Studies in Practical Laboratory Medicine

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By

Aurelian Udristioiu, Liviu Martin
and Manole Cojocaru

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NOTE FROM THE AUTHOR

The readers that will benefit from the information presented in the Book "Studies in Practical Laboratory Medicine", can find the practical works associated with the courses of Laboratory Medicine in General Medicine Assistance, with time of studies four years.The Book presents in practical mode, the exploration of lipid metabolism, protein metabolism, practical works with carbohydrates, the significance of blood cytological examination in peripheral blood, characteristics of acute and chronic anemia, functional exploration of the kidney, metabolic syndrome status, urine`s culture technique with antibiogram and the diagnosis of the main groups of microorganisms in the human organism, with significant image examples.

Also, the Book provides important benchmarks in the field, studying the hematologic and metabolic problems of the human body, has original content, deals with the practical part, highlighting the great need for new in the biological sciences, in general, and in medicine, in particular, for professionals in the field.

This considers the progress in the pathophysiology and biochemistry of laboratory medicine based on many years of experience in the medical field of this book's authors.

The new book, published this year by Cambridge Scholars Publishing, Great Britain, may be of interest for doctors, medical students, as well as staff working in clinical laboratories, from different countries presenting a large volume of data and experiments of works laboratory with a systematic and logical data display.

Author,
Dr. Aurelian Udristioiu, MD, Fellow PhD,

Associate University Professor, Titu Maiorescu University of Bucharest, Faculty of Medicine, General Medicine Assistant, Targu Jiu Township, Romania.

Member in American Association of Clinical Chemistry, AACC, & ADLM/National Academy of Biochemical Chemistry, USA, Member in

Society of Hematology Oncology, (SOHO), University Texas of Houston, USA and International Associate Member of ASH, American Society of Hematology, USA.

CHAPTER I

SIGNIFICANCE OF BLOOD CYTOLOGICAL EXAMINATION IN PERIPHERAL BLOOD

Normal Hemogram aspect

The first step is the evaluation of the hematological function and the diagnosis of hematological pathologies. The hemogram and the peripheral blood smear provide important diagnostic information and allow differential diagnosis and the opportunity for additional tests. Blood elements: erythrocytes, leukocytes and platelets. The quantification of these elements is done by manual and automatic methods. Blood samples must be obtained under optimal conditions. Factors that can influence the results: degree of activity, level of hydration, medication, sex, age, race, smoking, and age of the sample.

The blood is collected on an anticoagulant, preferably EDTA (ethylenediaminetetraacetic acid) - it removes calcium and prevents the formation of blood clots, with minimal morphological and physical effects on the cells.

Erythrocytes are red cells with a major role: they contain hemoglobin, which transports oxygen to the tissues and participates in the transport of carbon dioxide (they contain carbonic anhydrase). They are an-nucleated cells, with the shape of a biconcave disc, very elastic, with dimensions approx. 7.5 microns with a lifespan of approx. 120 days. LPR synthesis occurs in the hematogenous bone marrow and is controlled by erythropoietin from the kidneys.

Hemoglobin (Hb) is the main constituent of red blood cells and is determined by the photometric method. Normally, 16 g/100 ml of blood is found in men and 14 g/100 ml in women. Hemoglobin decreases in anemia. Normally, peripheral blood contains the following types of leukocytes that can be evaluated by the automatic analyzer: Neutrophils (segmented granulocytes), Eosinophils, Basophils Lymphocytes Monocytes, [**Figure 1 A, B**].

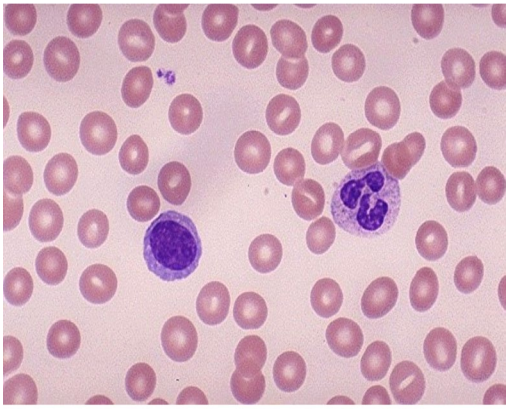

Figure 1 A. Lymphocyte, (left), Neutrophil, (center), Eosinophil (right).

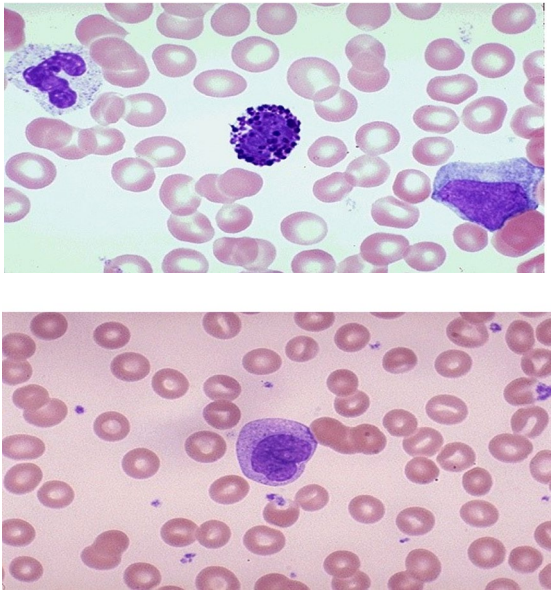

Figure 1 B. Neutrophil, (left), Basophil,(center), Reactive lymphocyte, (rigt), a Monocyte and Platelet in right photo.

In practice, granulocytes, lymphocytes and monocytes are called leukocytes. Their normal number is 5,000 – 8,000/mm3. Their count (leukocyte formula or leukogram) is done by reading the blood smear. Normal values are: Neutrophil granulocytes 60 - 70% 3,000 - 6,000/mm3; segmented 40 - 65%; unsegmented 2 – 5%; eosinophils 1 - 4%; 50-300 mm3; basophils 0 - 1% 0 - 50/mm3; Lymphocytes 20 - 30% 1,000 - 3,000/mm3; Monocytes 2 - 6% 100-600/mm3

The increase in leukocytes above 9,000 – 10,000 = leukocytosis (or hyperleukocytosis) and the decrease below 4,000 – 5,000 = leukopenia.

Thrombocytes are small an-nucleated cells, with granulations and rich enzymatic equipment. They play an important role in hemostasis, protect the vascular endothelium, form the primary white clot, contribute to plasma coagulation and produce clot retraction (thrombasthenia) [**Figure 2].**

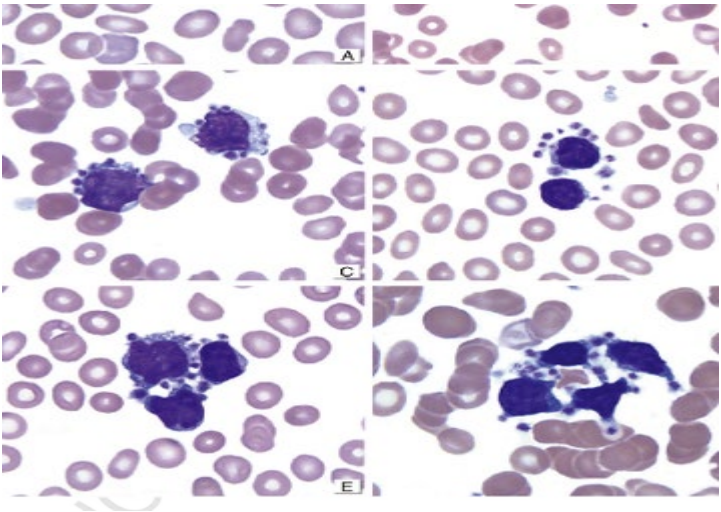

Figure 2. Samples collected in EDTA. PLT satellitism has been seen with in basophils

They are found in numbers of approximately 250,000/mm3. The decrease in the number occurs in medullary lesions, splenomegaly, immunological factors and the increase in thrombocytosis and thrombocythemia. There are also qualitative alterations of platelets (thrombasthenia).

Patient can came with platelet counts of 30,000, with no history of bleed with agrgate formes, **[Figure 3].**

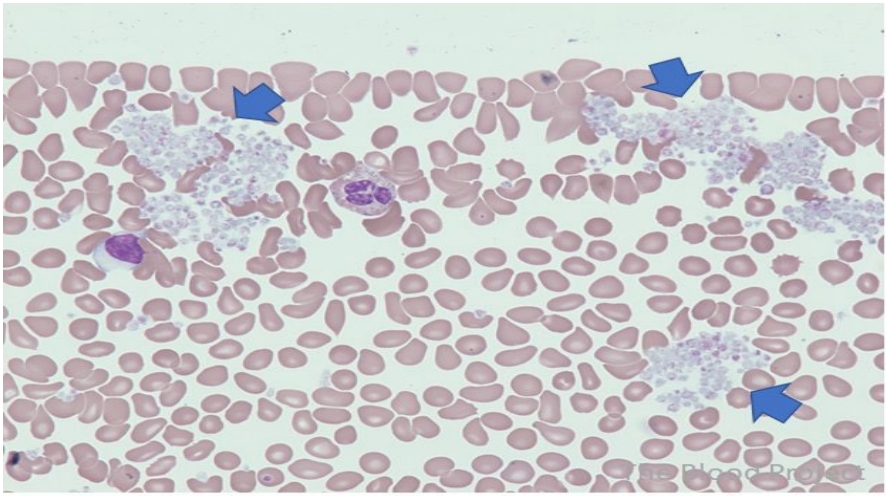

Figure 3. Platelets agrgate formes in cloting EDTA anticoagulant.

Red blood cells contain substances called agglutinogens and plasma substances called agglutinins, which are natural antibodies, also called isoantibodies. According to the distribution of agglutinogens and agglutinins, four blood groups are distinguished. Those from group 0 (I) are called universal donors and those from group AB (IV) are universal recipients. apart from these antigens, the Rhesus (Rh) factor is also known, an antigen found in approximately 85% of people. If the blood groups are not respected, hemolysis accidents can occur during transfusions, sometimes fatal. Hemolytic accidents can also occur in a newborn whose father is Rh-positive and mother Rh-negative.

Iron deficiency anemia

Moderate decrease in the number of red blood cells = 3000,000/mm3 with corresponding hematocrit = 30-40%, Hb. = 6-8g/dl; on the smear, anisocytosis with aniso-poikilocytosis is visible when anemia is pronounced and red blood cells in a sign of shooting at the target =5-6%. The leukocyte formula can sometimes have a moderate neutropenia with a relative lymphocytosis and a slight eosinophilia 6 8%, **{Figure 4].**

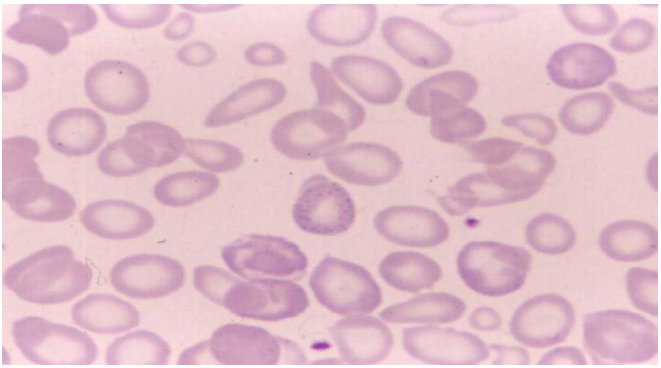

Figure 4. Erythrocyte forms in Iron deficiency anemia on smear of peripheral blood

Hypochromic Thalassemic Anemia

Thalassemias are hereditary hemolytic anemias in which the viability of red blood cells is disturbed due to the inadequate synthesis of normal hemoglobin - major deficiency of hemoglobin A. Thalassemia major, B-thalassemia, is due to the inhibition of the synthesis of the Beta chain, characteristic of hemoglobin A of the adult and further formation of gamma chains specific to fetal hemoglobin gamma Hb.F. In the peripheral blood, anemia is found with <2500 000 red blood cells/mm3, intense hypochromia with a Globular Value <0.7, (norm VG=Hemoglobin/Number of erythrocytes x $3x10^{-3}\times10^{-3}$ = <1 [example 14/3x 4.5= 1.02); red blood cells have a target appearance >50% of the smear; on the smear, oxyphilic erythroblasts appear in 10-20%; reticulocytosis exceeds 80-100%; there is an increase in globular resistance, hemolysis starts at 0.30; hemoglobin Hb electrophoresis. F is in a concentration of 80%.

Thalassemia Minor and B thalassemia heterozygous

The peripheral blood in an adult has a normal appearance of asymptomatic hypochromic anemia and red blood cells in the target = 10-20%; moderate hypochromic of erythrocytes that contrasts with the growth of siderocytes and sideroblasts

Hypersideremia with discrete hemosiderosis. During Hb Electrophoresis, there is an increase in Hb. A2 .5-6% and Hb. F 3-10%, **[Figure 5].**

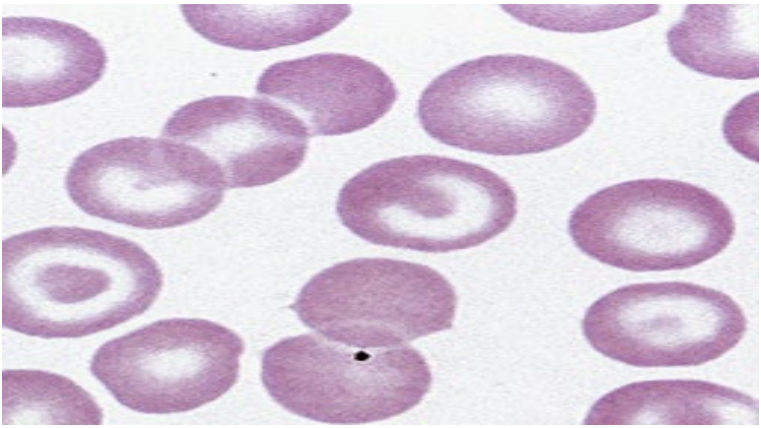

Figure 5. Red blood cells in the target, (Thalassemia major).

Hemolytic anemias

Hemolytic anemias are pathological conditions in which the destruction of red blood cells exceeds the capacity of marrow regeneration. Anemia occurs when the average life span of red blood cells falls below 20 days, exceeding the medullary production of red blood cells. It is known that the average life span of a red blood cell is 120 days and that the normal marrow can increase its rate of red blood cell formation 5-6 times.

Classification: hemolytic anemias are grouped into hemolytic anemias with genetic defect erythrocyte, which may be due to abnormal shape, hemoglobin or enzymes and hemolytic anemias with acquired defect.

Hemolytic anemia with a genetic defect, for example, Spherocytic Hemolytic Anemia, which is a genetically transmitted disease, with a chronic evolution, that affects both sexes equally. Hemolysis is due to the spherical shape of the erythrocytes which generates increased osmotic fragility, sequestration and premature destruction in the spleen **[Figure 6].**

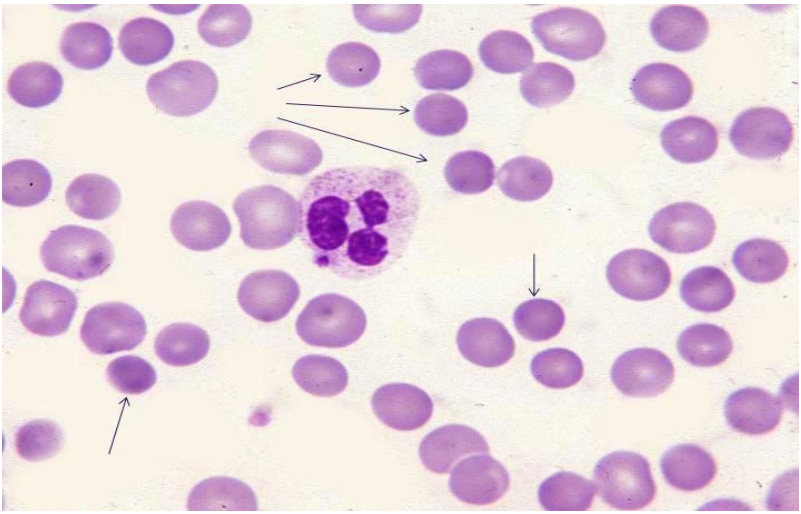

Figure 6. Spherocytic Hemolytic Anemia

The blood count shows anemia, low blood cell resistance, microspherocytosis (transverse diameter decreases and red blood cells increase in thickness). The number of reticulocytes is greatly increased, **[Figure 7].**

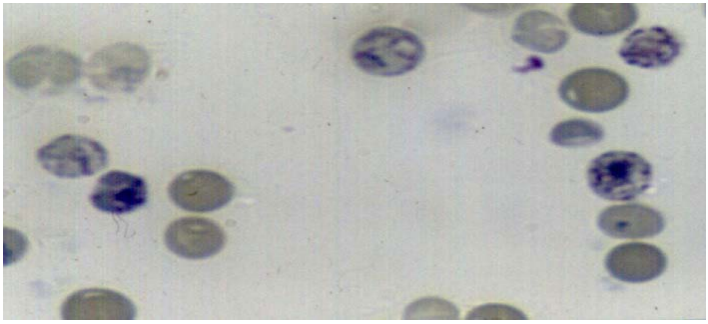

Figure 7. The number of reticulocytes increased in hemolytic anemias

The most common are immune hemolytic anaemias. Immune hemolytic anaemias can be autoimmune, so with autoantibodies (active in heat and active in cold), which react with antigens on the surface of their erythrocytes (direct positive Coombs test); there are also hemolytic anaemias in which the hemolysis is due to some drugs or chemical substances. Immune hemolytic anaemias with heat-active autoantibodies are idiopathic and

secondary (after lymphoproliferative diseases, lupus, infections, etc.).

Autoantibodies are fixed on the surface of erythrocytes, but they are also free in the serum, giving a positive direct and indirect Coombs test. They occur more frequently in the elderly. In some patients, the acute onset is febrile. Anaemia is macrocytic with ferrocytosis, reticulocytosis, leukocytosis, sometimes jaundice, hemoglobinuria and splenomegaly. Immune hemolytic anaemias with cold-active autoantibodies and acquired, drug-induced immune hemolytic anaemias occur in patients treated with penicillin, quinine, quinidine, and alpha methyldopa.

Paroxysmal nocturnal hemoglobinuria is a chronic, acquired hemolytic anemia, characterized by crises of acute hemolysis with hemoglobinuria, which occur at night during sleep and are due to the fragility of erythrocytes in the acidic environment. The clinical picture is rarely dramatic, with hemolytic shock, most cases being discrete. Pancytopenia is common.

- Post-transfusion hemolytic anaemia occurs in group transfusions incompatible.

Posttransfusion hemolytic accidents, depending on the intensity of hemolysis evolves in three phases: the shock phase, the hemolysis phase with jaundice hemoglobinuria and the anuria phase (tubal nephritis), in which case the prognosis is very serious.

Megaloblastic Anemia

It occurs in adults >40 years old. The hemogram shows a sharp decrease in red blood cells <2000 000/mm³ with VG=1.2-1.4; Ht.<, VEM.>; reticulocytes 0.2-1%; leukopenia = 3000-40000/mm3 and platelets are in normal number. Pronounced erythrocyte anisocytosis with macro and megalocytosis is evident in the peripheral blood.

On the smear, only 30-40% of the red blood cells have an increased size above 80μ³; neutrophils can be low <50%, with the predominance of hyper-segmentation stages, deviation to the right. Lymphocytes can reach 30-40%. Red blood cells appear well loaded with hemoglobin with a narrowed center, different from red blood cells in hypochromic anemia **[Figure 8].**

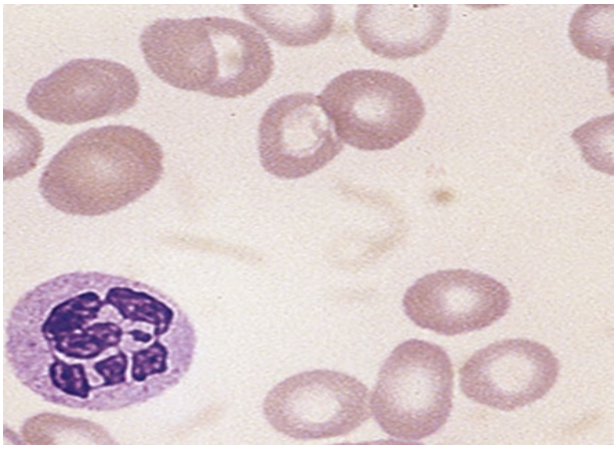

Figure 8. Leukocyte Polylobate Neutrophil in megaloblastic anemia

Sideroblastic anemia

It is a deficit in the union of Fe with Protoporphyrin 9 and thus the protoporphyrin remains dispersed in the cytoplasm. It also occurs in Pb poisoning. Chloramphenicol alcohol deficiency of vitamins B6, B12, folic acid, neoplasms, porphyrins, alcohol, autoimmune hemolytic anemias. The diagnosis is based on hypochromia, anisocytosis, accentuated poikilocytosis and reticulocytopenia. Secondary sideroblastic anemia is secondary primarily to chloramphenicol which blocks heme synthesis and leads to saturnism.

The diagnosis consists of hypochromic anemia with uniform erythrocytes, without anisocytosis but with a high percentage of basophilic dots, >6 thousand of erythrocytes **[Figure 9].**

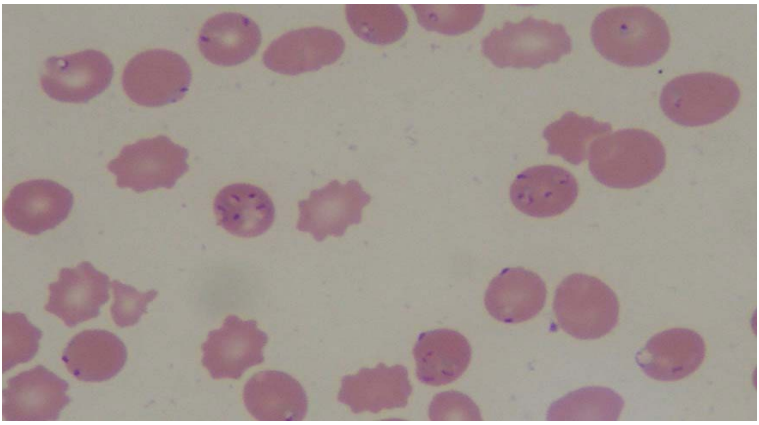

Figure 9. Basophilic punctations in sideroacrestic anemia (Lead Poisoning)

Hypochromia and other disorders of red blood cells in the peripheral blood:

- annulocytes = iron deficiency anemia,
- microcytes = iron deficiency anemia, thalassemia, sideroblastic anemia
- macrocytes=/megalocytehepatopathies, pernicious anemia,- ovalocytes, spherocytes,=hemolytic anemia
- polychromatophilic and basophilic dots = poisoning (lead.)
- spherical cells = hereditary spherocytosis
- target cells = thalassemia, liver cirrhosis
- hereditary elliptocytosis, **[Figure 10]**

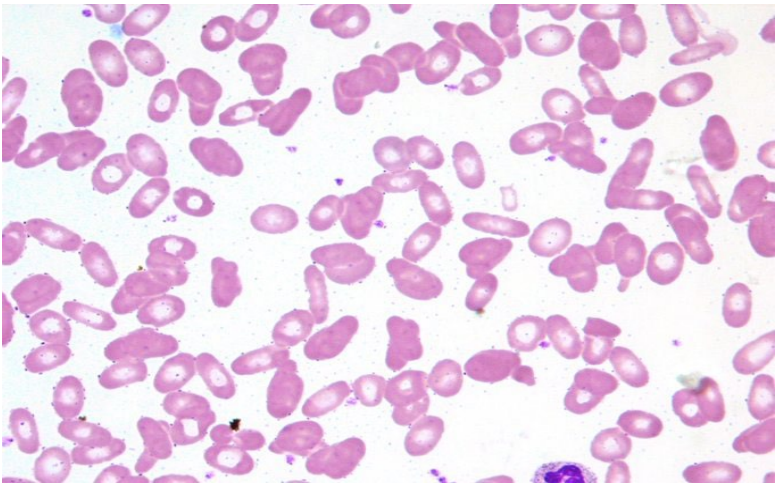

Figure 10. Hereditary elliptocytosis

- Howell Joly corpuscles => after splenectomy, Biermer anemia, Cabot ring (center), schizont trophozoites=malaria. Red blood cells in the body=monoclonal gammapathies [Figure 11].

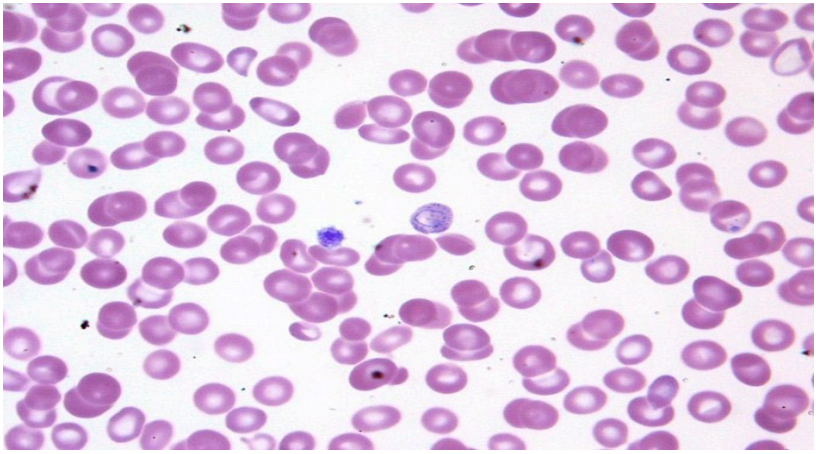

Figure 11. Cabot rings in accelerated hematopoiesis

Fragmentocytes = hemolytic syndrome, uemia, disseminated vascular coagulation, (CID) **[Figure 12].**

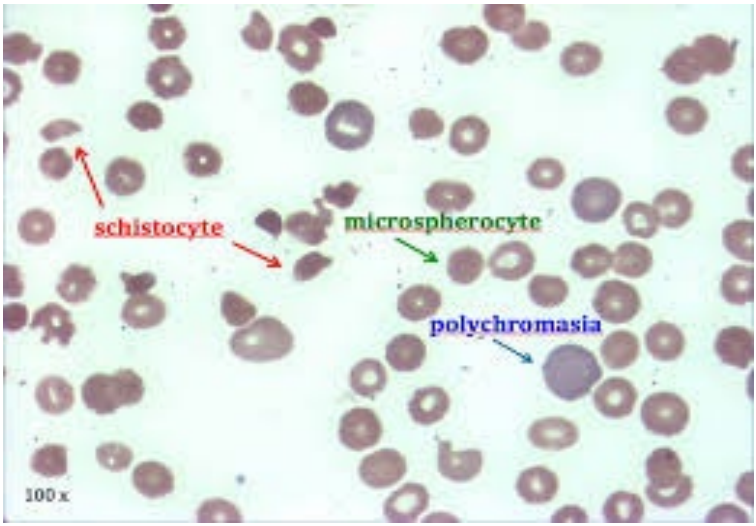

Figure 12. Fragment cells, hemolytic, uremic syndrome, CID

Morphological characterization of leukemias in peripheral blood

Chronic Granulocytic Leukemia

Chronic Granulocytic Leukemia, (LGC), is a slowly progressive neoplasia that preserves the maturation capacity of cells of the granulocytic series until the adult stages with qualitatively characteristic changes: modification of the karyotype, disappearance of alkaline phosphatase.

Peripheral blood count

Hemogram with leukocytosis >50 0000-100 000/mm³ with sub leukemic, leukemic forms. In the absolute predominant formula of the granulocytic series in the majority of cases are found: myelocytes + metamyelocytes + segmented = 80%; rare promyelocytes <20%; myeloblast in the period of activity = 3-4%; eosinophils = 8-10% and basophils 3-4%.

Adult granulocytes are hypo or agranular. If eosinophils >50%=LGC with eosinophilia; basophils >20%=LGC with basophils, the Presence of monocytoid cells and myeloblasts = acute myelomonocytic leukemia, different

from chronic monocytic leukemia; erythroblasts are rare, oxyphilic, polychromatophilic = 3-4%. The number of platelets is increased, >300,000/mm, **[Figure 13].**

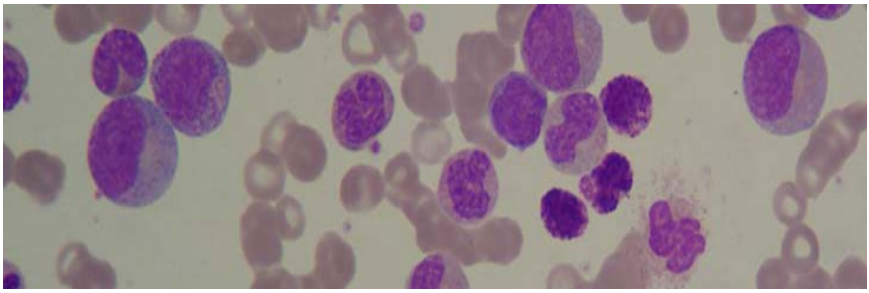

Figure 13. Aspect of chronic granulocytic leukemia (CGL). Myelocytes and metamyelocytes

MMM – Myeloid metaplasia with myelofibrosis

MMM is a clonal disease of pluripotent stem cells from the MO that evolves with medullary osteo-myelofibrosis simultaneously with the development of extramedullary hematopoiesis, predominantly splenic.

1. The phase is established in 1-2 years with myeloid hyperplasia both in the bone marrow (BM) and the spleen, less in the liver, poly globulins, leukocytosis, thrombocytosis, global myeloproliferative.

2. The second phase evolves with the fibrosclerotic transformation of the MO, the disappearance of blood elements with the maintenance of Megakaryocytes.

From the MO and the extramedullary territories, leukocytes migrate with Leukocytosis 50,000/mm, due to the disorganized cyto-diabase. With reactive fibrosis, pancytopenia, collagen fibers and reticulin due to fibroblastic transforming factor B and platelet factor 4F with a specific picture in the peripheral blood; moderate, normal or hypochromic anemia; poikilocytosis, extramedullary red blood cells; reticulocytosis 2.5% due to hyper-hemolysis; young cells from the granulocytic series 15-20%, (promyelocytes), rare polychromatophilic and oxyphilic erythroblasts, thrombocytopenia with aniso-thrombocytes >40%., **[Figure 14].**

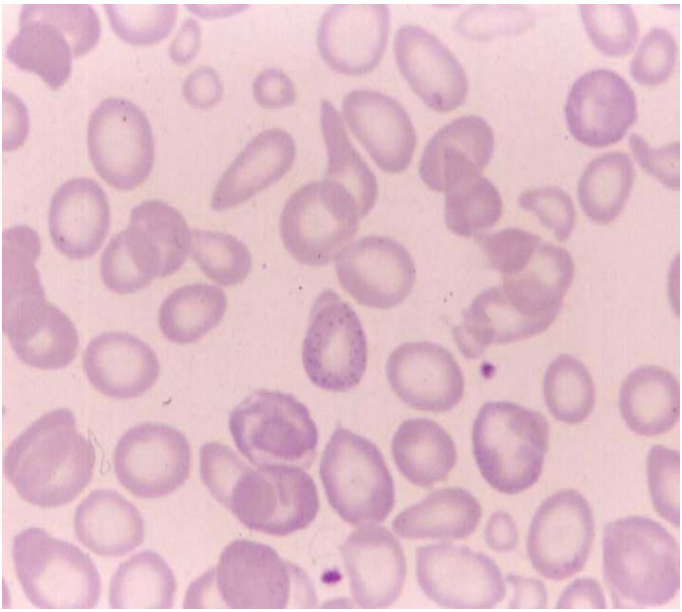

Figure 14. Erythrocytes in the tear and in the eggshell, (Severe Pokilocytosis)

Polycythemia Vera

Primary poli-globulia, characterized by global myeloid hyperplasia, with a predominance of erythrocytes, is characterized by an exaggerated increase in red blood cells, Hb, EVM.

Appearance of the Hemogram: the number of red blood cells => 5000000 /mm³; Hematocrit >60-70%, Hemoglobin >17-18g/dl, Leukocytes=10,000-20,000/mm; Platelets>300,000/mm, hematuria with normal appearance of smaller size. In the leukocyte formula, a few myelocytes may appear when we have leukocytosis; increased basophilia, and eosinophilia; the volume increases due to the large number of red blood cells; the viscosity is very high. O2 saturation is normal in arterial blood established by oximetry **[Figure 15]**.

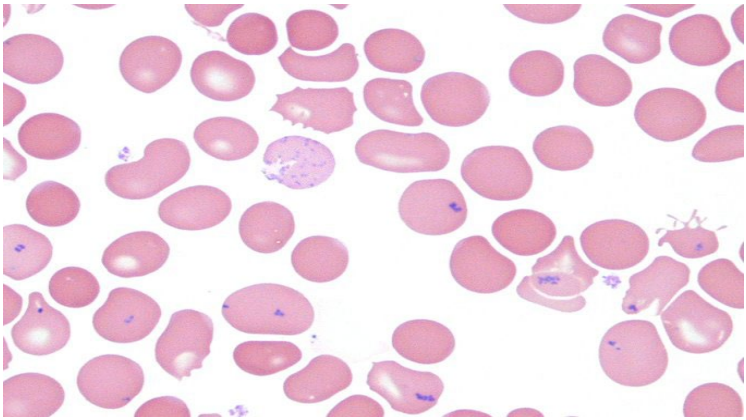

Figure 15. Basophilic stippling in PB of MDS patient

Hemorrhagic thrombocythemia

The diagnosis is established by counting platelets >1000 000/mm3, with normal morphology, the number of leukocytes >, after bleeding, otherwise normal. Frequent hypochromic anemia.

In the bone marrow, MO, we find a large number of megakaryocytes, mostly thrombocytopenic. On the smear from the peripheral blood, we find: pronounced thrombocythemia, erythrocytes in the target, erythrocytes with Joly bodies, erythroblastic picture with haemorrhages, and increased bilirubin. Splenectomy is contraindicated and iron deficiency is associated with thrombocytosis through consumption, **[Figure 16**].

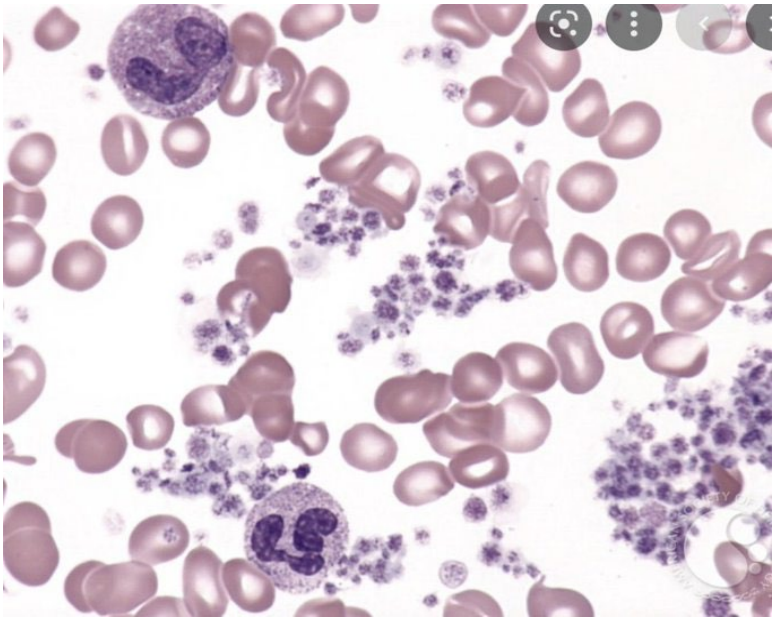

Figure 16. Platelets in increased number on the smear (thrombocythemia)

Chronic Lymphoproliferative Syndromes

Slow neoplastic processes of the cells of the lymphoid series that retain their ability to mature, but are inert from an immunological point of view. HEMOGRAM: Leukocytes >10,000-50,000/mm. In the formula, numerous adult cells are 70-80%, but numerous Gumprecth shadows, lymphocytes large and prolymphocytes indicate an accelerated evolution. In 10-15% of patients, the number of leukocytes can be normal. Anaemia is frequent, normal or hypochromic, and sometimes decreases in red blood cells, <2000 000/mm, from marrow suppression.

Platelets <, often low, <100,000, = thrombocytopenia **[Figure 17].**