

Duffield AS, Mullighan CG, Borowitz MJ. International Consensus Classification of Acute Lymphoblastic Leukemia/Lymphoma. Virchows Arch. 2023;482:11–26. doi:10.1007/s00428-022-03448-8

Choi JK et al. WHO Classification of Haematolymphoid Tumours: Acute Lymphoblastic Leukaemias and related entities. Mod Pathol. 2024;37:100466. doi:10.1016/j.modpat.2024.100466

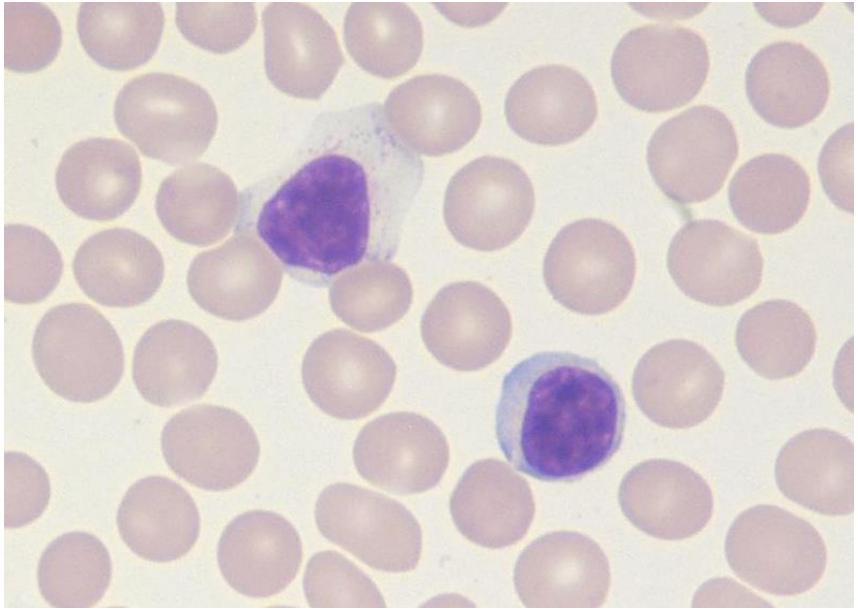
National Cancer Institute. Childhood ALL Treatment (PDQ) and Childhood AML Treatment (PDQ).

NORMAL LYMPHOCYTE MORPHOLOGY

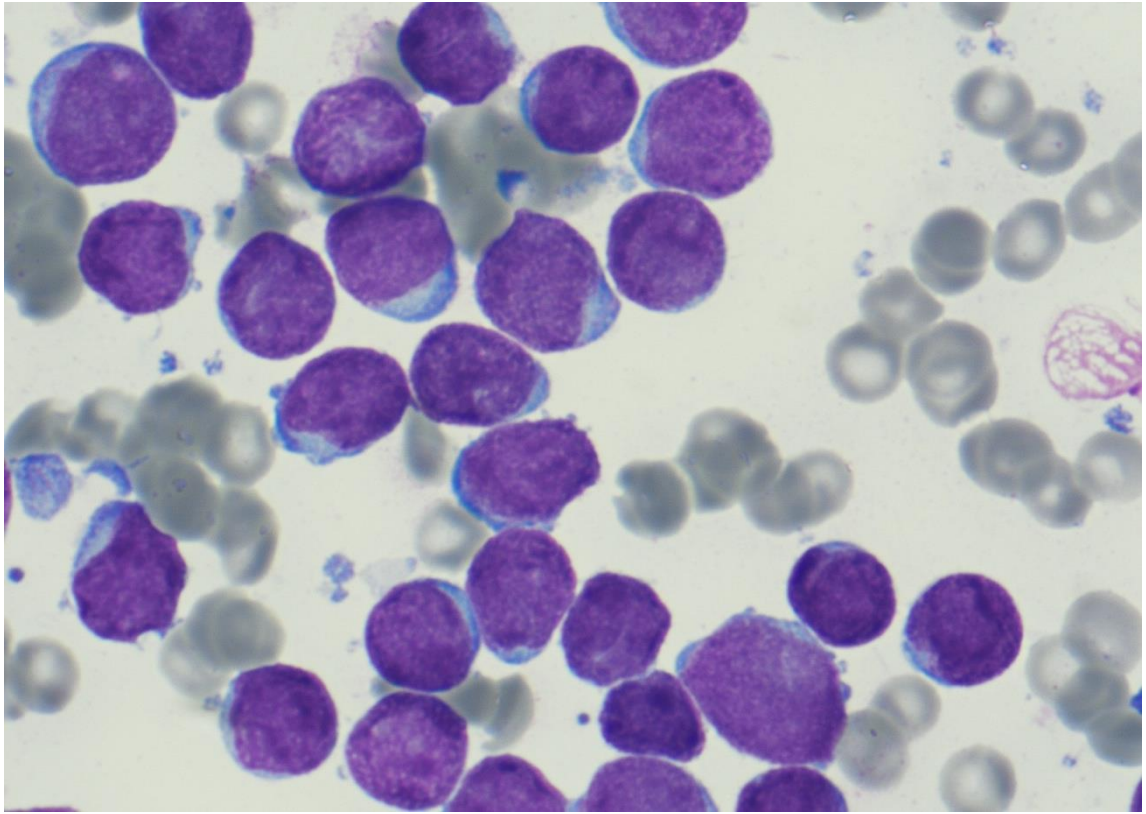
Lymphocyte

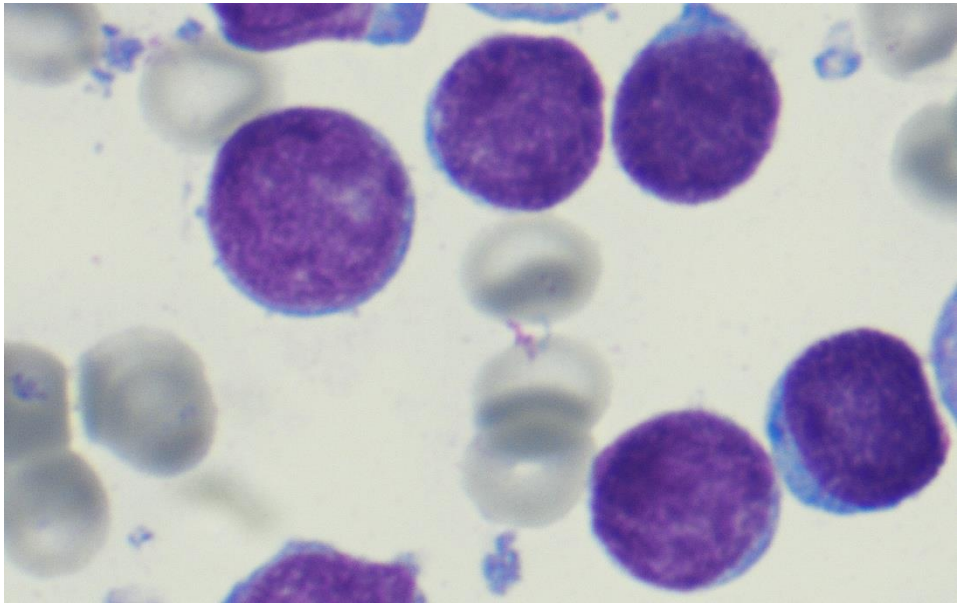
Lymphocytes seen in the peripheral blood are predominantly small (10–12 µm), or, less frequently large (12–16 µm).

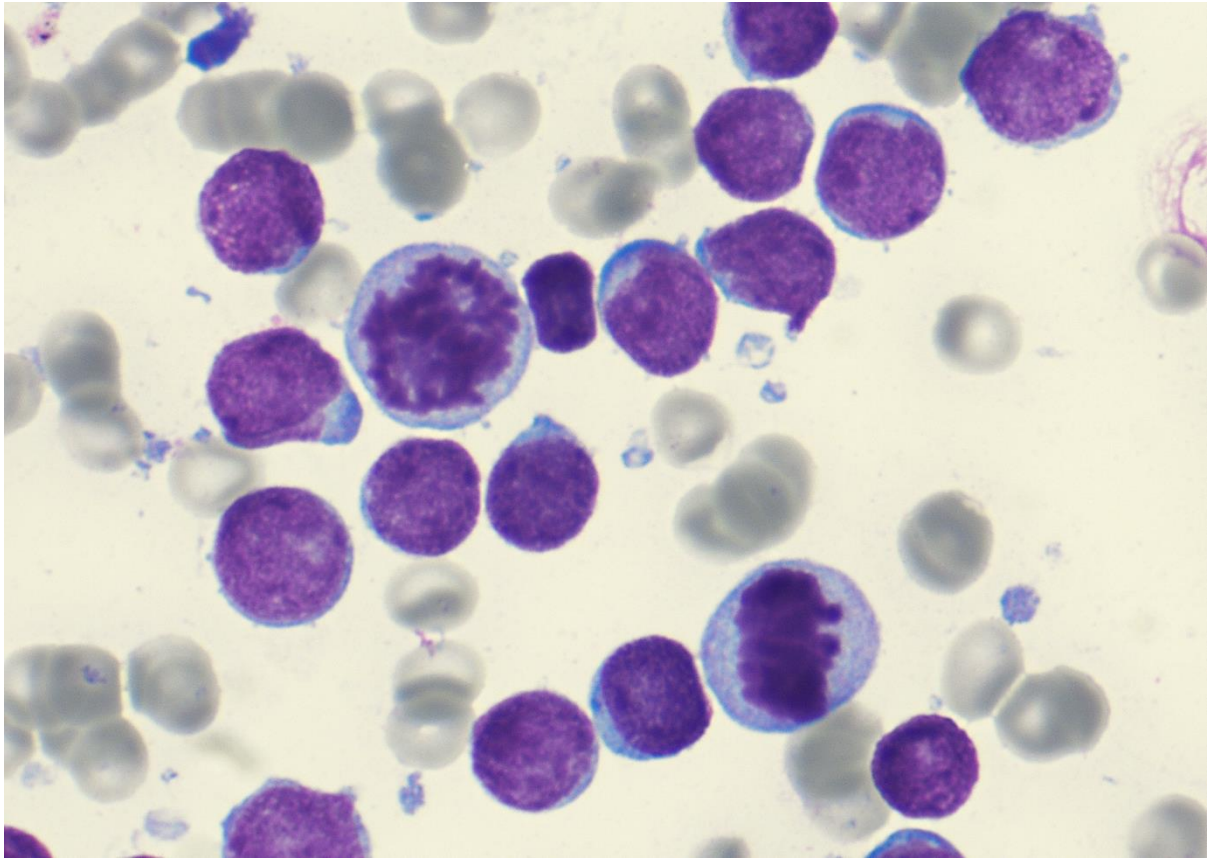
Small lymphocytes are usually round in outline, and the nucleus is round with coarse, densely staining chromatin. Cytoplasm is scanty.
Large lymphocytes are usually irregular in outline, and the nuclear chromatin is not as coarse as in small lymphocytes. Cytoplasm is abundant and tends to be light sky blue in colour.
Large granular lymphocytes (LGLs) are of the same appearance as large lymphocytes but the cytoplasm contains prominent small red-violet granules. These cells can comprise up to 10–20% of the peripheral blood lymphocytes in normal subjects. LGLs are not routinely counted as a separate lymphocyte population.



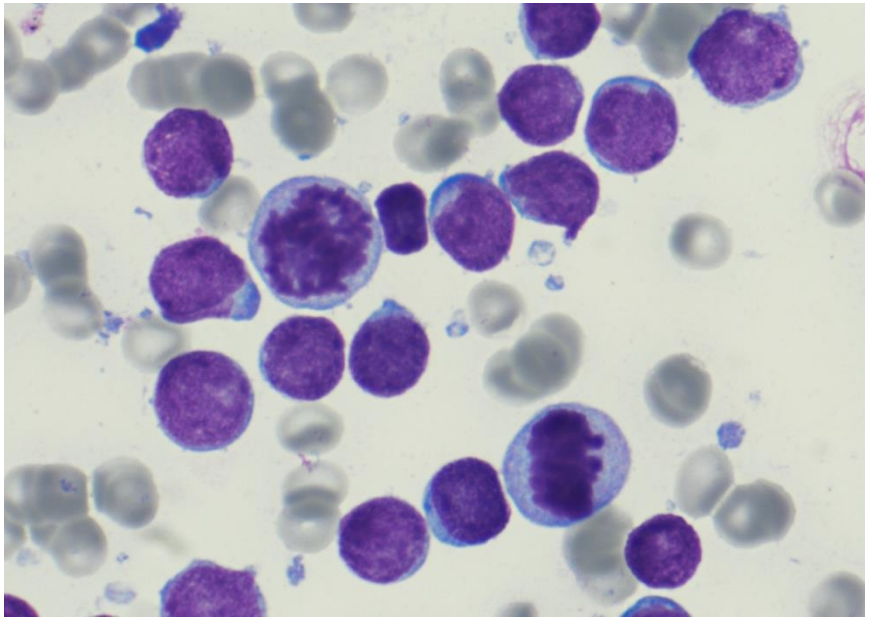
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

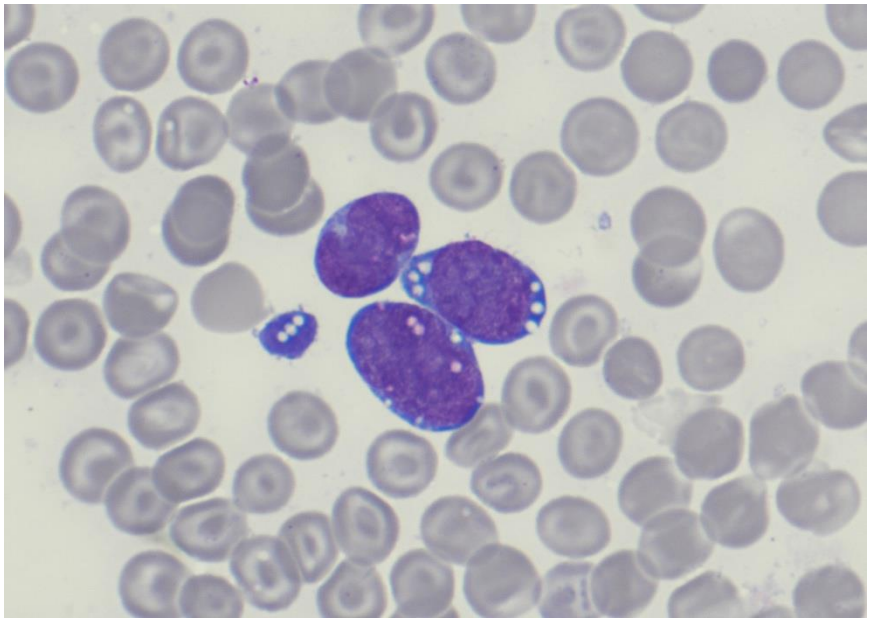


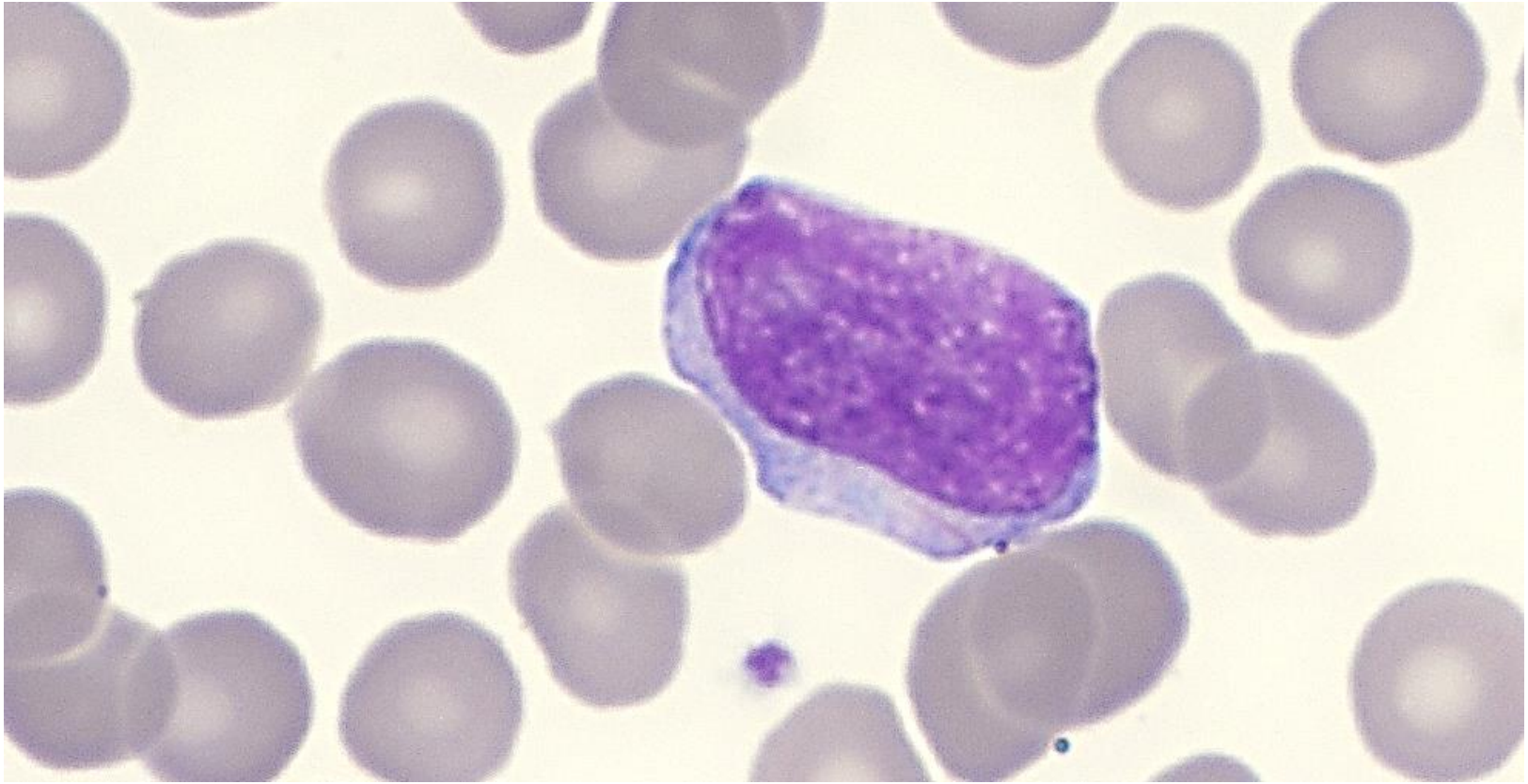


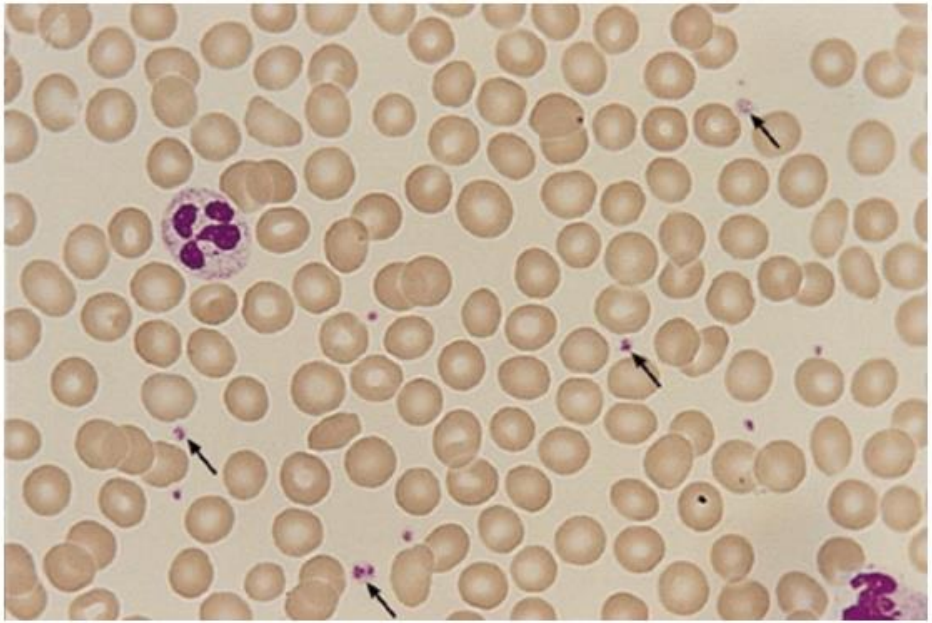


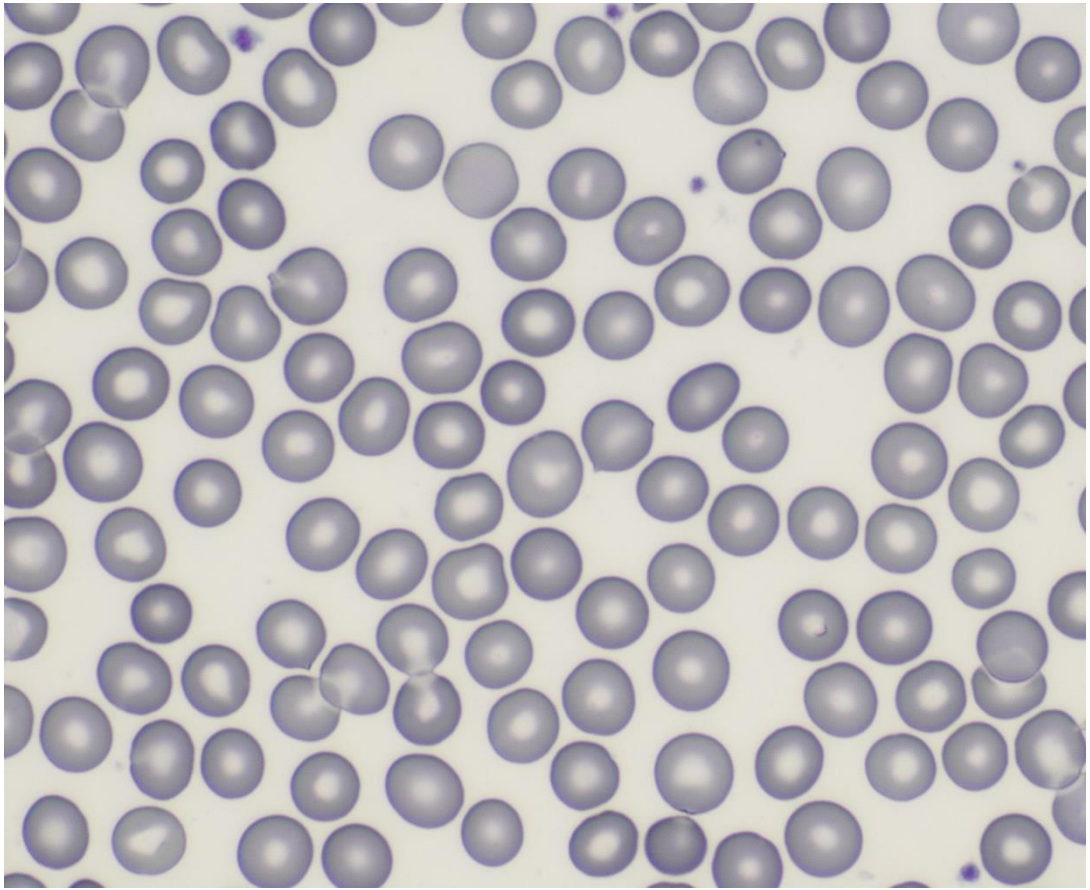
PERIPHERAL BLOOD MORPHOLOGY: LEUKAEMIA SUSPECT ALERTS

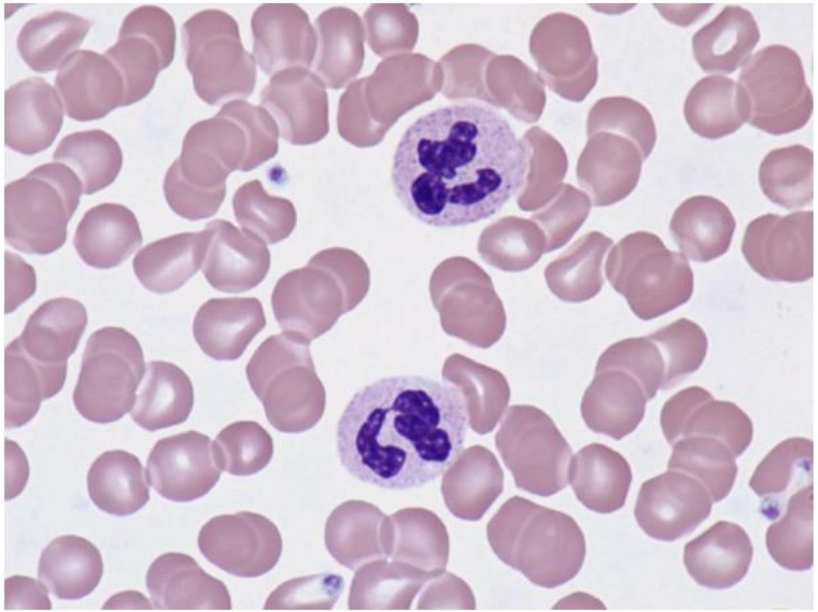








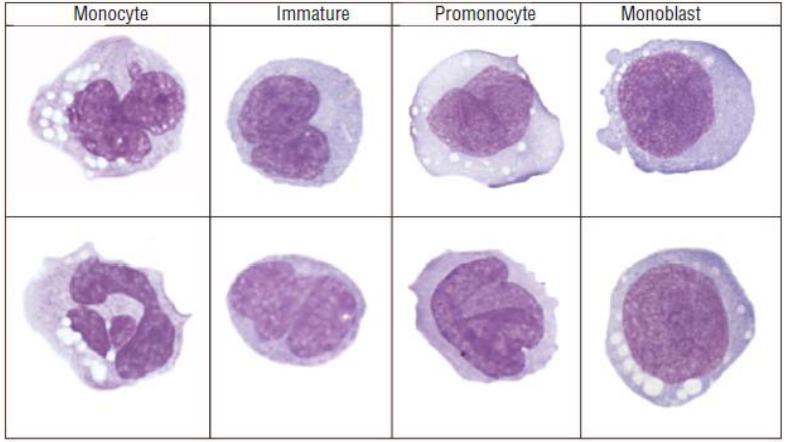




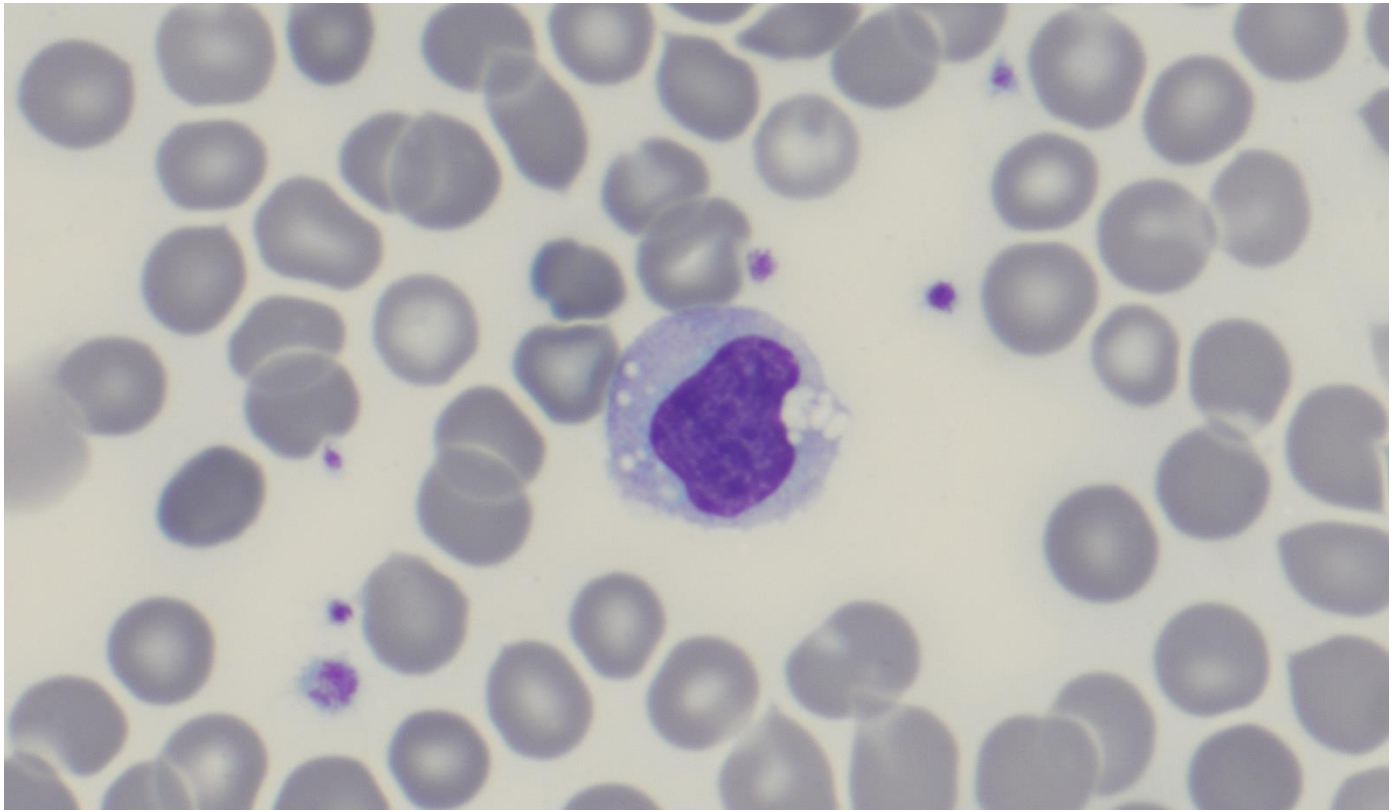
Morphological evaluation of monocytes and their precursors

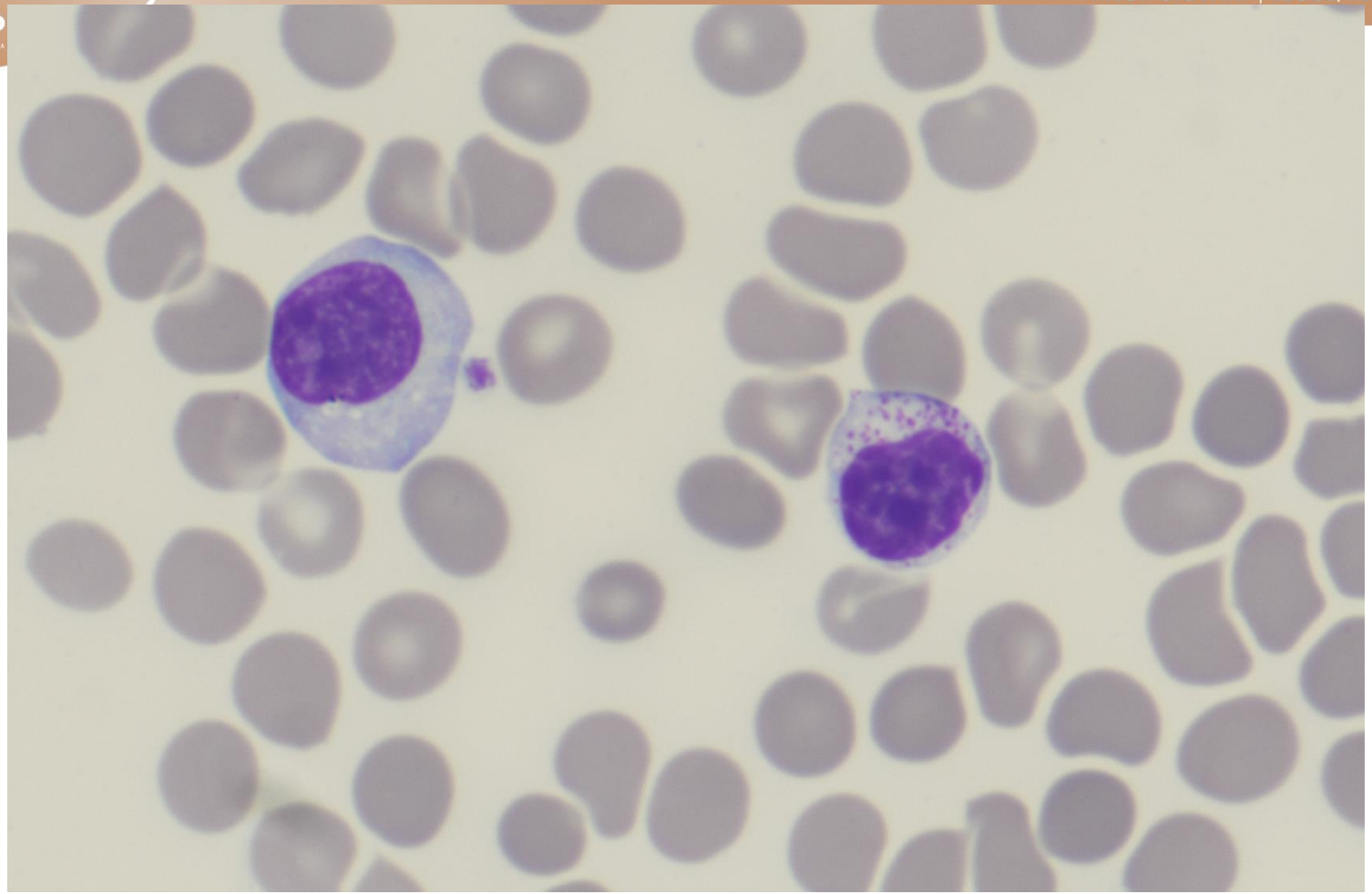
Jean E. Goasguen,[1] John M. Bennett,[2] Barbara J. Bain,[3] Teresa Vallespi,[4] Richard Brunning,[5] and Ghulam J. Mufti[6]
for the International Working Group on Morphology of Myelodysplastic Syndrome (IWGM-MDS)

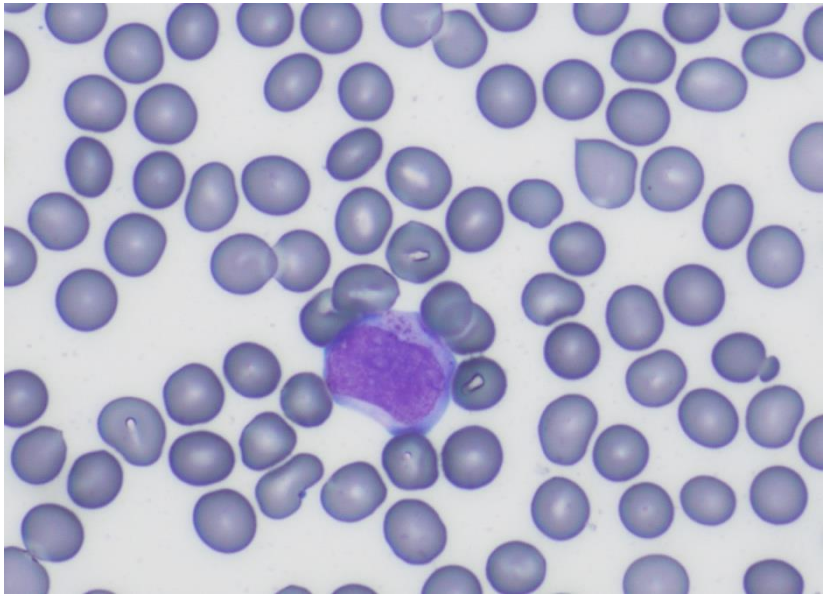
	Nuclear shape	Chromatin	Cytoplasm	Comments
Monoblast	Round/oval	Delicate / lace-like Nucleolus prominent	Basophilic Rare azurophilic, Granules	Large: 20-30 µm
Promonocyte	Convoluted / indented	Delicate / lace-like Nucleolus prominent	Variably basophilic Variable azurophilic Granules	Except for nuclear shape, very similar to monoblast
Immature monocyte	Convoluted / indented	More condensed Rare nucleolus	Less basophilic than promonocyte or blast, but more basophilic than mature monocyte	Resemble monocytes but less mature and smaller
Monocyte	Lobulated/ indented	Condensed No visible nucleolus	Gray Occasional azurophilic granules. Occasional vacuole	Large : 20-25 µm



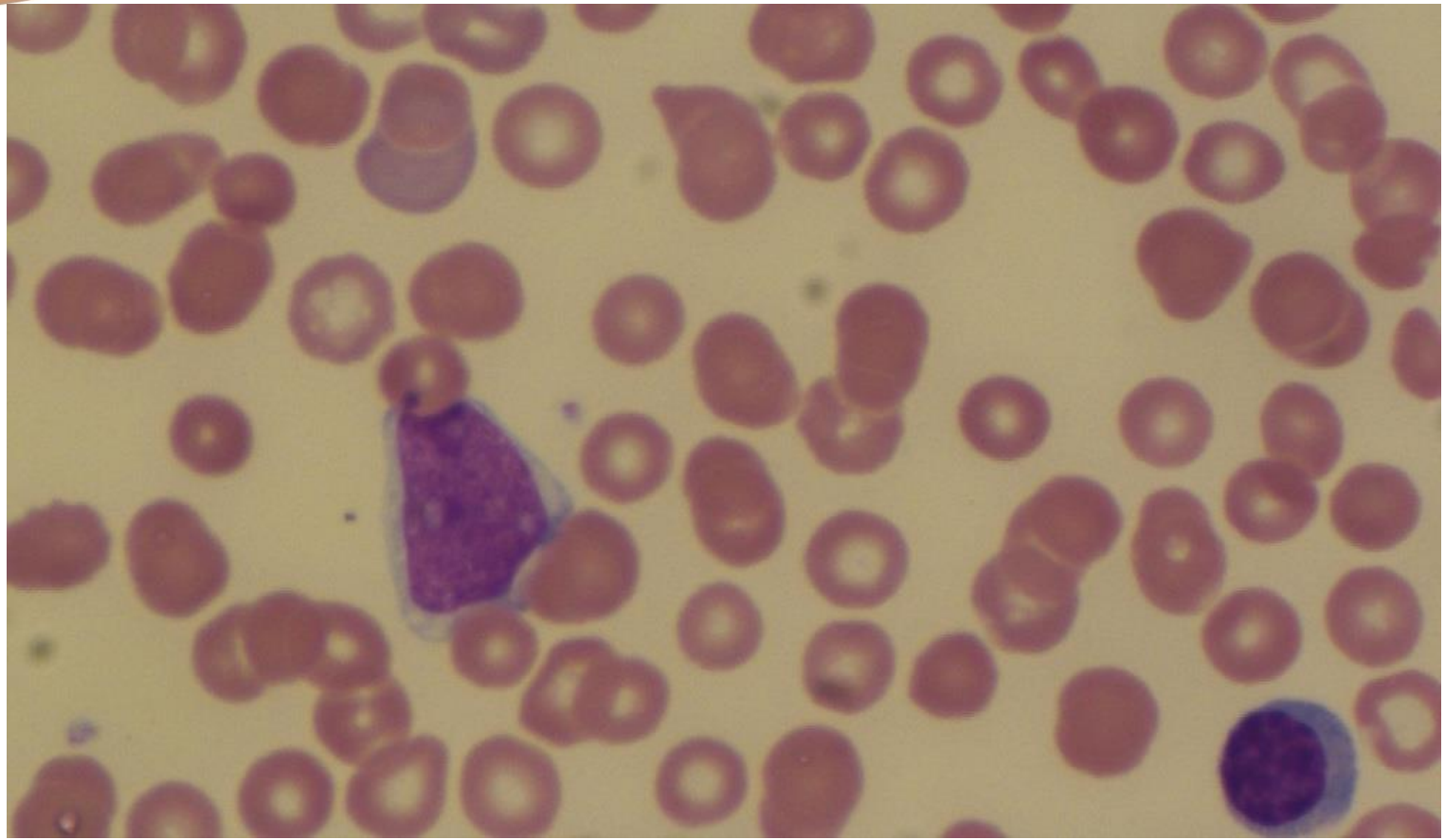
Goasguen JE et al. Haematologica. (2009) 94:994–7



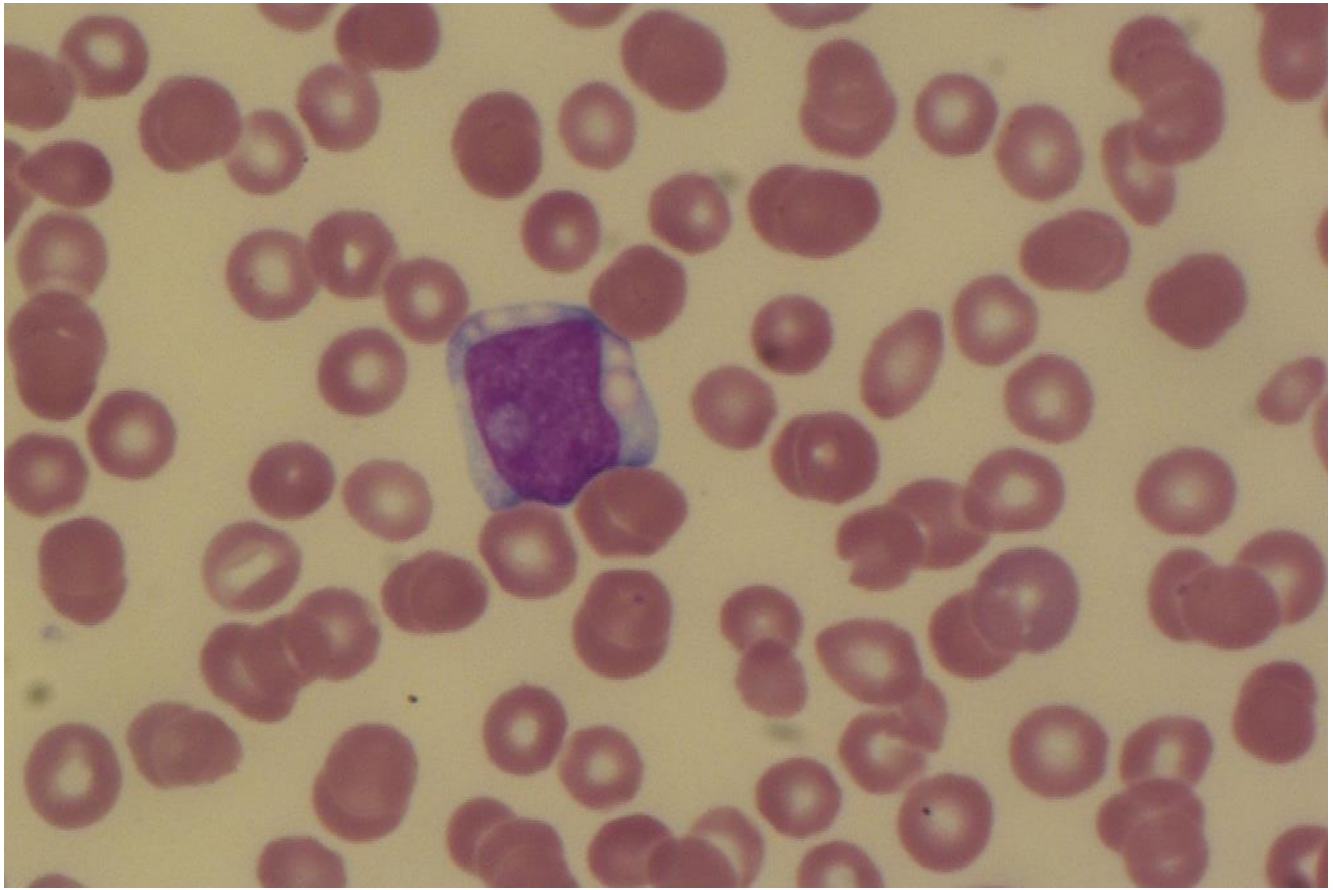


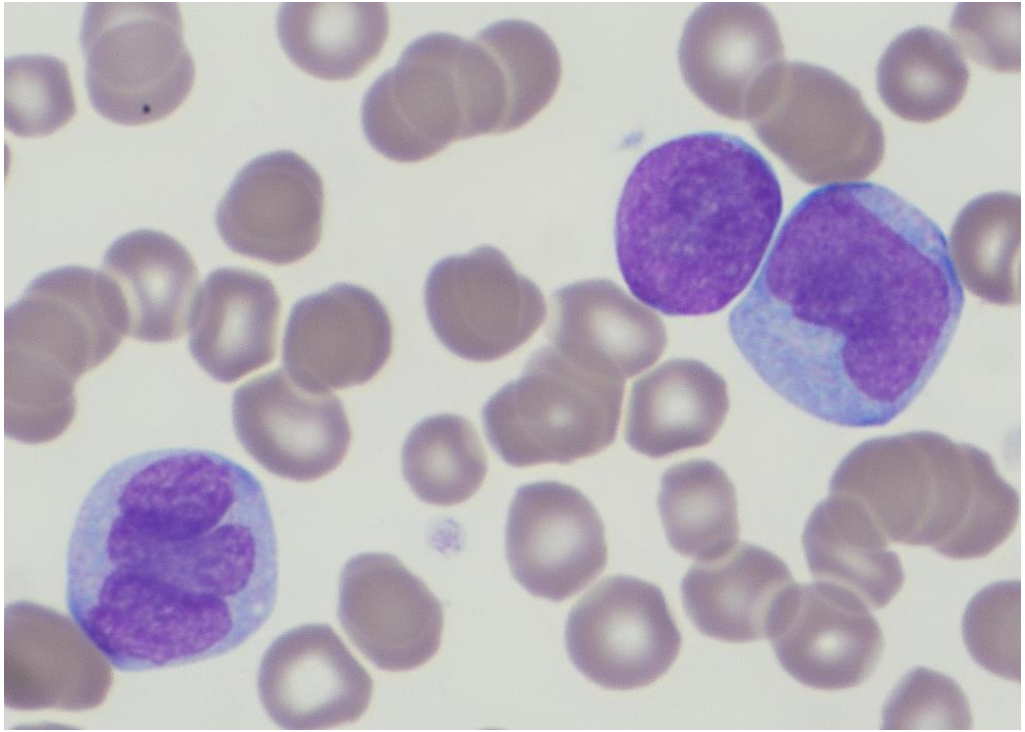


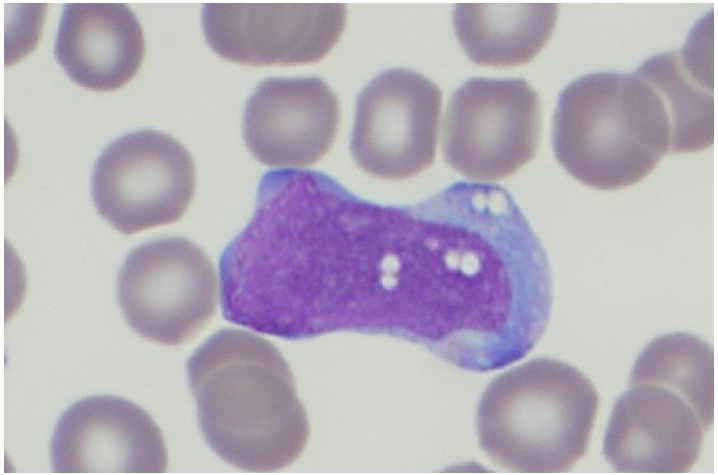
PERIFERICO ???

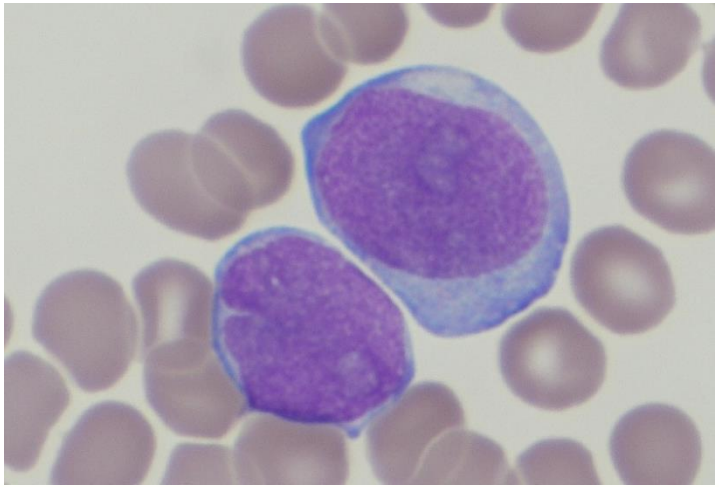


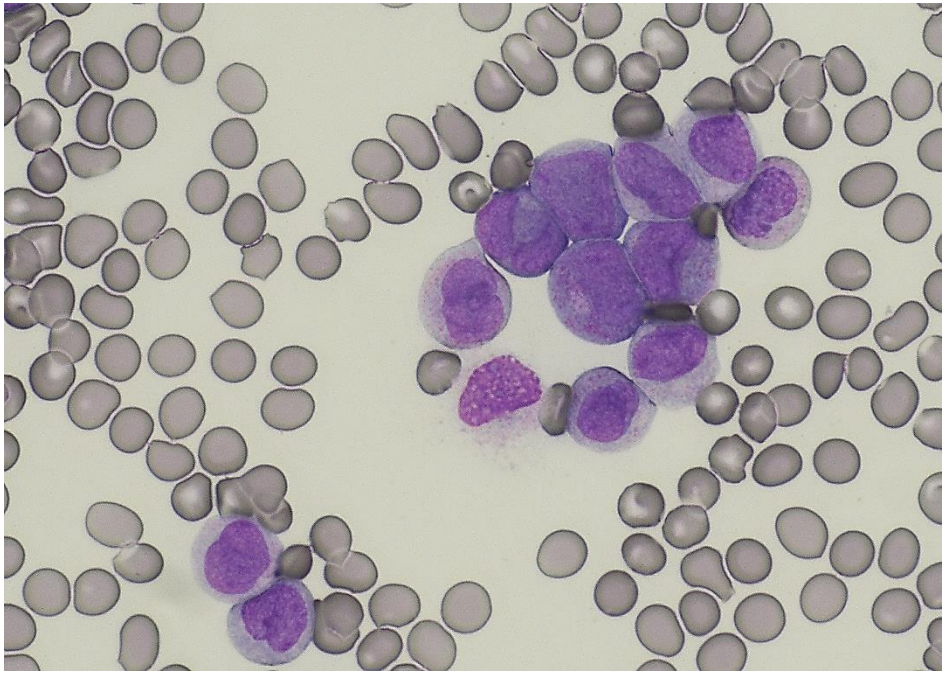
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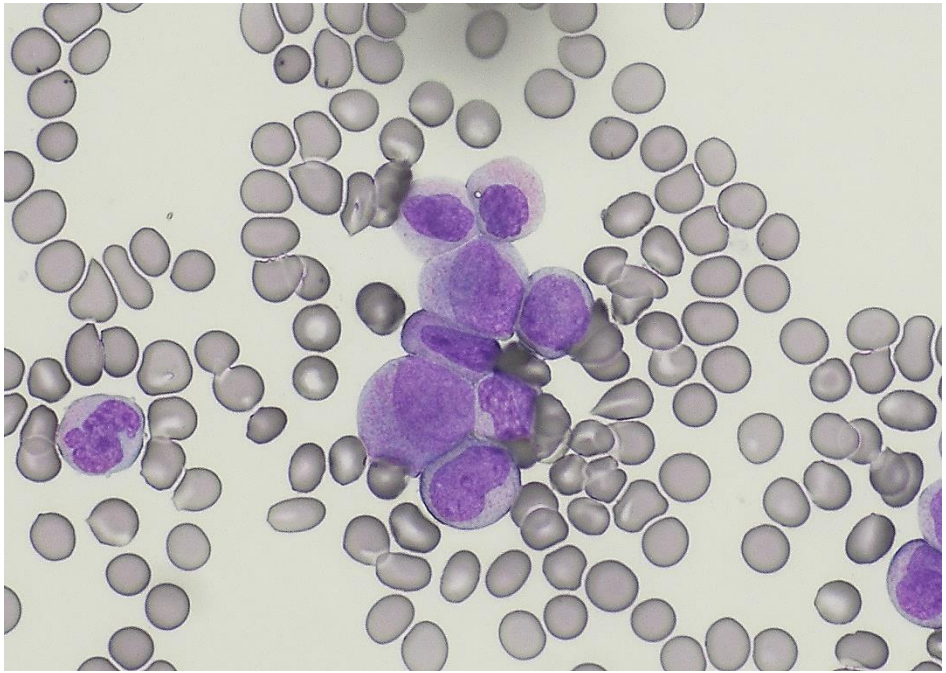


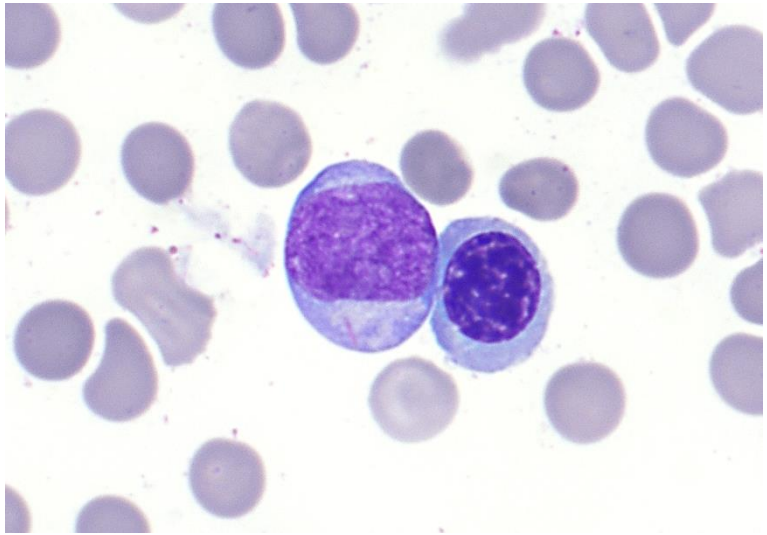


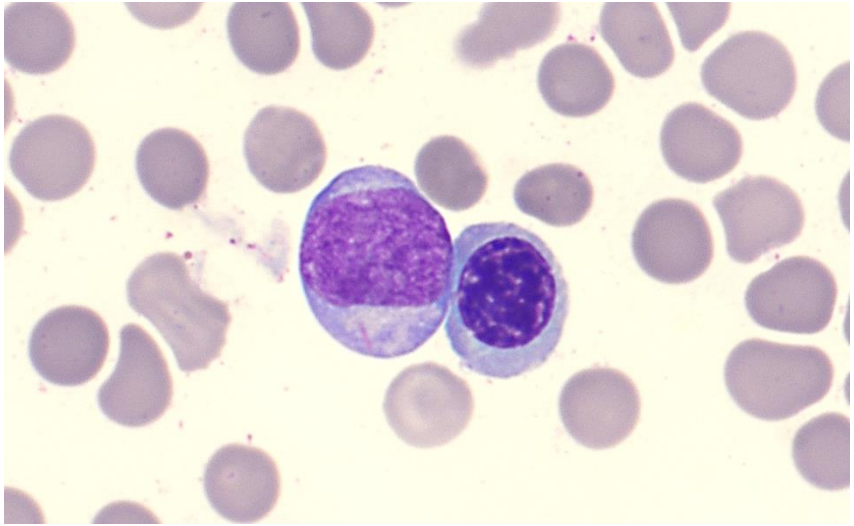


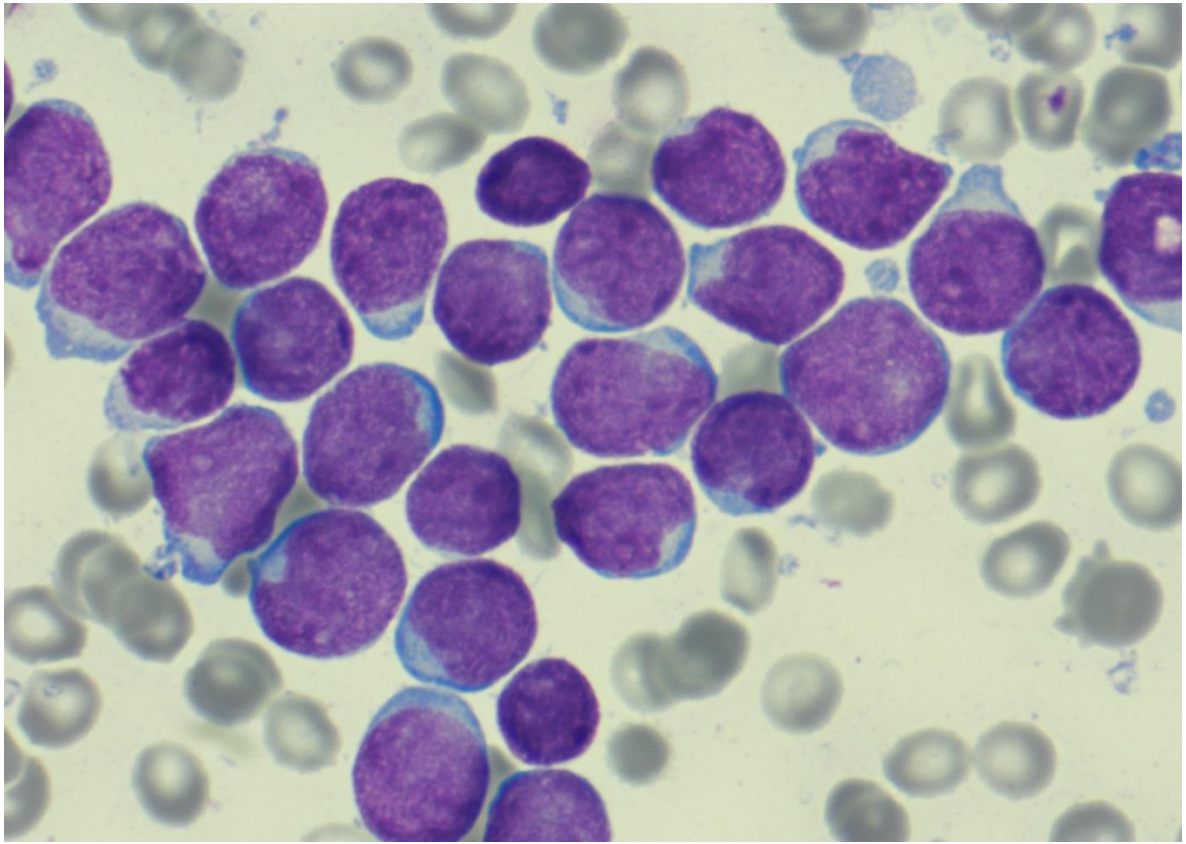












TECHNIQUE OF EXAMINATION OF BLOOD FILMS

- **High-Resolution Analysis (x100 Oil Immersion)**: *should be used only for the final examination of unusual cells and for looking at fine details such as basophilic stippling (punctate basophilia) or Auer rods*. Utilization of the x100 objective is non-routine.
- Because the diagnosis depends on **comprehension of the whole picture** the film presents, the red cells, leucocytes, and platelets must all be systematically examined.
- The film examination also serves **to validate the automated blood count**, distinguishing, for example, between true thrombocytopenia and factitious thrombocytopenia caused by platelet aggregation.

Dacie and Lewis, Practical Haematology 2011, 11th Edition

💡 Morphological Differentiation: Acanthocytes vs. Echinocytes

📋 Diagnostic Criteria & Clinical Correlates

Feature	Acanthocytes (Spur Cells) 💥	Echinocytes (Burr Cells) 🦔
Spicule Morphology	**Club-shaped** (blunt, rounded tips), variable length.	**Sharp or blunt**, short, highly uniform.
Distribution	**Asymmetric**, randomly protruding from the cell.	**Symmetric**, evenly spaced across the entire membrane.
Spicule Count	Few per cell (usually **2 to 10**).	Numerous per cell (usually **10 to 30**).
Cell Core	Often lacks central pallor, dense contracted center.	Retains central pallor, disc-like global shape.
Reversibility	**Irreversible** (due to fixed lipid/structural defects).	**Reversible** (can revert to discocytes in normal plasma).
Top Pediatric Causes	Severe liver disease, abetalipoproteinemia, McLeod syndrome.	**Uremia**, **Pyruvate Kinase deficiency**, artifact (glass effect).

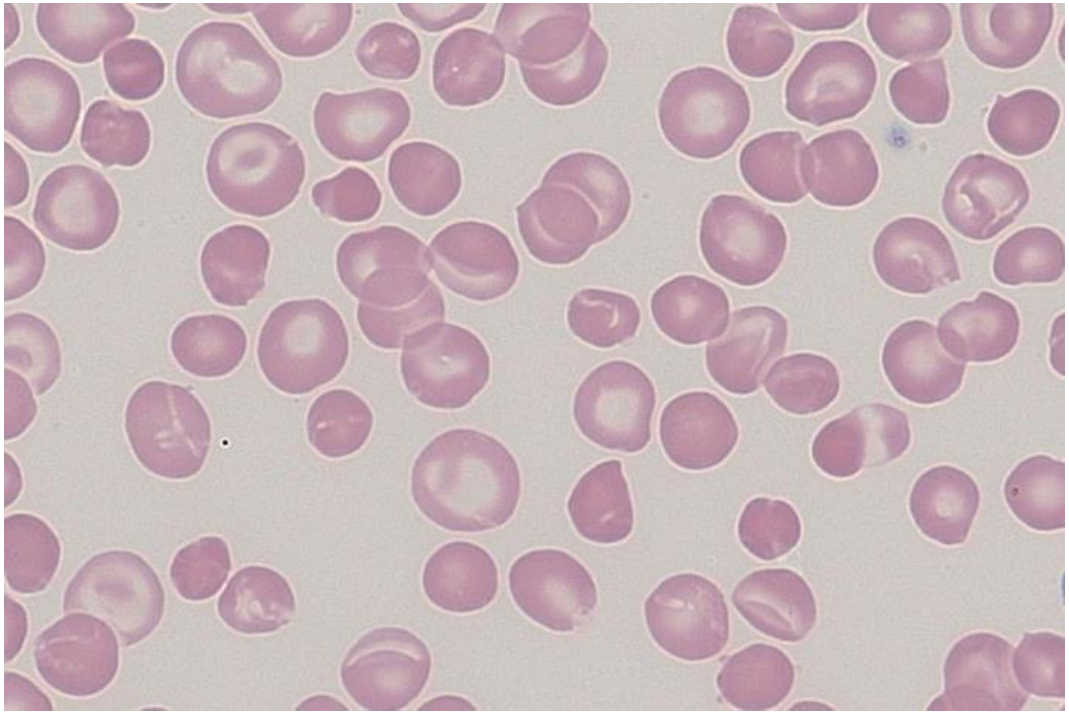
ABNORMALITIES OF RBC SHAPE

Irregularly contracted cells

Irregularly contracted cells are smaller and denser RBC which lack an area of central pallor but are not as regular in shape as spherocytes.

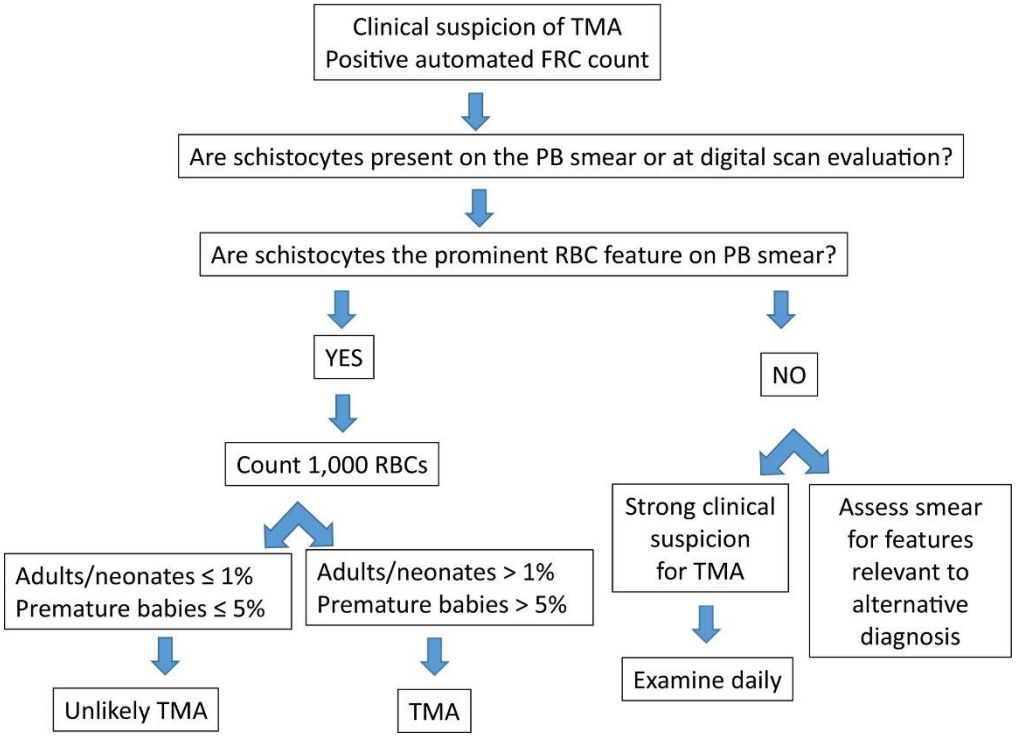
G6PD deficiency, haemoglobinopathies

Unstable haemoglobin



Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

2021 UPDATE OF THE 2012 ICSH RECOMMENDATIONS FOR IDENTIFICATION, DIAGNOSTIC VALUE, AND QUANTITATION OF SCHISTOCYTES

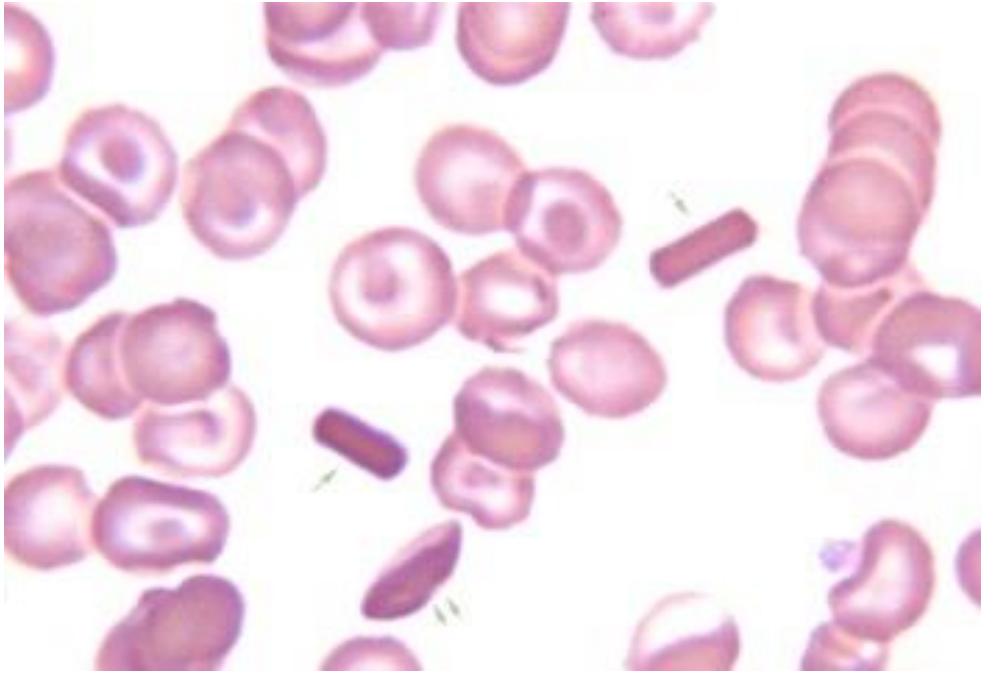


Zini G, et al. ICSH recommendations for identification, diagnostic value and quantitation of schistocytes. Int J Lab Hematol. 2012;34(2):107–116. Update 2021: Int J Lab Hematol. 2021;43(6):1256–1262

INCLUSION IN RBC

Intracellular haemoglobin crystals

Crystalline aggregates of haemoglobin may be seen in HbC and HbSC disease. These crystals stain densely, vary in size and have straight edges with pointed ends.

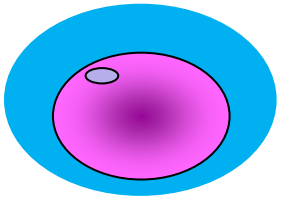


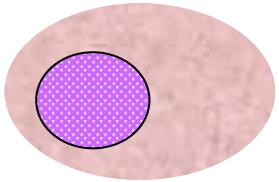
Hemoglobin C - ASH Image Bank

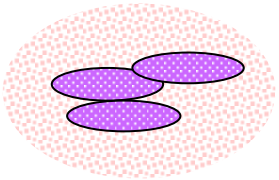
Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

MORPHOLOGICAL CHARACTERISTICS OF THE WHITE BLOOD CELLS

- **SIZE:** large – medium – small
- **N/C RATIO:** high – medium – low
- **CYTOPLASM:** basophilic – acidophilic
- **INCLUSIONS:** presence / absence of ***GRANULATIONS, AUER RODS***
- **NUCLEUS POSITION:** central – eccentric
- **NUCLEAR MEMBRANE:** regular, indented, etc.
- **CHROMATIN:** fine, dispersed, condensed
- **NUCLEOLUS:** present (one or more), absent

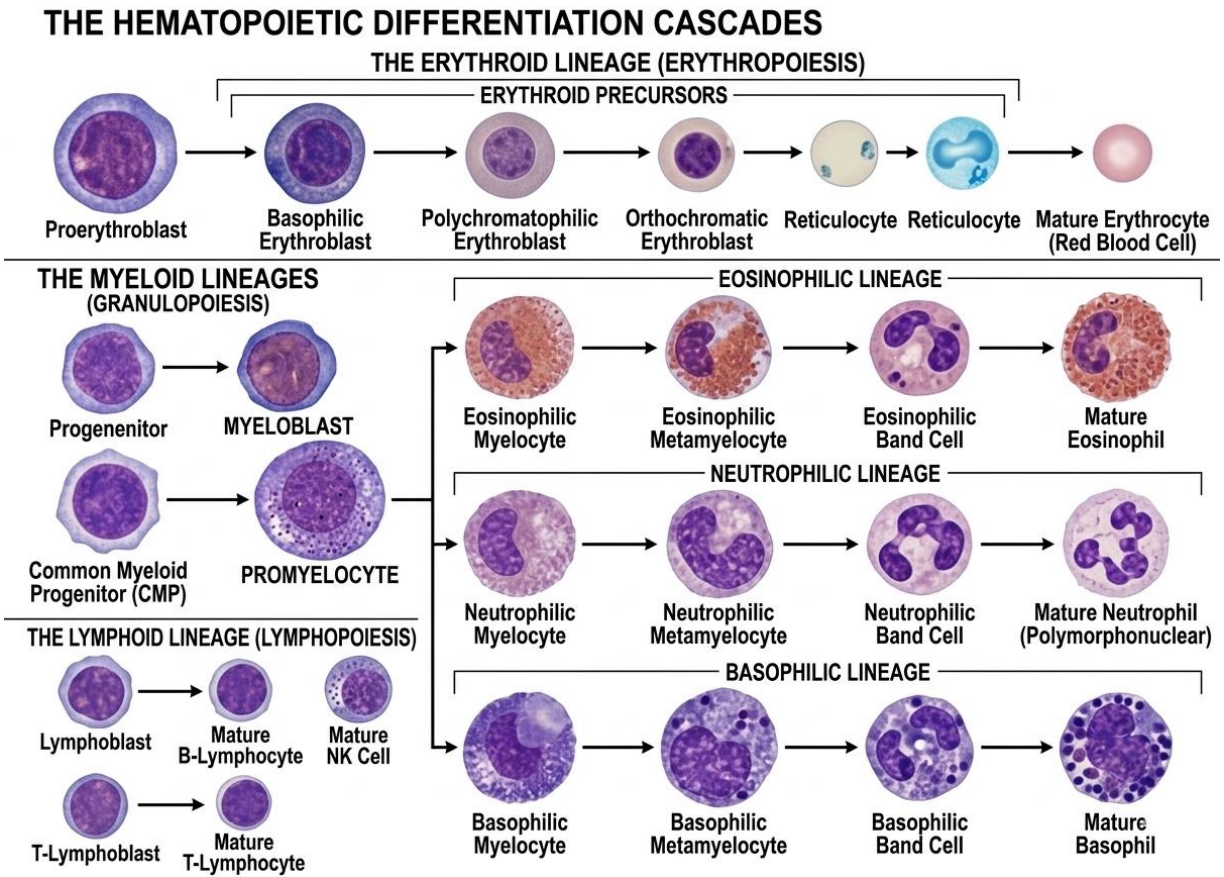






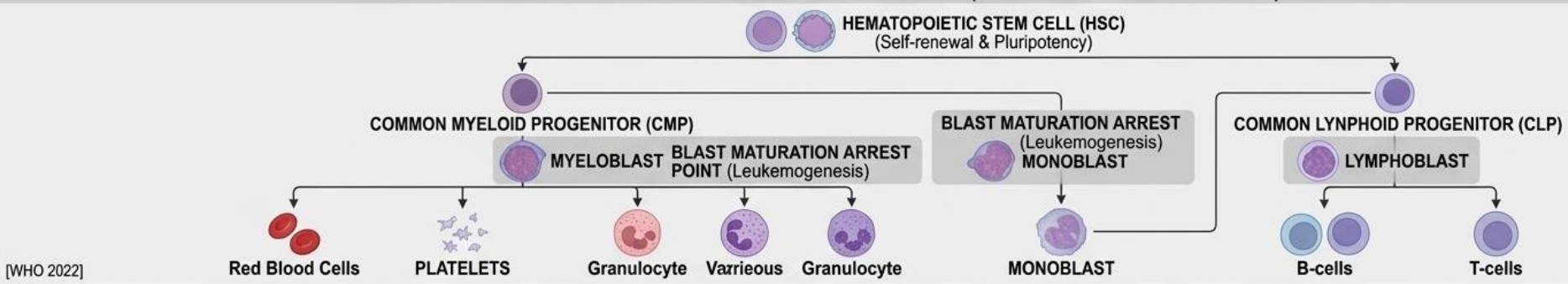
Must be evaluated on 200 cells on a peripheral blood smear

Courtesy Dr. Susanna Fenu


THE HEMATOPOIETIC DIFFERENTIATION CASCADES
THE ERYTHROID LINEAGE (ERYTHROPOIESIS)
ERYTHROID PRECURSORS
Proerythroblast
Basophilic Erythroblast
Polychromatophilic Erythroblast
Orthochromatic Erythroblast
Reticulocyte
Reticulocyte
Mature Erythrocyte (Red Blood Cell)
THE MYELOID LINEAGES (GRANULOPOIESIS)
Progenenitor
MYELOBLAST
Common Myeloid Progenitor (CMP)
PROMYELOCYTE
EOSINOPHILIC LINEAGE
Eosinophilic Myelocyte
Eosinophilic Metamyelocyte
Eosinophilic Band Cell
Mature Eosinophil
NEUTROPHILIC LINEAGE
Neutrophilic Myelocyte
Neutrophilic Metamyelocyte
Neutrophilic Band Cell
Mature Neutrophil (Polymorphonuclear)
BASOPHILIC LINEAGE
Basophilic Myelocyte
Basophilic Metamyelocyte
Basophilic Band Cell
Mature Basophil
THE LYMPHOID LINEAGE (LYMPHOPOIESIS)
Lymphoblast
Mature B-Lymphocyte
Mature NK Cell
T-Lymphoblast
Mature T-Lymphocyte

HEMATOPOIETIC STEM CELL DIFFERENTIATION, BLAST MATURATION ARREST, AND WHO/ICC LINEAGE DEFINITION

PART 1: UPDATED HEMATOPOIETIC STEM CELL HIERARCHY (WHO/ICC 2022/2023 CANON)



[WHO 2022]

PART 2: COMPARATIVE BLAST MORPHOLOGY AND WHO/ICC RIGID LINEAGE CRITERIA

CELL TYPE & PATHWAY	MICROSCOPIC PHOTOMICROGRAPH (High-Resolution)	MORPOLOGY & ELEMENTS	WHO/ICC MINIMAL LINEAGE REQUIREMENTS (Immunophenotype)
MYELOBLAST (AML Pathway)	• **Fine, lacy chromatin** • **Visible nucleoli** • **Small red Auer rods**	• **Fine, lacy chromatin** (fine, lacy-anny chromatin • **Visible nucleoli** • Small nucleoli **red Auer rods** • **Small red rods and small red Auer rods**	**Mieloperossidasi (MPO)+** (Cytometry/Cytochemistry) • Strong expression of **myeloid antigens** (e.g., CD13, CD33, CD117)
MONOBLAST (AML M5 Pathway)	• **Massive cell** • **Irregular nuclear folds, 'ground glass' cytoplasm**	• **Massive cell** • **Irregular nuclear folds**, and cytoplasm, 'ground glass' **cytoplasm** • Irregurlasm 'ground glass' cytoplasm	**Aspecific Esterase (NSE)+** (Sodium fluoride sensitive) • Strong expression of **monocytic markers** (CD14, CD64, CD11c)
LYMPHOBLAST (ALL Pathway)	• **Massive nucleus** • **Scanty cytoplasm** • **Clumped chromatin**	• **Massive nucleus** • **Scanty cytoplasm** • **Clumped chromatin**	• **CD19+** (Lineage B) • **cCD3+** (Cytoplasmic CD3, Lineage T) [ICC 2023]

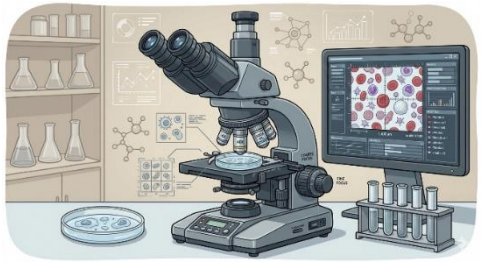
PART 3: INTERNATIONAL CLASSIFICATION CRITERIA (WHO 2022 vs. ICC) FOR BLAST COUNT AND MPAL

	BLAST THRESHOLD	MPAL DEFINITION
Nature Reviews [WHO 2022]	1.0 – 20 2.0 – 20 ≥ 10+	• MPAL • MPAL and strow Haematologica [2002] • MPAL nIIL. definition and Haematologica [2023]
Nature Reviews [ICC 2023]	> 10 2.0 - 30 > 10+	• MPAL (ord./Haematologica [2027] • MPAL (eg., cytormetric and Haematologica [1133] • MPAL MPAL: asociation and Haematologica [20112]

[Nature Reviews et al. 2022]

Haematologlca et al. 2023], Haematclogica et al. 2023]

PERIPHERAL BLOOD MORPHOLOGY



- **CONFORM OR DISMISS ALARMS FROM AUTOMATED CYTOMETRY** (e.g., cell clusters);
- **EVALUATE KEY PATHOLOGICAL ALTERATIONS:**
  - Size and shape variations of Red Blood Cells (RBC), White Blood Cells (WBC), and Platelets.
  - Cytoplasmic and nuclear abnormalities;
- **DISCRIMINATE FALSE POSITIVES VS. FALSE NEGATIVES**
- **GUIDE SECOND-LEVEL DIAGNOSTIC WORK-UPS** (e.g., flow cytometry, molecular genetics);
- **CAUTIONAY NOTE: A NORMAL RESULT DOES NOT EXCLUDE CONSTITUTIONAL OR ACQUIRED HEMATOLOGICAL PATHOLOGY (Clinical integration essential).**

Courtesy Dr. Susanna Fenu

EFFECTS OF STORAGE ON BLOOD CELL MORPHOLOGY

- **Immediate Action**: *preparing blood films immediately from non-anticoagulated blood is the absolute gold standard* for preserving optimal cellular morphology free from chemical artifacts.
- **Permissible Time Limit**: when utilizing EDTA tubes, the blood smear must be done within a maximum delay of 3 to 4 hours. Beyond this window, irreversible storage artifacts appear (e.g., RBC crenation, neutrophil nuclear swelling).
- **Temperature Effect** (Storage Conditions): if analyzed within 3 hours, the EDTA tube should remain at room temperature (18–22°C). Refrigeration at 4°C retards morphological degradation but does not entirely eliminate it.

Dacie and Lewis, Practical Haematology 2011, 11th Edition

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PERIPHERAL BLOOD MORPHOLOGY IN PEDIATRIC LEUKEMIA

WHY THE SMEAR MATTERS:

Early recognition

Blasts may be the first visible clue to acute leukemia.

Morphology can trigger urgent review even before marrow results.

Rapid clinical context

Interpret the film alongside symptoms and the full blood count.

Look across all three cell lines—not only the white-cell count.

A first step, not the final diagnosis

Morphology raises suspicion and helps prioritize next tests.

Flow cytometry and genetic studies establish classification.

Absence of circulating blasts does NOT exclude acute leukemia

EFFECTS OF STORAGE ON BLOOD CELL MORPHOLOGY

- **Immediate Action**: *preparing blood films immediately from non-anticoagulated blood is the absolute gold standard* for preserving optimal cellular morphology free from chemical artifacts.
- **Time-dependent changes:** morphological alterations in stored samples begin within a few hours of blood collection.
- **Golden rule:** blood smear must be done within a maximum delay of 3 to 4 hours; films prepared **within 1 hour** at room temperature are indistinguisable from immediate films.

Dacie and Lewis, Practical Haematology 2011, 11th Edition

EFFECTS OF STORAGE ON BLOOD CELL MORPHOLOGY

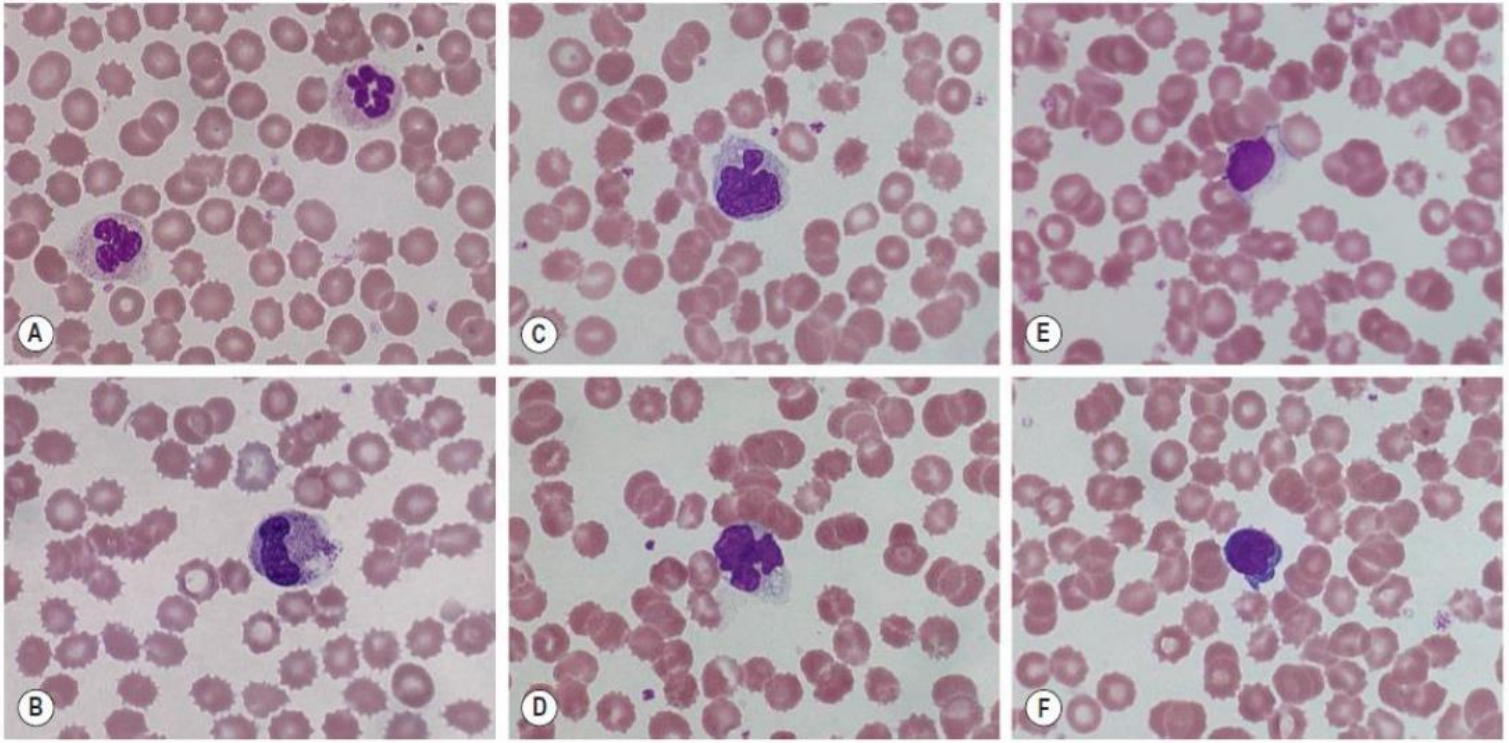


FIGURE 1-2 Effect of storage on blood cell morphology. Photomicrographs from films made from ethylenediaminetetra-acetic acid (EDTA) blood after 24 h at 20°C. (**A, B**) Polymorphonuclear neutrophils; (**C, D**) monocytes; (**E, F**) lymphocytes. Red cell crenation is prominent in all images.

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TECHNIQUE OF EXAMINATION OF BLOOD FILMS

- **Macroscopic Evaluation**: preliminary assessment to verify the adequacy of the smear preparation, staining quality
- **Microscopic evaluation:** must follow a strict sequential protocol, advancing from macroscopic inspection to low-power and subsequently high-power microscopy. Random application of immersion oil is strictly contraindicated.
- **Low-Power Screening (x10/x20/x40/x60 objectives)**: utilized for comprehensive specimen triage and initial qualitative assessment.
- **High-Resolution Analysis (100x Oil Immersion):** evaluation of morphological features and differential cell counts (PB & BM), essential for fine detail assessment (e.g., Auer rods, basophilic stippling/punctate basophilia) and for the final characterization of atypical or unusual cells.

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RED CELL MORPHOLOGY

- Microscopic slide review of red cells and the identification of abnormalities **in size, shape and staining and the presence of inclusions**, remains a fundamental way of identifying morphological abnormalities useful in the diagnostic work-up

- **At least 1,000 RBCs should be evaluated** to provide an accurate quantification of the morphological abnormalities

- **Schistocytes should be counted and reported as a percentage**

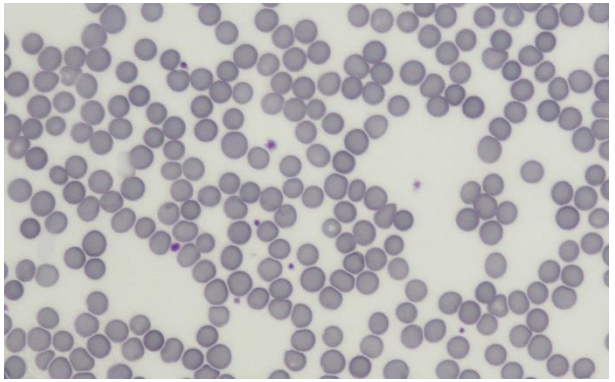
Palmer L et al. ICSH recommendations Int. Jnl. Lab. Hem. 2015, 37, 287–303

RED CELL MORPHOLOGY

Artifactual Distortion: Normal and pathological red blood cells are highly susceptible to mechanical distortion during smear preparation; comprehensive scanning is mandatory to locate the least distorted area prior to detailed analysis (*false impression of spherocytosis).*

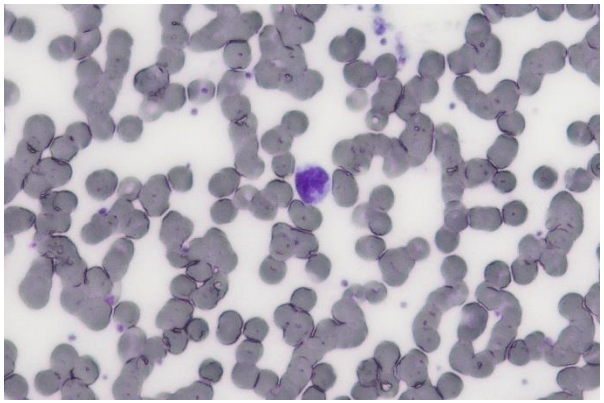
Rouleaux Formation: Rouleaux occur rapidly post-withdrawal—even in bedside films—and predominate in slower-drying, **thicker** sections; morphological assessment must strictly avoid these areas.

Optimal Examination Field: The ideal zone can usually be found toward the tail of the film, where there are no rouleaux and the red cells are touching but with little overlap.



too thin

too thick



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INTERNATIONAL COUNCIL FOR STANDARDIZATION IN HAEMATOLOGY RECOMMENDATIONS FOR SCHISTOCYTE COUNTING

Table 1. International Council for Standardization in Haematology recommendations for schistocyte counting

1. Schistocytes should be evaluated on peripheral blood smears using an optical microscope at medium magnification and estimated as a percentage after counting at least 1000 red blood cells
2. A schistocyte count should be requested and carried out when a diagnosis of thrombotic microangiopathies caused by red cell mechanical damage is suspected, usually in patients with thrombocytopenia
3. Schistocytes should be identified by specific positive morphological criteria. Schistocytes are always smaller than intact red cells and can have the shape of fragments with sharp angles and straight borders, small crescents, helmet cells, keratocytes, or microspherocytes*
4. A schistocyte count should be considered clinically meaningful if schistocytes represent the main morphological red blood cells abnormality in the smear (other than signs of erythropoietic regeneration)
5. A robust morphological indication for the diagnosis of thrombotic microangiopathic anemia in adults should be recognized when the percentage of schistocytes is above 1%
6. Fragmented red cell enumeration by automated blood cell counters should be considered a useful complement to microscopic evaluation, as it provides rapid results with a high predictive value of negative samples. A microscope check is needed for positive and macrocytic samples†

*Microspherocytes only in the presence of other mentioned RBC shapes.
†Macrocytic samples are at risk of underestimation or absence of flag ('false-negative' test).

WHITE BLOOD CELLS

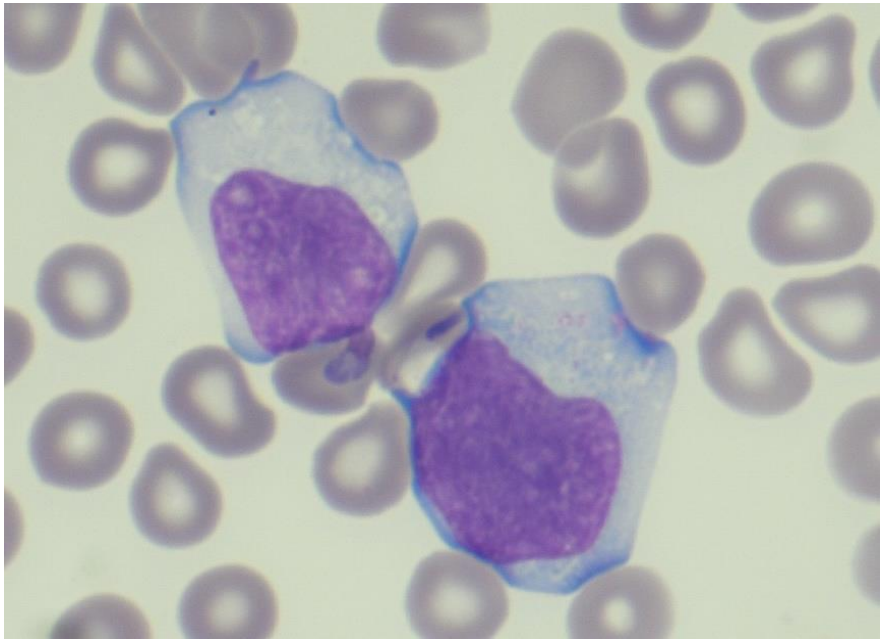
- White cell differentiation involves the classification of white cells based on size, nuclear shape, chromatin pattern and cytoplasmic appearance and content. Morphological qualitative abnormalities of the cell nucleus or cytoplasm and/or the size of the white cells can be congenital or acquired in the course of various diseases.

- The 2008 World Health Organisation (WHO) Classification of Tumours of Haemopoietic and Lymphoid Tissues recommends a 200 white cell peripheral blood cell differential be performed as part of the diagnostic work-up in acute myeloid leukaemia (AML) and myelodysplastic neoplasms.

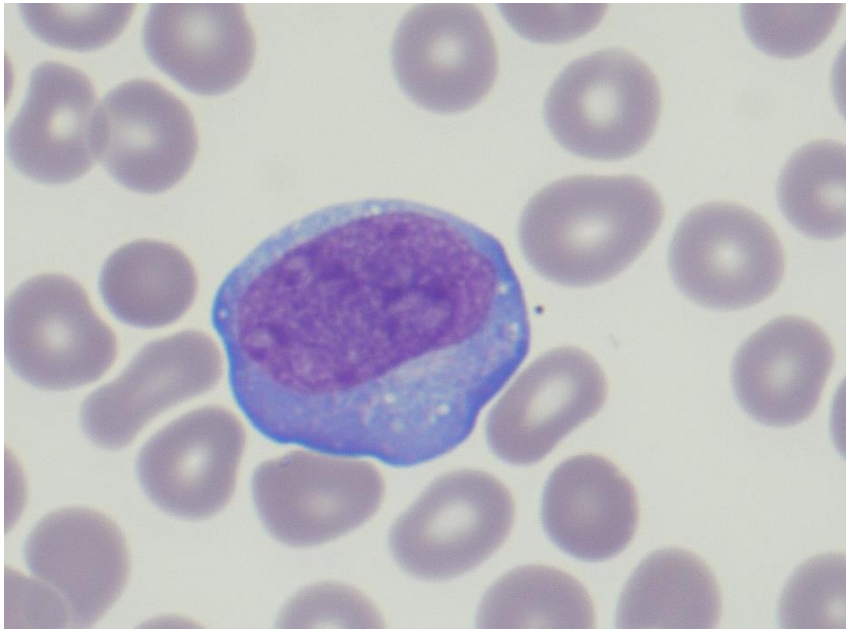
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DOWNEY CLASSIFICATION OF REACTIVE LYMPHOCYTES



Downey cells type II

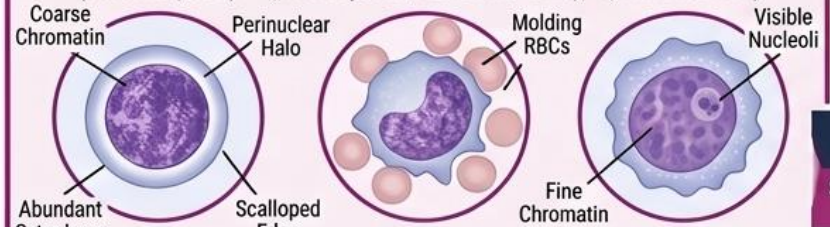


Downey cells type III

EVOLUTION OF LYMPHOCYTE CLASSIFICATION: FROM MORPHOLOGY TO DIAGNOSIS

THE PAST / "The Downey Era"

PAST / THE DOWNEY ERA
(Morpfologia-Driven)



TYPE I
(Plasmacytoid)
- Coarse Chromatin
- Perinuclear Halo
- Abundant Cytoplasm
- Scalloped Edge

TYPE II
(Monocytoid / Ballerina Skirt)
- Molding around RBCs

TYPE III
(Immunoblast)
- Large nucleus and
- Visible nucleoli
- Fine Chromatin
- Visible Nucleoli

OVER 7 HISTORICAL CELL TYPES (Morfologia-Driven)



VIROCYTES | TÜRK CELLS | IMMUNOBLASTS ATYPICAL LYMPHOCYTES

THE TRANSITION
Standardization → Etiology Focus

NOW CONSOLIDATED INTO ONLY 2 CATEGORIES

THE PRESENT / "The Modern Era"

THE MODERN BIVIO (ICSH/ELN)
(Diagnosis-Driven)

1. ABNORMAL LYMPHOCYTES
(Suspect Neoplastic / Malignant)



- Monomorphic blast-like population
- Nucleoli

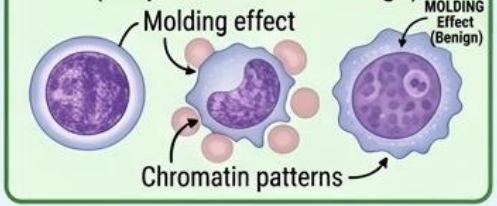
- Blasts
- Prolymphocytes
- Cancer Diagnosis

CRITICAL CLINICAL ALERT

INTO ONLY 2 CATEGORIES

This single category incorporates and consolidates **all** historical 'Reactive' cell types (e.g., all 3 Downey Downey Types) into a clear diagnostic pathway.

2. REACTIVE LYMPHOCYTES
(Suspect Reactive / Benign)



INCLUDES: Downey Types I, II, III; Historical synonyms

Takeaway/Key Concept

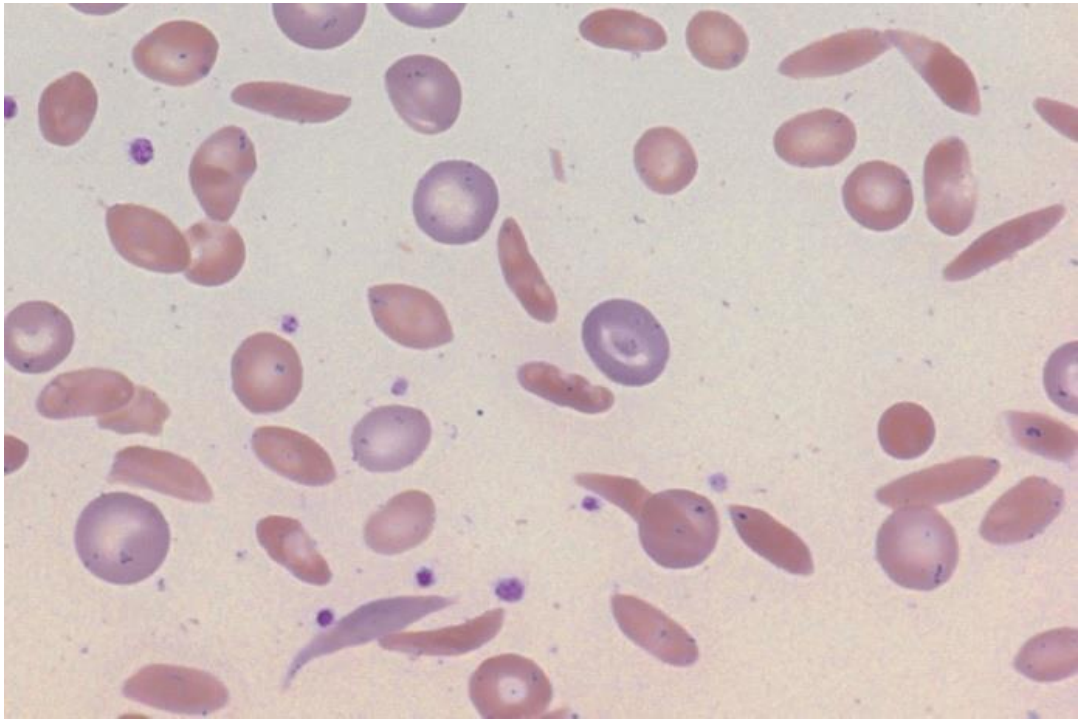
KEY MESSAGE

- MODERN LABS USE 'REACTIVE' vs. 'ABNORMAL' BINARY SYSTEM.
- DOWNEY TYPOLOGY HELPS PREVENT TYPE III (Immunoblast) BEING MISIDENTIFIED AS MALIGNANT BLASTS.

ABNORMALITIES OF RBC SHAPE

Sickle cells

Sickle cells are red cells that become crescent or sickle-shaped with pointed ends as a result of polymerization of HbS.
A sickle screen or haemoglobinopathy screen is recommended.



Sickle cell disease

Palmer L, et al. Int J Lab Hematol. 2015;37(3):287-303

PERIPHERAL BLOOD MORPHOLOGY: SPOT THE CLUES

What is the most likely diagnosis?

