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ASTEREOLOGICAL EXAMINATION OF IMMATURITY OF MEGALOBLAST CELL NUCLEI OF BONE MARROW IN PERNICIOUS ANEMIA

Lana Mačukanović-Golubović[1], Gorana Rančić[2], Mladen Milenović[1], Dragan Mihailović[3], Gordana Kostić[4]

[1]*Clinic of Haematology,*
[2]*Institute of Histology and Embryology*
[3]*Institute of Pathology*
[4]*Clinic of Pediatrics, Medical Faculty, Niš, Serbia and Montenegro*

Summary. *In pernicious anemia (PA) proliferation of hematopoetic cells is disturbed due to impaired DNA synthesis. The bone marrow is usually hypercellular with dominant megaloblast transformation of erythrocyte precursors. Megaloblastic changes are present in all stadiums of erythrocyte development. The aim of our study was to apply astereological methods in order to estimate morphological immaturity of megaloblastic cell nuclei in the bone marrow in PA. Patients diagnosed with PA (58) and treated at the Clinic of Hematology, Niš, during the period 2000-2004 were divided into three groups according to the pathological finding of the gastric mucosa. Astereological examination of megaloblastic cells of the bone marrow was performed using a B 100 grid system. The absolute nuclear surface, absolute contour nuclear density and form factor of megaloblastic nuclei was determined. A significant increase in the absolute surface and contour density of nuclei of the examined cells was found in all groups, while the nuclear form factor showed a statistically significant reduction. It could be concluded that astereological changes in erythroid elements can be used for detection of cells with impaired function.*

Key words: *Pernicious anemia, bone marrow, erythroid elements, astereological analysis*

Introduction

In pernicious anemia (PA) proliferation of hematopoetic cells is disturbed due to impaired DNA synthesis. This is most expressive at the level of erythrocyte stem cells. The bone marrow is usually hypercellular and megaloblast transformation of erythrocyte precursors dominates. Megaloblastic changes are present in all stadiums of erythrocyte development.

Megaloblasts are related to the normal erythroblasts of larger size, and have a huge nucleus with loose chromatin; asynchronism is also noticed in the course of maturation of the nuclei and cytoplasm (1,2,3).

Aim

The aim of the study was to perform a quantification of morphologically visualized immaturity of nuclei of megaloblastic cells of the bone marrow in pernicious anemia by applying astereological methods (4,5,6).

Patients and Methods

During the period 2000-2004, 58 patients with pernicious anemia and 30 control patients were examined at the Clinic of Hematology, Niš. The patients with PA were divided into three groups according to the pathological finding of the gastric mucosa (7,8):

Group I – 6 patients with chronic atrophic gastritis (CAG) grade I with intestinal metaplasia (inflammatory infiltration destroyed the neck zone of gastric glands in the superficial part of tunica mucosae);

Group II – 40 patients with CAG grade II (inflammatory infiltration destroyed 2/3 of gastric glands); and

Group III – 13 patients with CAG grade III (inflammatory infiltration destroyed nearly all glands or a few groups of glands are present in the basal part of tunica nucosae).

The group of patients with dyspeptic syndrome had no pathohistological lesion in the gastric mucosa, while the finding of normal blood and marrow was considered an inclusion criterion for controls. In all the examined patients the following parameters were analyzed:

- clinical picture;
- condition of corporal and antral gastric mucosa;
- bone marrow; and
- astereological parameters.

In the study we used the following methods:

- methods of clinical examination (anamnesis, objective status, gastroscopy);
- methods of histological examination of the gastric mucosa – Haematoxilyn Eosin staining;
- methods of hematological examination of the bone marrow according to May-Grünwald- Giemsa staining; and
- methods of astereological examination (4,5,6).

An astereological examination of megaloblastic cells of the bone marrow was done using a double ocular grid system B 100 (d = 9.3 μm) and an ocular micrometer made by Opton (1:100). Cells were examined with a Carl Zeiss (Jena) microscope with a 100 X lens (oil immersion, numerical aperture 1.25) and 10 X eyepiece magnification, according to the astereological rules. Referential space were megaloblastic cells, and fields were selected systematically-zonal, until obtaining the adequate number of cells (for promegaloblast n = 36; for basophilic megaloblast n = 38; for polychromatophilic megaloblast n = 30; for orthochromatic megaloblast n = 38). Points and intersections were counted and calculated using conventional formulas (6). We determined: the absolute nuclear surface (An = Aan × Ao); the absolute contour nuclear density (Bn = Ban × Ao); and the form factor of nuclei (FOn = Bn/An). Abrevations are as follows: An – absolute nuclear surface of the examined element; Aan – relative nuclear surface; Bn – absolute contour nuclear density; and Ban – relative contour nuclear density.

Results

We have determined the absolute nucleus surface of the erythroid cell line as there is a distorted DNA synthesis in PA that leads to distorted nucleus maturity (Table 1).

The absolute surface of the nucleus (An) of all examined cells shows statistically significant growth ($p < 0.001$) in all three groups graded according to pathological changes in the gastric mucosa.The most expressive growth of An was registered in the third patients group for polychromatophilic megaloblast (3.2 times higher than in controls).

In all erythrocytic cells of the bone marrow, contour density of the nucleus was determined (Table 2).

The growth of contour (Bn) density of the nucleus of the examined cells was determined, too, and was shown statistically significant ($p<0.001$) in all three groups of patients compared to controls. The most expressive growth of Bn was observed at the level of basophilic megaloblast.

Table 1. Absolute areal surface of the nuclei of erythroid elements of the bone marrow ($\overline{X}$ ±SD)

Erythroid elements of bone marrow	control group n = 30	I group n = 6	II group n = 40	III group n = 30
Proerythroblast / promegaloblast	242.19 ± 34.57	416.74*** ± 9.90	533.19*** ± 32.15	714.72*** ± 38.21
Basophilic erythroblast / megaloblast	173.02 ± 31.60	283.06*** ± 38.53	419.66*** ± 41.33	465.42*** ± 62.81
Polychromatophilic erythroblast / megaloblast	126.81 ± 43.18	196.54*** ± 38.58	278.21*** ± 74.20	407.18*** ± 39.58
Orthochromatic erythroblast / megaloblast	86.50 ± 0.00	102.14 ± 33.39	108.10*** ± 37.44	129.73*** ± 43.2

An –μm^2, *** $p < 0.001$

Table 2. Absolute contour nuclear density of erythroid elements of the bone marrow ($\overline{X}$ ± SD)

Erythroid elements of bone marrow	control group n = 30	I group n = 6	II group n = 40	III group n = 13
Proerythroblast / promegaloblast	56.13 ± 7.49	59.45* ± 2.71	71.14*** ± 7.8	76.58*** ± 5.21
Basophilic erythroblast / megaloblast	41.65 ± 5.60	53.40*** ± 4.66	62.42*** ± 3.43	67.02*** ± 6.09
Polychromatophilic erythroblast / megaloblast	37.07 ± 5.11	41.60* ± 5.4	46.09*** ± 5.64	54.44*** ± 3.86
Orthochromatic erythroblast / megaloblast	26.87 ± 3.32	29.04*** ± 0.03	29.04*** ± 0.03	32.67*** ± 3.64

Bn - μm, * $p<0.05$, ** $p< 0.01$, *** $p< 0.001$

Table 3. Form factor of the nuclei of erythroid elements of the bone marrow ($\overline{X}$ ± SD)

Erythroid elements of bone marrow	control group n = 30	I group n = 6	II group n = 40	III group n = 13
Proerythroblast / promegaloblast	0.23 ± 0.05	0.14*** ± 0.01	0.13*** ± 0.01	0.10*** ± 0.00
Basophilic erythroblast / megaloblast	0.24 ± 0.04	0.19*** ± 0.02	0.15*** ± 0.01	0.15*** ± 0.02
Polychromatophilic erythroblast / megaloblast	0.31 ± 0.08	0.21*** ± 0.02	0.17*** ± 0.04	0.13*** ± 0.01
Orthochromatic erythroblast / megaloblast	0.31 ± 0.03	0.30 ± 0.06	0.29 ± 0.07	0.27* ± 0.07

F0n – μm^{-1}, *** $p <0.001$

Table 4. Correlation of erythrocyte count, serum vitamine B12 and absolute nuclear surface of the bone marrow erythroid elements

Erythroid elements of bone marrow	control group n = 30	I group n = 6	II group n = 40	III group n = 13
Erythrocyte count ×10^{12} /L	4.25 ± 0.41	3.24 ± 0.20	2.53 ± 0.42	1.87 ± 0.39
Serum vit. B12 pg/ml	525.19 ± 117.21	160.06 ± 10.20	110.31 ± 30.79	71.75 ± 29.81
Proerythroblast / An promegaloblast	242.18 ± 34.56	416.74 ± 49.89	533.19 ± 32.14	714.72 ± 38.21
Basophilic An erythroblast / megaloblast	173.03 ± 31.59	283.06 ± 38.53	419.66 ± 41.32	465.41 ± 62.81
Polychromatophilic An erythroblast / megaloblast	126.81 ± 43.18	196.54 ± 38.58	278.21 ± 74.20	407.18 ± 39.58
Orthochromatic Erythroblast/megaloblast	86.50 ± 0.0	102.14 ± 33.39	108.10 ± 37.44	129.72 ± 43.23

The form nucleus factor of erythrocyte cells of the bone marrow (Table 3) was estimated on the basis of the absolute nucleus surface and contour density of the nucleus. It shows a statistically significant reduction for all three patients groups ($p < 0.001$), but the highest reduction was observed at the level of the polychromatophilic megaloblast where the decrease was by 2.37 times more pronounced compared to controls.

A corellation between erythrocyte number, vitamin B_{12} and the absolute nucleus surface of erythroid elements of the bone marrow was examined (Table 4).

Statistical processing of data confirmed a significant correlation (–0.504) between erythrocyte number and vitamin B12 levels, suggesting that decreased levels of vitamin B12 are followed by reduction of erythrocyte number. A similar correlation was observed between vitamin B12 levels and the absolute areal nuclear surface of promegaloblasts, that is, low levels of vitamin B12 lead to enlarged areal surface of nuclei. A negative correlation was registered between vitamin B12 levels and the absolute areal nuclear surface of other erythroid precursors in the bone marrow (basophilic, polychromatophilic and acidophilic megaloblasts) in patients with the most extensive pathologic changes in the gastric mucosa.

Discussion

Stereologic studies are still a rare kind of examination of the bone marrow. Only a few data have been obtained based on stereological studies, but astereological data are even fewer. We have not found any data about astereological analyses in pernicious anemia.

By examining An of erythrocytes of bone marrow cells in PA, data about the changes present at the nucleus level have been obtained (9, 10). The most expressive growth of An is at the level of polychromatophilic megaloblast where distortion in the maturing of the nucleus has advanced. These findings correlate with the grade of atrophic gastritis.

The examination of Bn of erythroid elements of the bone marrow shows an increase in this stereological parameter, i.e., the excess membrane compared to the surface, which correlates with the grade of atrophic gastritis. The most explicit growth of Bn is at the level of basophilic megaloblasts, which suggests that the existing process of nucleus maturing retains an amplified activity inadequate for that stadium - basophilic erythroblast.

F0n was decreased in all examined groups when compared to controls and estimated against the grade of the atrophic gastritis. Decreased F0n implies an intensive increase in An in relation to Bn. The most explicit F0n decrease is at the level of polychromatophilic megaloblast, which is explained by a slow maturation process in later stadiums of erythrocyte cells' development. In the course of maturation, the polychromatophilic erythroblast goes through two stadiums and changes the size of cells because of the mitosis of both the whole cell and the nucleus. During the astereological analysis, these stadiums cannot be easily singled out, which leads to the most explicit decrease in F0n of the polychromatophilic megaloblast (9, 10).

Conclusion

The most expressive megaloblastic changes of PA bone marrow erythroid elements, which have been found by examination of An, Bn and F0n, are at the level of polychromatophilic megaloblasts. This can be explained as a sign of a slow maturational process of the nucleus.

The examined astereological parameters suggest a correlation with the grade of atrophic gastritis.

The negative correlation between vitamine B_{12} values in serum and An of erythroid elements in the bone marrow is a valuable finding which can help clinical practitioners evaluate B_{12} deficiency by examining bone marrow elements.

The examination of An, Bn and F0n of megaloblastic cells in PA contributes to a better understanding of the cause of their minor values and of an attempt to compensate for the insufficiency of those cells by enlarging the surface. These morphologic changes can account for the inpaired function of the examined cells.

According to the morphologically visualized immaturity of nuclei and disturbed function of the cells, an astereological analysis can help define and select a risk group of PA patients in precancerous condition of the gastric mucosa.

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ASTEREOLOŠKO ISPITIVANJE NEZRELOSTI JEDARA MEGALOBLASTA KOSTNE SRŽI U PERNICIOZNOJ ANEMIJI

Lana Mačukanović-Golubović[1], Gorana Rančić[2], Mladen Milenović[1], Dragan Mihailović[3], Gordana Kostić[4]

[1] Klinika za hematologiju
[2] Institut za histologiju i embriologiju
[3] Institut za patologiju
[4] Dečija interna klinika Medicinskog fakulteta, Niš

Kratak sadržaj. *Proliferacija hematopoetskih ćelija u pernicioznoj anemiji (PA) je poremećena zbog neadekvatne sinteze DNA. Kostna srž je obično hipercelularna i dominira megaloblastna transformacija eritrocitnih prekursora. Megaloblastne promene su prisutne u svim stadijumima eritrocitnog razvića. Cilj rada je bio kvantifikovati morfološki vizuelizovanu nezrelost jedara megaloblastnih ćelija kostne srži u PA korišćenjem astereoloških metoda. Obuhvaćeno je 58 pacijenata sa PA u periodu 2000-2004. godine koji su podeljeni u tri grupe prema patološkom nalazu želudačne sluzokože. Astereološko ispitivanje megaloblastnih ćelija kostne srži obavljeno je korišćenjem mrežastog sistema B100. Određivani su apsolutna nuklearna površina, apsolutna konturna gustina i oblikovni faktor jedra megaloblasta. Naši rezultati ukazuju da apsolutna površina i konturna gustina jedra ispitivanih ćelija rastu statistički značajno u svim grupama, dok oblikovni faktor jedra pokazuje statistički značajno smanjenje. Uzimajući u obzir astereološke promene eritroidnih elemenata može se diskutovati i zaključiti da je funkcija ovih ćelija neadekvatna.*

Ključne reči: *Perniciozna anemija, kostna srž, eritroidni elementi, astereološke analize*