The reduction of the cell nuclear size in the cell body space during the differentiation might be cell lineage specific (A retrospective morphological note)

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Short title: Nucleus size, cell space, cell lineage specificity

Summary

The cell body space occupied by the nucleus decreased during the cell differentiation of the granulocytic cell lineage in CML (Chronic Myeloid Leukemia) patients. In contrary, in patients suffering from CLL (Chronic Lymhocytic Leukemia), the cell body space occupied by the nucleus during the cell differentiation of the lymphocytic lineage did not decrease despite the reduction of the cell size. Thus, the cell body space occupied by the cell nucleus during the differentiation was characteristic for each of these cell lineages.

Key words: Nucleus size, cell space, cell lineage specificity

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Introduction

Since the onset of the last century the equilibrium between the nucleus and cytoplasm was expressed by the "Kern-Plasma-Relation", i.e. nuclear-cytoplasmic index **[1, 2, 3]**. In the classical cytology the nuclear-cytoplasmic index was calculated by dividing the nuclear volume by the cytoplasmic volume subtracted from the nuclear one. It should be mentioned that that index was mostly based on direct nuclear and cell diameter measurements which were followed by volume or area calculations using various geometric models resembling the cell appearance. To simplify such calculations in the clinical and hematological cytology, the calculated nucleus to cell body ratio was based just on the simple nuclear and cell body diameter measurements. In addition, largest – maximal – nuclear body diameter or axis measurements frequently provided satisfactory and informative results utilizing the cell viscoelastic properties **[4, 5, 6, 7, 8]**. Such calculations also appeared to be very useful in both light and electron microscopy if the number of measured cells was adequate to reduce the variability **[9, 10].** At this occasion it should be mentioned that the nucleus to cell body ratio multiplied by 100 might also reflect the rough cell space estimate occupied by the nucleus **[8]**. It should be added that in the clinical cytology or histology the simple nuclear to cytoplasmic ratio in specimens stained with current panoptic staining procedures appeared to be a very helpful tool to estimate the cell proliferation potential and cell malignancy [**11]**. It should be also mentioned that the changing equilibrium of both these main cell compartments expressed by the nucleus-cytoplasmic or nucleus – cell body ratios were considered to precede the cell division. Moreover, the changing proportion of the nucleus in the cell body was also apparent in the course of the cell differentiation and maturation or aging **[2, 12, 13]**.

The present retrospective study was undertaken to provide more information on the nucleus to cell body maximal diameter ratio that reflected a rough estimate of the cell space occupied by the nucleus during the cell differentiation of selected cell lineages. The human granulocytic and lymphocytic lineages in bone marrow or peripheral blood smears of patients suffering from chronic phase of myeloid (CML) and chronic lymphocytic (CLL) and leukemias were very convenient models for such study. The differentiation steps of these lineages are easily identified and are present in these specimens in a satisfactory number for measurements at the single cell level **[8, 13]**. In addition, the peripheral blood and bone marrow samples were

originally used for the routine laboratory control of these patients with the approval and supervision of the Institutes medical authorities.

Material and methodical notes

The differentiation steps of the lymphocytic and granulocytic cell lineages in the peripheral blood and bone marrow smears of patients suffering from CML and CLL were stained with May-Grünwald – Giemsa-Romanowsky panchromatic procedure (MGGR) and acidified methylene blue cytochemical method for RNA **[14, 15]**. 3 patients in each group of these patients were untreated and 3 were treated with the current antileukemic therapy (Imatinib for CML, Fludara and Leukeran for CLL,) at the time taking samples for the present study. The peripheral blood and bone marrow samples of studied patients were originally taken for diagnostic purposes with the supervision and approval of the Institute authorities.

Digitized micrographs captured with a Camedia digital camera C4040 ZOOM (Olympus, Japan) on Jenalumar microscope (Zeiss, Germany) were processed and nuclear and cell body diameters were measured using Quick Computer Photoprogram (Olympus, Japan). Nucleus to cell body diameter ratio was calculated by dividing the maximal nuclear by the maximal cell body diameter for each measured cell. The calculated ratio multiplied by 100 estimated the approximate size – proportion – of the cell body space occupied by the nucleus **[8]**. The results of all measurements and calculations at the single cell level such as mean and standard deviation followed by the t-test were evaluated using Primer of Biostatistic Program, version 1 developed by S.A. Glantz (McGraw-Hill, Canada, 1968).

Results

CML proliferating compartment of the neutrophilic lineage. During the differentiation the terminal dividing differentiation step – myelocyte – was characterized by the decreased cell body space with the nucleus (Tab. 1, Fig. 1, a, b). In contrary, the reduction of the cell body diameter was less apparent. On the other hand, the cell body diameter appeared to be slightly but significantly reduced in myelocytes of patients treated with the cytostatic therapy. (Tab 1). The small variation coefficient supported the significance of measured and calculated data.

CLL lymphocytic cell lineage. According to the measurements and calculations (Tab. 1, Fig. 1, c, d, e) the cell body space estimate occupied by the nucleus during the cell differentiation did not diminish despite the marked reduction of the cell size. The slightly decreased estimate of the cell space with the nucleus in lymphocytes of patients treated with the cytostatic therapy

was not significant. Thus, the cell body space occupied by the cell nucleus was not substantially influenced by the anti-leukemic therapy. The presented data appeared to be significant because of the very small variation coefficient.

Discussion and conclusion

The cell body space occupied by the nucleus decreased during the cell differentiation of the granulocytic cell lineage in CML patients. In contrary, in the lymphocyte lineage of patients suffering from CLL, the cell body space occupied by the nucleus during the cell differentiation did not change despite the reduction of the cell size. Thus, the reduction of the cell body space occupied by the cell nucleus during the differentiation was characteristic for each cell lineage. At this occasion it should be mentioned that that conclusion might be useful because the very small variation coefficient for the nuclear and cell body measurements reflected by the rough estimate of the cell body space with the nucleus during the differentiation of both studied cell lineages [**9, 10]**.

The above presented conclusion is also supported by the differentiation of erythroid cell lineages in CML patients or patients suffering from the refractory anemia of the myelodysplastic syndrome **[8]**. In CML patients the advanced differentiation steps of the erythroid cell lineage were characterized by a significant reduction of the cell body space with the nucleus similarly as differentiation steps of the proliferative compartment of the granulocyte lineage. However, in patients suffering from the refractory anemia of the myelodysplastic syndrome, the less apparent reduction of the nuclear size in the cell body of "megaloblasts" resembled that of differentiated steps of the lymphocytic lineage.

The presented measurements also demonstrated a remarkable similarity of the cell body space occupied by the nucleus in early differentiation steps of neutrophil granulocytes and lymphocytes in both untreated and treated patients with the antileukemic therapy. Such similarity just suggested that the stability of the nucleus-cell body equilibrium of these cells reflected by the nucleus to cell body ratio in these differentiation steps was not influenced by the cytostatic therapy of studied patients. It should be also mentioned that the approach used in the present study is time consuming and requiring the knowledge of the cell identification. However, there is also a possibility that future cyto-flow measurements of a larger number of cells with specific cell markers together with the artificial intelligence would be simpler to

provide useful information on examined cell lineages under physiological as well as pathological or experimental conditions.

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Competing interests

The authors declared that no competing interests exist.

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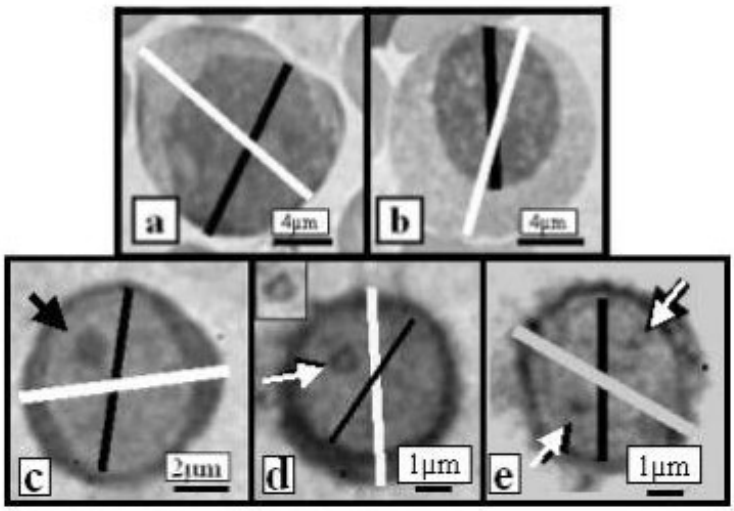
Table 1. The cell space occupied by the nucleus (Maximal Nuclear Diameter : Maximal Cell Body Diameter x 100) in differentiation steps of the granulocytic and lymphocytic cell lineage in patients suffering from CML and CLL*

Differentiation steps	Mx Nu Dm : Mx Cell Body Dm x 100	Mx Cell Body Dm	Th
Myeloblasts	76.0 ± 1.1 (1.4)	16.4 ± 1.1 (6.7)	0
	78.4 ± 1.6 (2.0)	16.3 ± 1.1 (6.7)	+
Myelocytes	65.8 ± 4.2§ (6.3)	16.3 ± 1.2 (7.3)	0
	65.1 ± 3.5§ (5.3)	14.3 ± 0.5§ (3.4)	+
Lymphoblasts	76.3 ± 2.5 (3.2)	12.7 ± 0.7 (5,5)	0
	76.4 ± 2.3 (3.0)	13.8 ± 2.1 (15.2)	+
Lymphocytes 1	77.0 ± 0.3 (0,3)	10.6 ± 0.4§ (3.7)	0
	76.3 ± 2.3 (3.0)	11.0 ± 0.6 (5,4)	+
Lymphocytes 2	77.6 ± 4.4 (5.6)	10.6 ± 0.3§ (2.8)	0
	75.5 ± 2.4 (3.1)	10.9 ± 0.5 (4.5)	+

Legend

*Mean and standard deviation of ≈ 100 measurements of lymphocytes or myelocytes and ≈50 lymphoblasts or 30 myeloblasts in 3 untreated and 3 treated patients with the cytostatic therapy at the time of taking samples for control examinations. Myeloblasts – early differentiation steps of the neutrophil granulocytic lineage, myelocytes – terminal differentiation steps of the mitotic compartment of the neutrophil granulocytic lineage. Lymphoblasts – early differentiation steps of the lymphocytic lineage, Lymphocytes 1 – differentiated mature lymphocytes with characteristic ring-shaped nucleoli, Lymphocytes 2 – differentiated terminal lymphocytes with micronucleoli, § - significantly different from myeloblasts (treated and untreated with antileukemic therapy) or lymphoblasts (untreated with antileukemic therapy) using t-test ($2\alpha=0.05$). Numbers in brackets represent the percentage of the calculated variation coefficient multiplied by 100, Th + - therapy with Imatinib for CML, Fludara and Leukeran for CLL .

Fig. 1



Legend

The neutrophil granulocytic cell lineage: a – early differentiation step (myeloblast) with a nucleus occupying ≈ 78.6% of the cell space, b – last dividing differentiation step (myelocyte) with a nucleus occupying 66.6% of the cell space. MGGR staining procedure **[14]**.

The lymphocytic cell lineage: c – lymphoblast (early differentiation step) with a nucleus occupying ≈ 75.7% of the cell space that contains a characteristic nucleolar body (arrow and insert), d – mature lymphocyte with a nucleus occupying ≈ 75.6% of the cell space that contains a characteristic ring-shaped nucleolus (arrow and insert), e – terminal lymphocyte with a nucleus occupying ≈ 76.1% of the cell space containing 2 micro-nucleoli (arrows). White lines – maximal cell diameter, black lines – maximal nuclear diameter. Cytochemical staining for RNA **[15]**).