MORPHOLOGICAL AND CHEMICAL CHANGES IN BLOOD CELLS IN ACUTE LEUKAEMIA IN HUMANS NOTE 2. MORPHOLOGICAL CHANGES OF FIGURATIVE ELEMENTS BLOOD AND BONE MARROW IN PATIENTS WITH ACUTE LEUKAEMIA SUSPICION

Olga-Alina RADA[1], Diana BREZOVAN[1], Corina Luminița MĂRAN[2], Mihaela OSTAN[1]

[1]Banat's University of Agricultural Sciences and Veterinary Medicine Timişoara, Faculty of Agriculture, Romania
[2]Municipal Clinical Emergency Hospital from Timisoara, Gheorghe Dima Street, no. 5, Romania
radaolga2005@gmail.com

Abstract: *capturing and highlighting morphological changes that occur in the examination of a peripheral blood smear or bone marrow are important in the diagnosis of acute leukaemia, motivating the goal of this paper. The examination of blood smears from all patients included in the study revealed the presence of blast cells (2-97% of total leukocyte cell line). Of total studied smears, most presented type I and II blasts, which pointed to acute lymphoblastic leukaemia (LAM) 2, 3, 4 and 5; lymphoblastic modest presence pointed to acute lymphoblastic leukaemia (LAL) (4%). About 36% of smears of blood blast cells analyzed showed a pronounced polymorphism, which could not be classified even after performing bone marrow aspirate, requiring further investigation to establish the diagnosis.*

Keywords*: CBC, blood smear, bone marrow smear, acute leukaemia, blast*

INTRODUCTION

Thorough examination of a well-done blood smear is an important part of assessing blood diseases. Though a specific diagnosis can be suggested by data supplied by automatic blood count, certain diseases can have a normal number of cells but abnormal cell morphology. Blood smear is a valuable means in diagnosing and assessing anaemia, hereditary erythrocyte anomalies, infections, inflammations, leukaemia and other lympho- and myeloproliferative diseases (DE MOTT *ET AL*., 1994).

Peripheral blood smear supplies indices on leukocyte morphology and distribution; the presence of morphological anomalies or of myeloid precursors (metamyelocyte, myelocyte, promyelocyte, blasts) is assessed based on literature (DE MOTT *ET AL*., 1994; MCKENZIE, 1996); if signalled, the investigation is completed with haematogenous marrow smear.

Establishing with accuracy the type of blast cell can be done only by examining peripheral blood and haematogenous marrow smears, which is indispensable for the classification in one of the acute leukaemia types: LAL (lymphoblast acute leukaemia) and LAM (myeloblastic acute leukaemia). Classification criteria follow reference data in two current classifications: FAB - French-American-British (BAIN *ET AL*., 2010; KINNEY AND LUKENS, 1999) and WHO (World Health Organization) (MUNTEANU *ET AL*., 1999; VARDIMAN, 2009; FEY AND BUSKE, 2013).

MATERIAL AND METHODS

Examining blood cells to describe morphology and confirm their belonging to a certain type of leukocytes was possible due to the investigation of peripheral blood smears with usual laboratory methods: monolayer display on port blades, fixing and colouring through May-Grünwald-Giemsa panoptical coloration. Haematogenous marrow smears were prepared

at the patient's bed to avoid coagulation. Puncture-extraction marrow was displayed on port blades while spreading and smashing marrow grains between the two blades. Smears were fixed and sent to the laboratory for coloration. We used the May-Grünwald-Giemsa panoptical coloration.

Later, peripheral blood and haematogenous marrow smears were analysed with an Olympus BX43 optic microscope; we used a SC100 camera to photograph and measure cells. Microscopic examination was initially done at low power (with a 20x lens) to assess cell coloration and distribution as well as the presence of abnormal cell elements (blasts, erythroblasts), thrombolytic aggregates, erythrocyte/thrombolytic agglutinations. Then the smears were examined with an immersion 100x lens; each cell type was assessed for quantitative and qualitative anomalies.

RESULTS AND DISCUSSIONS

Examining blood smears in all studied patients showed blast (immature) cells representing 2-97% of the total leukocyte cells. The presence of a single immature cell guides the diagnosis towards suspicion of leukaemia. Quantitative and qualitative changes identified upon examining peripheral blood smears are described below.

We noticed a high percentage of large blast cells with a high nucleus/cytoplasm ratio and a fine vacuole cytoplasm; the nucleus has visible nucleolus and the cytoplasm has inclusions called Auer rods. The aspect was completed by the presence of hypochromic erythrocytes and the lack of thrombocytes that added anaemia and thrombocytopenia to the initial diagnosis. These features of the blast populations made possible their classification within the type II blast cell typology and the identification of the disease as LAM 3 myeloid monoblast acute leukaemia (Figure 1 and Figure 2).

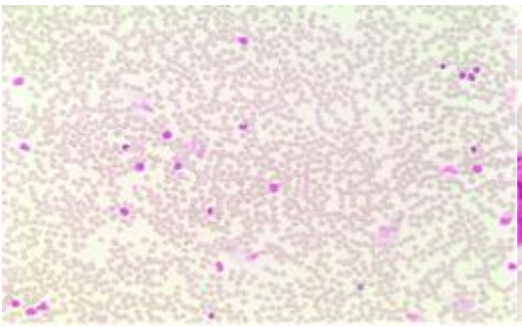

Figure 1. Smear with a high percentage of blasts (x20, original)

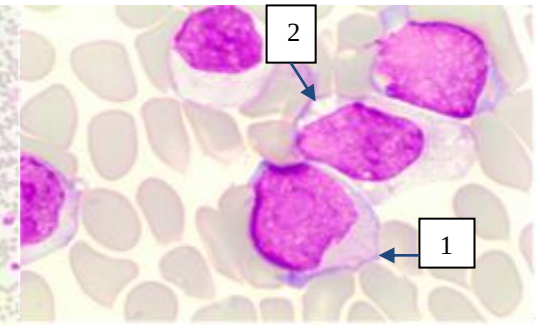

Figure 2. Blasts with multiple Auer rods (1) and intracytoplasmic vacuoles (2); visible nucleoli (x100, original)

In Figures 3 and 4, the percentage of blasts of a very large number of leukocytes is over 80% (significant leukocytosis), but these blasts have polymorphous features that do not allow their classification in a certain group; therefore, until we identify the phenotype, the suspicion is LAM 1, an extremely aggressive leukaemia evolving quickly to death. Figure 4 also shows the presence of neutrophil with pseudo-Pelger anomaly, hyposegmentation of granulocyte nucleus into one or two lobes, with dense chromatin and visible nucleoli, and fine, immature cytoplasm. This neutrophil appears in disgranulopoiesis and it is a pathological alteration of the nucleus or cytoplasm of granulocyte series. Erythrocytes are empty, hence the

very severe anaemia. There is also absence of thrombocytes. All the features of this image points to a high degree of malignity.

Figures 5 and 6 show the presence of myeloblasts, round immature cells with basophilic cytoplasm and few granulations, large euchromatic nucleus and visible nucleoli classified as type I blasts characteristic for LAM 1 and 3. The studied smears had, besides normoblasts (precursor cells of the red line) nuclear shadows (cells broken because of their frailty), erythrocyte and hypochromic and they have a strong anisocytosis. This myeloblast cell type points to a diagnosis of LAM 1, 3, 4.

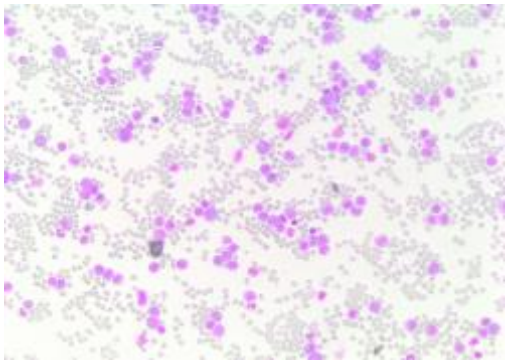

Figure 3. Leukocytosis with a high percentage of peripheral blasts (x20, original)

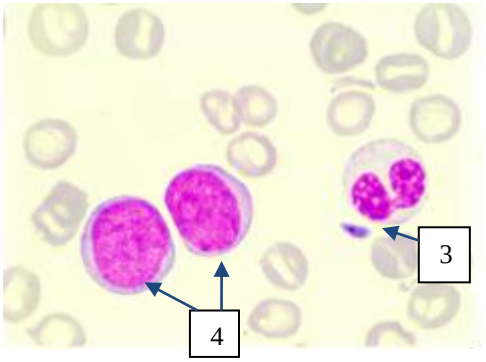

Figure 4. Undifferentiated blasts (4). Neutrophil with pseudo-Pelger anomaly (3) (x100, original)

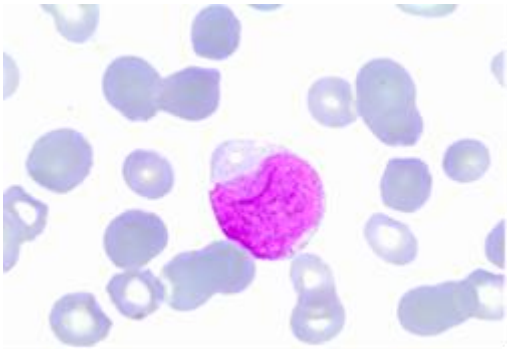

Figure 5. Large size myeloblast-blast, oval-like nucleus, incised; fine chromatin, basophile cytoplasm (x100, original)

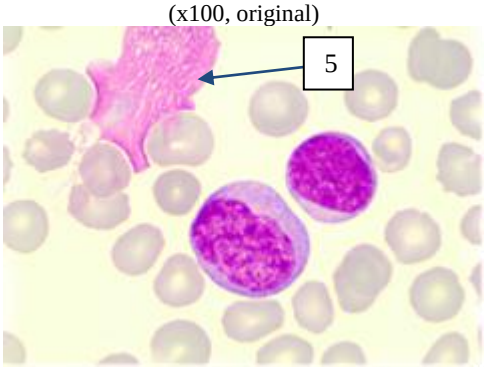

Figure 6. Myeloblast (centre); (upper left) nucleus shadow (5) (broken cell) (x100, original)

Monoblasts and promonocytes were present on four of the studied smears, confirming the diagnosis of LAM 4 or 5. The monoblast is a cell larger than the myeloblast with irregular contour nucleus, visible nucleoli with thickened nucleus membrane, intensely basophile agranular cytoplasm. It has intracytoplasmic vacuoles. Chromatin is dispersed (Figures 7 and 8).

Promonocytes have a low basophile abundant cytoplasm with intracytoplasmic vacuoles, condensed chromatin nucleus pointing to a nucleus maturation. The images show anisocytosis and erythrocyte hypochromia thrombocytopenia and the presence of macro thrombocytes pointing to anaemia and thrombocytopenia that are secondary to leukaemia (Figures 9 and 10).

The study showed a single peripheral blood smear with lymphoblasts confirming the diagnosis of lymphoblast acute leukaemia – LAL, because this type of leukaemia is more common in children (Figure 11).

Of the 25 smears analysed in this study, 9 had blasts that could not be classified because of their polymorphism. They had the features of a blast cell but, because of the combination of features, we could not identify their type. They were classified after supplementary analysis – immune-cytochemistry, immune-phenotypic, molecular biology (Figure 12).

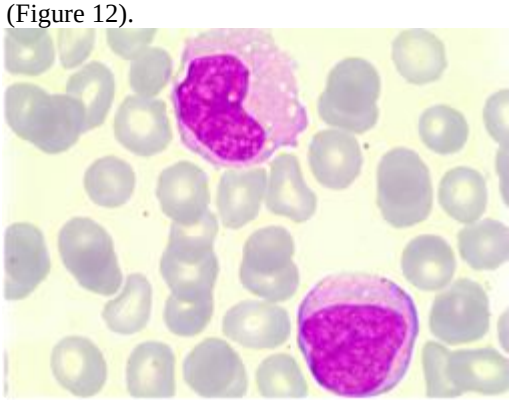

Figure 9. Up - promonocyte-nucleus with condensed chromatin, abundant cytoplasm and intracytoplasmic vacuoles; Down - monoblast (x100, original)

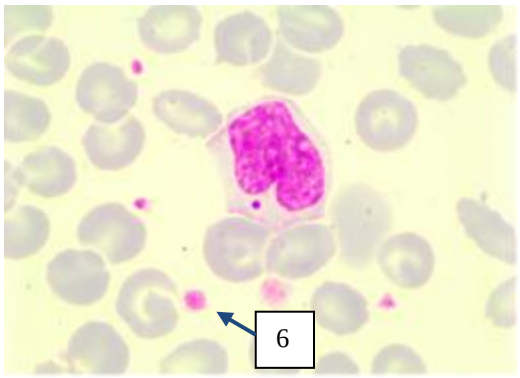

Figure 10. Promonocyte; macro thrombolytic (6) (x100, original)

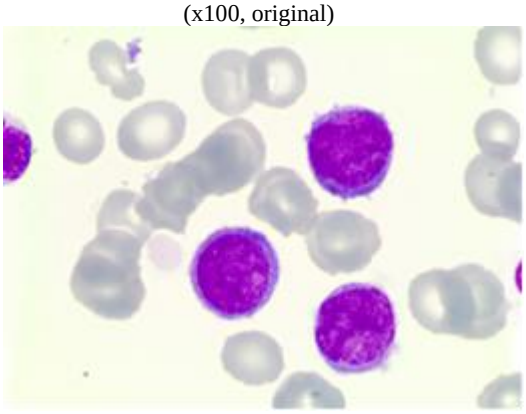

Figure 11. Lymphoblasts-monomorphic small blasts; round or oval-shaped nucleus; visible nucleoli; agranular and basophile cytoplasm (x100, original)

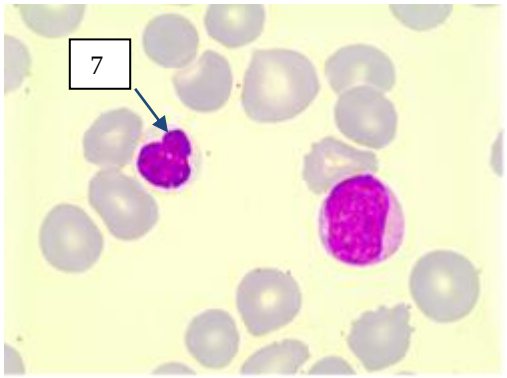

Figure 12. Undifferentiated blast; agranular unsegmented neutrophil (7) (x100, original)

After analysing the smears and crushed grains of marrow, we could see the same cell polymorphism (Figures 13 and 14). The marrow contained medullar infiltrate with blast cells, mainly variable size blasts, all with polymorph nucleoli and intensely basophile cytoplasm. Marrow cells were very rich in type.

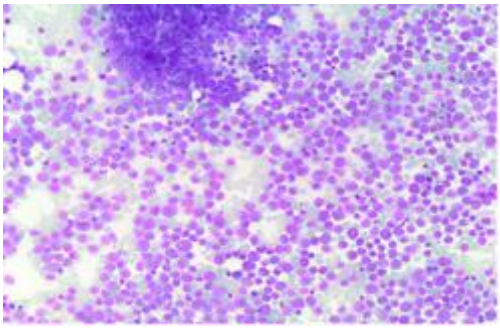

Figure 13. Marrow infiltrate of the blast-MO type (crushed grain present) (x20, original)

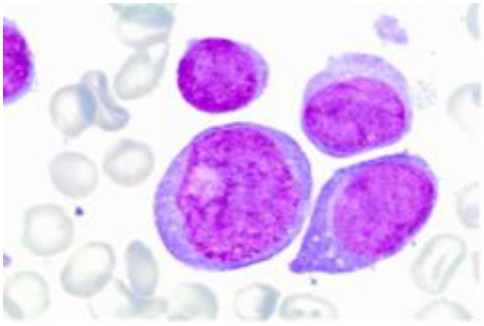

Figure 14. Bone marrow with blasts (x100, original)

CONCLUSIONS

After examining the 28 smears from acute leukaemia suspicion patients, we could see quantitative and qualitative changes at blood cell level; the share of blast cells was 2-97% of the total leukocyte cells.

4 smears (16%) could be classified as type I and II blasts, pointing to myeloid blast acute leukaemia (LAM) 2 and 3, 4; 5 smears (20%) supplied criteria of myeloid blast acute leukaemia (LAM) 5, i.e. type I blasts; 5 smears (20%) had undifferentiated or low-differentiated blasts pointing to myeloid monoblast acute leukaemia (LAM) 1; 1 smear (4%) showed type I blasts, pointing to a myeloid monoblast acute leukaemia (LAM) 4; 1 smear (4%) showed lymphoblasts, lymphoid blast acute leukaemia (LAL).

9 peripheral blood smears (36%) had blast cells with strong polymorphism, that could not be classified even after marrow examination. They needed supplementary analysis – immune-cytochemistry, immune-phenotypic, molecular biology.

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