



To the Maximal Nuclear Diameter and Cell Cycle Phase of Lymphoblasts or Myeloblasts in Patients Suffering from Acute Lymphoblastic and Myeloblastic Leukaemia. A Simple Morphological and Technical Note

(maximal nuclear diameter / cell cycle / leukaemic lymphoblasts / myeloblasts)

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Abstract. The present study was undertaken to provide complementary information on the cell cycle of leukaemic lymphoblasts and myeloblasts based on the computer-assisted maximal nuclear diameter measurements. The measurements were carried out in “one-size cell layer” loci of bone marrow smears of patients suffering from B-cell acute lymphoblastic leukaemia (B-ALL), acute myeloblastic leukaemia with minimal differentiation (M0 AML), acute myeloblastic leukaemia without maturation (M1 AML), acute myeloblastic leukaemia with maturation (M2 AML) and chronic myeloid leukaemia (CML). The maximal nuclear diameter was also measured in peripheral blood mature lymphocytes of B-cell chronic lymphocytic leukaemia (CLL), which are known to

be in the G0/G1 phase of the cell cycle. In contrast, the maximal nuclear diameter in lymphoblasts of patients with B-ALL was larger and reflected the S and G2 cell cycle phases. The largest incidence of myeloblasts with the smallest maximal nuclear diameter was in M0 AML. The smaller incidence of such cells was also noted in patients with M1 AML. Myeloblasts with larger nuclear bodies were present in M2 AML and CML. Thus, post-mitotic myeloblasts in G0 and G1 phases were characteristic of M0 and to a smaller extent of M1 AML. Myeloblasts with larger maximal nuclear diameter reflecting the S and G2 phases were dominant in M2 AML and CML. In summary, the nuclear size heterogeneity of leukaemic lymphoblasts and myeloblasts in bone marrow smears depends on various phases of the cell cycle classified according to the maximal nuclear diameter.

Received November 13, 2025. Accepted December 11, 2025.

This study was partially supported by the Ministry of Health of the Czech Republic Institutional grant DRO IHBT 00023736.

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Abbreviations: B-ALL – B-cell acute lymphoblastic leukaemia, CLL – chronic lymphocytic leukaemia, AML – acute myeloblastic leukaemia, CML – chronic myeloid leukaemia, G0 – zero “resting” post-mitotic gap, G1 – “activated growing” post-mitotic gap, G2 – pre-mitotic gap of the interphasic cell cycle, L3 – Burkitt-like acute lymphoblastic leukaemia, M0 – myeloid (myeloblastic) leukaemia with minimal differentiation, M1 – myeloid (myeloblastic) leukaemia without maturation, M2 – myeloid (myeloblastic) leukaemia with maturation.

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Introduction

It seems to be clear that the nuclear size reflects various phases of the cell cycle. The smallest nuclear size would correspond to the post-mitotic G0 or G1 phase. In contrast, the largest nuclear size is characteristic of pre-mitotic G2 phase, and the intermediate nuclear size reflects the S phase. On this occasion it should be mentioned that each phase of the cell cycle is involved in key biosynthetic activities necessary for both cell life and cell functions (Nagl, 1976; Fidorra et al., 1981; Thomas, 1996; Zetterberg, 1996; Maeshima et al., 2011; Keohane, 2016). The post-mitotic G0 phase reflects the resting state and the G1 phase represents the onset of the cell growth and biosynthetic activity necessary for replication. The S phase is characterized by DNA replication and continuing biosynthetic activity accompanied by the growing cell size. The G2 phase is reflected by nuclear and cell enlargement, gradual termination of the biosynthetic ac-

tivities preceding the mitotic process. On the other hand, the minimal, mean or maximal nuclear sizes of myeloblasts and lymphoblasts are known and very often mentioned in the literature, in various haematological publications including monographs (Undritz, 1972; Bessis, 1973; Cline, 1975). However, nuclear size histograms reflecting the cell cycle of early leukaemic differentiation precursors are less known, although they might provide useful complementary information potentially accessible for other more sophisticated diagnostic or research efforts. On this occasion it should also be mentioned that the “major nuclear diameter” (“maximal diameter” in the present study) was considered to be a morphometric prognostic marker for the malignant cell survival and might indicate cell cycle phases or maturing and aging states (Monge et al., 1999; Smetana et al., 2007).

Methodological Notes

The present study was undertaken to provide simple complementary information on the cell cycle phases of leukaemic lymphoblasts or myeloblasts based on the simple nuclear computer-assisted maximal diameter measurements and image processing (Olympus, Tokyo, Japan). The maximal nuclear diameter of these cells was measured in lymphoblasts of bone marrow smears of four patients suffering from B-cell acute lymphoblastic leukaemia (B-ALL), myeloblasts of two patients with acute myeloblastic leukaemia with minimal differentiation (M0 AML), three patients with acute myeloblastic leukaemia without maturation (M1 AML), four patients with acute myeloblastic leukaemia with maturation (M2 AML) and four patients with the chronic phase of chronic myelocytic leukaemia (CML). Bone marrow smears were stained by the May-Grünwald-Giemsa-Romanovsky staining in selected one-size cell layer loci of bone marrow smears without the cell shape and size morphological alteration of the measured cells. In addition, the control measurements for comparison with lymphoblasts and myeloblasts were carried out on differentiated and mature leukaemic lymphocytes in the peripheral blood of patients suffering from B-cell chronic lymphocytic leukaemia (CLL), because these cells are known to be in G0 and/or G1 post-mitotic phase of the cell cycle (Andreeff et al., 1980). Myeloblasts with the generally known differentiation potential were also measured in CML patients for comparison with these cells of AML, which are generally known for altered differentiation (Cáceres-Cortés, 2013). Burkitt-like lymphoblasts easily identifiable for the presence of characteristic multiple small vacuoles were measured in isogenic groups in bone marrow smears of B-cell acute lymphoblastic leukaemia (L3 ALL) to provide illustrative information on the cell cycle phase related to the nuclear size estimate (Fig. 1, Table 1).

Results

The results of control measurements of differentiated and mature leukaemic CLL lymphocytes demonstrated

that the maximal nuclear diameter in these cells was smaller than 11 μm and might reflect the G0/G1 post-mitotic phase of the cell cycle (Table 1). In contrast, the maximal nuclear diameter in lymphocytic early differentiation steps – lymphoblasts – of patients with B-ALL was mostly larger than 11 μm , and thus characteristic of these cells in the S and G2 cell cycle phases (Table 1). On this occasion it should be mentioned that lymphoblasts with the smaller maximal nuclear diameter ($< 11 \mu\text{m}$) were considered to be in the post-mitotic G0 or in G1 phase preceding the S phase (Table 1). The presence of lymphoblasts with variously sized nuclear bodies corresponding to various cell cycle phases was clearly seen in isogenic groups in bone marrow smears of the patients with Burkitt-like L3 ALL (Fig. 1). The Burkitt-like lymphoblasts with the smallest maximal nuclear diameter would correspond to G0/G1 phase of the cell cycle, intermediate maximal diameter to S phase, and largest maximal diameter to G2 phase (Fig. 1, Table 1).

The results of the maximal nuclear diameter measurements in leukaemic myeloblasts indicated that the largest incidence of these cells ($\sim 60\%$ of myeloblasts) with the smallest maximal nuclear diameter ($< 11 \mu\text{m}$) was noted in M0 AML. Such cells, but in a smaller number ($\sim 43\%$ of myeloblasts), were noted in patients suffering from M1 AML. Myeloblasts with nuclear bodies larger than 11 μm (about 70% of myeloblasts) possibly representing cells in S and G2 phases of the cell cycle were present in M2 AML or CML. Thus, post-mitotic myeloblasts in G0 or G1 phase were characteristic of acute myeloid leukaemia with minimal differentiation or without maturation (Table 1). In contrast (Table 1), myeloblasts with larger nuclear bodies possibly reflecting the S and G2 phases of the cell cycle were dominant in M2 AML and CML.

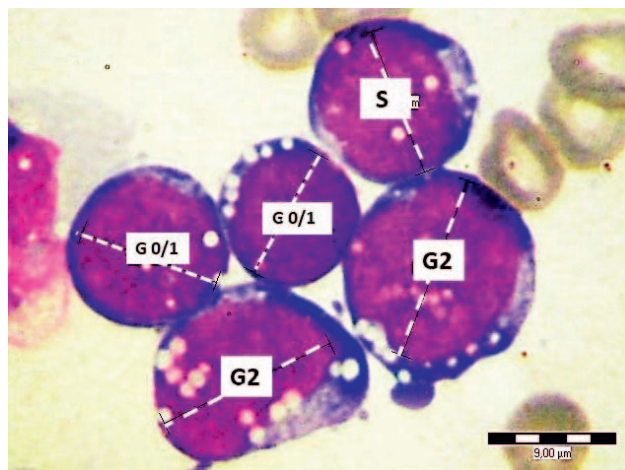


Fig. 1. Isogenic group of Burkitt-like lymphoblasts with characteristic multiple vacuoles selected at random in the bone marrow smear of a patient with L3 ALL. The maximal nuclear diameter: smaller than 11 μm would reflect the G0/1 phase, larger than 11 μm would reflect the S phase and larger than 13 μm would reflect the G2 phase of the cell cycle. MGGR staining.

Table 1. Histograms of maximal nuclear diameter and cell cycle phase estimate of leukaemic cells in peripheral blood (PB) or bone marrow smears (BM) stained with MGGR procedure

μm	< 9 + 10	11 + 12	13 + 14	(11 + 12) + (13 + 14)
cell cycle phase estimate	(G0/G1)	S	G2	S + G2
CLL lymphocytes (PB) %	92.1 ± 21.7	0	0	0
B-ALL lymphoblasts (BM) %	39.8 ± 7.7	44.2 ± 6.6	15.5 ± 9.3	59.7
B-ALL lymphoblasts Burkitt (BM) %	30.3 ± 1.2	40.3 ± 1.1	29.4 ± 2.7	69.4
M0 AML myeloblasts (BM) %	60.1 ± 3.5 (!)	28.8 ± 2.6	11.7 ± 1.3	40.5 (!)
M1 AML myeloblasts (BM) %	43.4 ± 6.5	37.9 ± 4.0	18.7 ± 6.9	56.6
M2 AML myeloblasts (BM) %	30.1 ± 10.0	46.6 ± 3.3	23.3 ± 8.3	69.9
CML myeloblasts (BM) %	26.6 ± 6.5	45.9 ± 15.9	27.5 ± 11.5	73.4

% Mean and standard deviation based on 20 (CLL, CML) or 40 and more (B-ALL, AML) measured agranular blast cells (lymphoblasts or myeloblasts) without the cell morphological alteration in one-cell layer portions of peripheral blood or bone marrow smears. Red lettering – lymphoid cell lineage, black lettering – myeloid cell lineage, (!) marked difference from M2 AML and CML.

Discussion and Conclusion

In conclusion, the nuclear size heterogeneity of early differentiation steps of leukaemic lymphoblasts and myeloblasts in bone marrow smears depends on the various phases of the cell cycle classified according to maximal nuclear diameter. In addition, the computer-assisted measurements of the maximal nuclear diameter are very easy but must be carried out in one-cell layer portions of the bone marrow or peripheral blood smears without deforming cells by the smearing procedure. The largest number of cells such as AML myeloblasts with the smallest maximal nuclear diameter, i.e., in G0/G1 phase of the cell cycle, was noted in patients suffering from M0 AML. The large number of myeloblasts with a small nuclear diameter in G0/G1 AML is in harmony with previous reports on the altered differentiation of AML myeloblasts and may reflect the protraction of the G1 phase of the cell cycle (Craddock and Nakai, 1962; Gavosto et al., 1969; Darzynkiewicz et al. 1980; Andreeff, 1986). In contrast, the prevalent incidence of B-ALL lymphoblasts with the smallest nuclear diameter, i.e., in G0/G1 phase, was not apparent. Moreover, the dominant and characteristic large incidence of small nuclear bodies in resting differentiated mature leukaemic lymphocytes in CLL in the G0/G1 cell cycle phase (Andreeff et al., 1980) supports such suppositions similarly as the small number of lymphoblasts with small nuclear bodies in B-ALL (the present note). On this occasion it should be noted that the simple maximal nuclear diameter measurements of lymphoid or neutrophil early precursors in currently stained bone marrow smears completed and verified numerous notes about the size diversity of these cells in leukaemic patients.

Acknowledgment

The authors would like to express their gratitude to physicians and technicians of the Institute of Hematology and Blood Transfusion for their support of the study.

Competing interests

The authors declare no competing interests.

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