MORPHOLOGICAL CHANGES OF ERYTHROCYTES IN HYPOCHROMIC ANAEMIA, IN HUMANS

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Abstract: *hypochromic anaemia cases are characterised by a deficit in synthetizing and then depositing of haemoglobin in erythroblasts and may result from metabolism disorders or poor synthetizing of any haemoglobin component (iron, protoporphyrin, globin). The microscopic exam of peripheral blood smears correlated with the study of automated complete blood cell counts (CBC) leads to the establishment of an efficient treatment which should correct the occurring deficit. The peripheral blood smears were examined under the microscope, establishing erythrocyte size, aspect and haemoglobin load. We observed shape variations (dacrocytes, ovalocytes, stomatocytes) in variable proportions (12.5-43.75%) as well as erythrocyte diameter variations (anisocytosis) associated with hypochromia. The study of automated complete blood cell counts supported the microscopic observations; the erythrocyte number was 38.5% lower, and the haemoglobin quantity and haematocrit value were 26.84%, respectively 23.75% lower than the minimum values of the reference biological interval.*

Key words**:** *hypochromic anaemia, haemoglobin, blood smear, complete blood cell count (CBC)*

INTRODUCTION

Anaemia is a state resulting from the disruption of the homeostatic balance between erythrocyte formation and their loss/destruction. This is associated with the decrease under a certain normal limit of the total haemoglobin quantity and/or of the haematocrit (2; 14).

Hypochromic anaemia cases (or haemoglobin synthesis deficits) can be caused by a protoporphyrin synthesis block (sideroblastic anaemia) (9; 5; 17), anomalies in iron metabolism (iron deficiency anaemia, simple chronic anaemia) (10; 8; 6; 13; 12) or disorders regarding globin synthesis (thalassemias) (11). Age groups affected by this type of anaemia are most often children (premature or twins, because of reduced iron quantities in deposits) (15; 14; 7; 16) teenagers (through exaggerated consumption or/and insufficient iron intake) (1) (but also pregnant women) (4; 16).

Aside from clinical signs (paleness of teguments and mucous, palpitations, tachycardia, asthenia, chephalalgia, dizziness, balance disorders, etc.), haematological tests are defining for the precise establishment of the anaemia type.

The execution of an automated complete blood cell counts (CBC) represents the primary mandatory investigation, to which, in some cases (established by the laboratory doctor depending on the blood cell count aspect), the examination of the peripheral blood smears is added.

The automated complete blood cell count is carried out in order to determine the haemoglobin concentration, the haematocrit, leucocyte, erythrocyte, thrombocyte number, erythrocyte indices (VEM/MCV-average erythrocyte volume, HEM/MCH-average erythrocyte haemoglobin, CHEM/MCHC- average erythrocyte haemoglobin concentration) and absolute values and percentages of lymphocytes, monocytes and granulocytes (neutrophils, eosinophils,

basophils) in the blood. The automated blood cell analysis renders rapid and correct information about the blood cells, including parameters with diagnosis usefulness (2).

Microscopic examination of the peripheral blood smears offers information on shape variations, erythrocyte aspect (poikilocytosis) associated with hypochromic anaemias, erythrocyte diameter (anisocytosis) and haemoglobin load, as it is known the this type of anaemia is associated with hypochromia.

MATERIALS AND METHODS

The erythrocyte study was carried out on blood collected through venepuncture in vacutainers for the automated complete blood cell count with anticoagulant EDTA without anterior centrifugation or through puncturing the finger pulp.

Automated complete blood cell counts were executed with the cu the automated analyser *SYSMEX XN-1000 series* in the Laboratory Haematology Compartment of the Medical Analysis Laboratory of SCMUT. The device executes automated complete blood cell counts, measuring a series of haematological parameters from venous blood integrally collected on K2EDTA as anticoagulant, in the relation blood:anticoagulant - 9:1.

Blood smears were carried out using the May-Grünwald-Giemsa (MGG) coloration and were examined in immersion at the microscope *OLYMPUS CX 41*. For erythrocyte photographing and measuring we used the statistical capturing and measuring soft *QUICKPHOTO MICRO 2.2*, of the *OLYMPUS CX 41* microscope.

RESULTS AND DISCUSSION

1. The examination of peripheral blood smears rendered the following morphologic erythrocyte variations:

- erythrocyte shape variations (poikilocytosis): erythrocytes with rouleau aspect („intense roll" and „short roll"), dacrocytes (erythrocytes shaped as a "tear"), stomatocytes (erythrocytes with the paler central region shaped as a "mouth"), ovalocytes (oval erythrocytes) in variable proportion (12.5-43.75 %) (fig. 1).

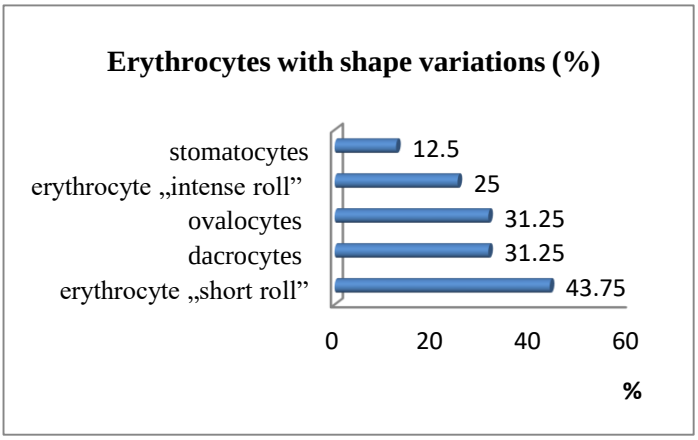

Figure 1. Proportion of erythrocytes with shape variations present in hypochromic anaemia

The “short roll” aspect of erythrocytes, where 3-5 erythrocytes overlap on the smear is frequently observed in cases of anaemia (fig. 2); this aspect can either be associated with hypochromia or anisocytosis or not. The “intense roll” aspect occurs when all erythrocytes overlap; this aspect is characteristic for monoclonal gammopathy (immune system disease characterised by anomalies of the lymphoplasmacytoid cells) and can be considered a sign of malignancy (fig. 3). Dacrocytes are presented in 31.25 % of the samples. They have also been called “drop” erythrocytes (fig. 4). Ovalocytes are oval shaped erythrocytes; they can suffer changes through longitudinal elongation and become elliptocytes (elliptical erythrocytes) (fig. 5). Ovalocytes can be associated with macrocytosis and were described in the vitamin B12 deficit. Stomatocytes are erythrocytes with colour and shape defects in the central, paler, region, their shape resembles a mouth, slit, shell, etc. (fig. 6).

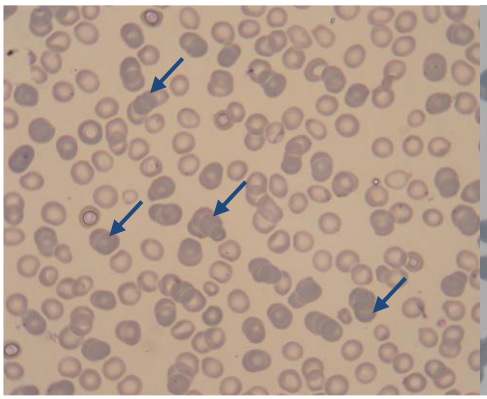

Figure 2. The aspect of erythrocytes displayed in “short roll” associated with erythrocyte hypochromia (x100, original)

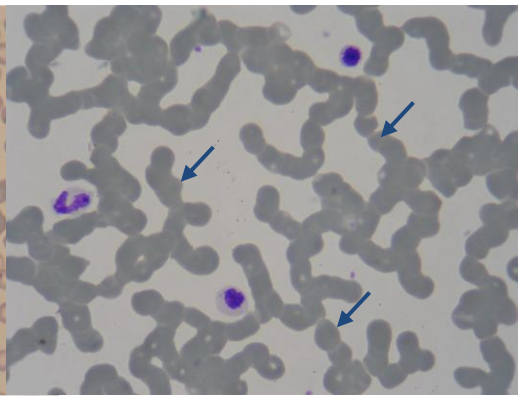

Figure 3. The aspect of erythrocytes displayed in “intense roll” (x100, original)

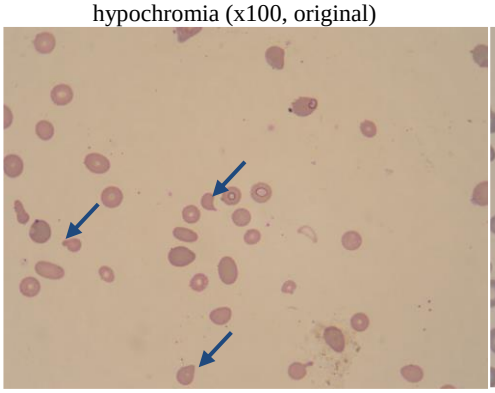

Figure 4. The aspect of dacrocytes upon blood smear examination (x100, original)

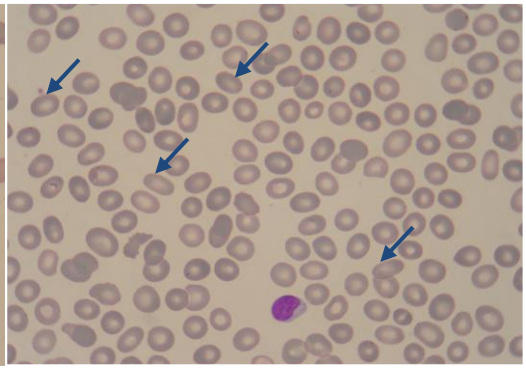

Figure 5. Oval erythrocytes (ovalocytes) present when examining the blood smear (x100, original)

- erythrocyte size (diameter) variations (anisocytosis) – determine the occurrence of microcytes, with a smaller diameter than normocytes (under 7.5µm, in dry state) associated with values lower than 80 fL of VEM, or that of macrocytes, with a very wide diameter and VEM values exceeding 100 fL. Blood smear examination showed that approximately 1/3 of the slides present microcytosis aspects, probably due to Fe deficit, with which this type of anaemia is associated (fig. 7). Macrocytosis aspects were more numerous; the specialty literature states

that this size variation is due to the vitamin B_{12} deficit, but also folate deficit, both deficiencies leading to the impossibility of synthetizing a normal quantity of haemoglobin; the presence of macrocytes may indicate hypochromic anaemia (fig. 8). Upon examining the blood smears, we also observed anisocytosis aspects, on the same smear occurring microcytes as well as macrocytes (fig.9).

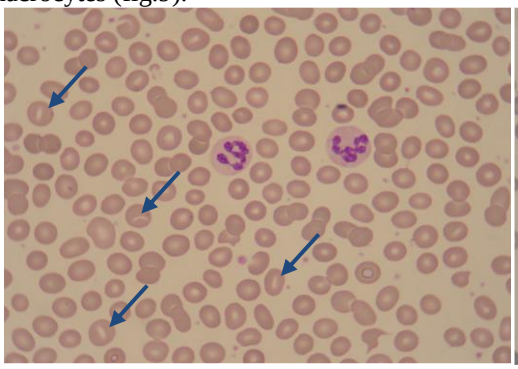

Figure 6. Stomatocytes present when examining the blood smear (x100, original)

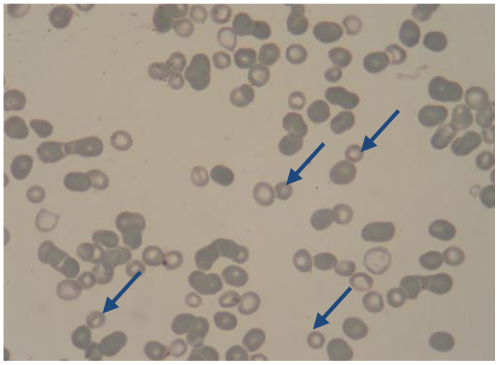

Figure 7. The aspect of microcytes, associated with hypochromia and displayed as a short roll (x100, original)

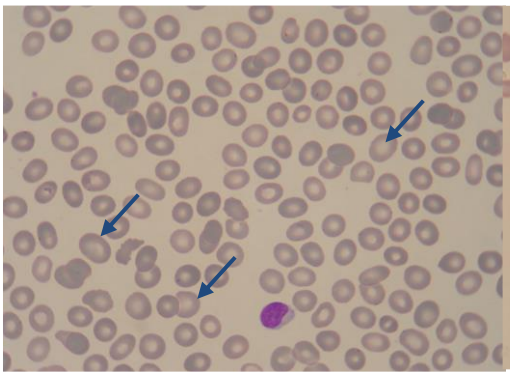

Figure 8. The aspect of hypochromic macrocytes on the blood smear (x100, original)

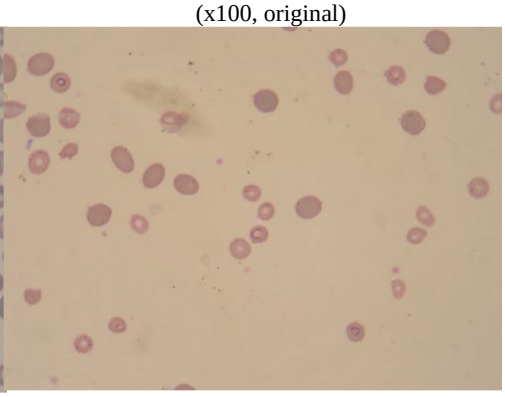

Figure 9. Microscopic field with anisocytosis aspect (microcytes and macrocytes are present) (x100, original)

2. The study of automated complete blood cell counts

The blood cell count study rendered the average erythrocyte number value as 2.86±0.94 which represents a percentage of 61.5 % from the minimal reference value, that is 38.5 % lower than normal. These results were correlated with the haemoglobin and haematocrit quantity, both displaying lower values than the minimal biologic reference interval: 8.78±1.96 g/dL (73.16 % of the minimal normal value that is 26.84 % lower than normal) and 27.45±5.74 % (76.25 % of the minimal normal value, 23.75 % lower than normal) (table 1).

The haemoglobin and haematocrit values, and the erythrocyte number are the base of the erythrocyte indices calculus. The average erythrocyte volume (VEM/MCV) indicates the average volume of individual erythrocytes (measured in femtolitres-fL or cube micrometre-µm3); it classifies erythrocytes in normocytes (80-100 fL), microcytes (sub 80 fL) and macrocytes (over 100 fL). The average value of the average experimental erythrocyte volume

was of 100.48±19.47, which indicates macrocytosis. Usually VEM is correlated with the erythrocyte diameter measured on the microscope examined blood smears.

The medium erythrocyte haemoglobin concentration (CHEM/MCHC) represents the medium haemoglobin concentration in erythrocytes, taken as a whole (g/dL or %) and is used to classify the erythrocyte population as: normochromic (32-36 g/dL) or hypochromic (under 32 g/dL). CBC processing showed that the average value of the average erythrocyte haemoglobin concentration was of 31.47±2.3 g/ dL, that is a percentage of 99.90 % displayed a lower CHEM than the minimal reference value, which indicates hypochromia, a clear indices of hypochromic anaemia (table 1).

Table 1

The study of automated complete blood cell counts

	Erythrocyte number (RBC)	**Haemoglobin (HGB)**	**Haematocrit (HCT)**	**Average erythrocyte volume VEM (MCV)**	**Average erythrocyte haemoglobin HEM (MCH)**	**Medium erythrocyte haemoglobin concentration CHEM (MCHC)**
Average	**2,86±0,94**	**8,78±1,96**	**27,45±5,74**	**100,48±19,47**	**32,44±7,67**	**31,47±2,43**
% of normal minimumm	**61,5 %**	**73,16 %**	**76,25 %**	**125,6 %**	**120,14 %**	**99,90 %**
Minimal biologic reference interval */unit	4-5,2/*100^3/µL	12-15 g/dL	36-46/%	80-100/fL Microcytes: < 80 Macrocytes: > 100	27-32/ pg	31,5-34,5 g/ dL normochromic erythrocytes: 32-36 hypocromic erythrocytes: <32

*- (3)

Average erythrocyte haemoglobin (HEM/MCH) measures the average weight of erythrocyte haemoglobin when taken individually; it is expressed in picograms (pg). The HEM value resulting after the CBC processing was of 32.44±7.67 pg, meaning 120.14 % in relation to the minimal reference value (table 2). HEM and VEM did not correlated with CHEM, but CHEM, correlated with the reduced haematocrit and haemoglobin, alongside with the small number of erythrocytes, indicates the occurrence of hypochromic anaemia (figure 10).

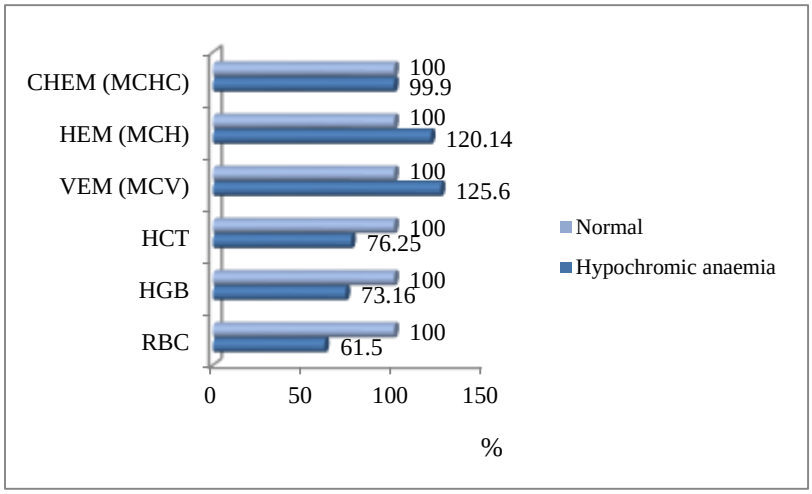

Figure 10. Percentage comparison of the erythrocyte number, haemoglobin, haematocrit quantity and experimental erythrocyte indices with minimal values from the biologic reference interval

CONCLUSIONS

Haematological tests revealed the existence of morphological changes, but also regarding the haemoglobin, haematocrit quantity and erythrocyte indices;

The most frequent morphological erythrocyte variations were the following: shape variations (poikilocytosis): erythrocytes crowded in short or intense roll, dacrocytes, ovalocytes and stomatocytes, in variable proportions (12.5-43.75 %), erythrocyte diameter variations (anisocytosis), the blood smears observing most frequently macrocytosis, all these changes being associated with hypochromia;

The complete blood cell counts (CBC) revealed that the average value of the erythrocyte number is 2.86±0.94 which frepresents a percentage of 61.5 % of the minimal reference value, that is 38.5 % lower than normal;

The haemoglobin quantity and the haematocrit value were lower than the minimal values of the biologic reference interval, as follows: haemoglobin: 8.78±1.96 g/dL (73.16 % of the minimal normal value, that is 26.84 % lower than normal) and the haematocrit: 27.45±5.74 g/dL (76.25 % of the minimal normal value, 23.75 % lower than normal); these observations were correlated with the average erythrocyte haemoglobin concentration (CHEM/MCHC) which was of 31.47±2.43 g/ dL, that is 99.90 % of the minimal reference value, a clear indices of hypochromia.

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