# Original Article

# A Short Retrospective Cytometric Note on Myeloblasts in Patients Suffering from Acute Myeloblastic Leukaemia, Myelodysplastic Syndrome and Chronic Myelocytic Leukaemia: Myeloblasts with a Narrow Cytoplasm without Azurophilic Granules Are Less Differentiated Committed Progenitor Stem Cells

(cytometry / nuclear body in cell space / leukaemic myeloblasts)

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**Abstract. The maximal nuclear and cell body diameters of leukaemic agranular myeloblasts ("type 1" without azurophilic granules) were measured in bone marrow smears of patients suffering from acute myeloblastic leukaemia with minimal differentiation (M0 AML), without and with maturation (M1 and M2 AML), refractory anaemia with excess of myeloblasts (RAEB) and chronic phase of the myeloid leukaemia (CML) to provide more information on the cytoplasmic space estimate occupied by the nucleus. The largest size of the cytoplasmic space occupied by the nucleus in agranular myeloblasts was noted in M0 and M1 AML in comparison with M2 AML or RAEB, and especially with CML. Similarly, agranular myeloblasts with nuclear bodies occupying more than 90 per cent of the cell space were most frequent in M0 and M1 AML (> 50 %), less frequent in M2 and RAEB (~ 20 %) but very rare in CML (~ 5 %). According to electron microscopy, the very narrow cytoplasmic shell surrounding the nucleus in such myeloblasts did not possess any space for structural components characteristic of the granulocytic cell lineage. Thus, the basic morphology of these myeloblasts would correspond to morphological features of a less differentiated committed stem cell. It should be noted that such cells may be easily recognized in currently stained bone marrow or peripheral blood smears.**

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Abbreviations: AML – acute myeloid leukaemia, CML – chronic myeloid leukaemia, FAB – French-American-British classification of acute myeloid leukaemias, M0 – myeloid (myeloblastic) leukaemia with (without) minimal differentiation, M1 – myeloid (myeloblastic) leukaemia with (without) minimal maturation, M2 – myeloid (myeloblastic) leukaemia with differentiation, MDS – myelodysplastic syndrome, MxCellDm – maximal cell diameter, MxNuDm – maximal nuclear diameter, RAEB – refractory anaemia with excess blasts (myeloblasts).

## Introduction

The present study was undertaken to provide additional information on myeloblasts of patients suffering from acute myeloblastic leukaemia with minimal differentiation (M0 AML), without (M1 AML) or with maturation (M2 AML) and refractory anaemia with excess of myeloblasts of the myelodysplastic syndrome (MDS RAEB). Conventionally stained bone marrow and peripheral blood specimens of patients suffering from acute myeloblastic leukaemias and myelodysplastic syndromes are characterized by the incidence of three types of myeloblasts (Mufti et al., 2008; Roquiz et al., 2016a, b; Invernizzi, 2020). In the first type of myeloblasts, nuclear bodies are large and surrounded by a narrow cytoplasmic shell. In the second type, the cytoplasmic shell surrounding the nucleus is larger and may possess a few azurophilic granules. The third type resembles more differentiated granulocytic precursors with larger cytoplasmic space with azurophilic granules. According to classical haematology, the two latter types with azurophilic granules might be considered to represent a further differentiation step, i.e., early promyelocytes (Naegeli, 1931; Whitby and Britton, 1947; Undritz, 1972; Bessis, 1973; Cline, 1975; Marmont et al., 1981; Laszlo and Rundles, 1983).

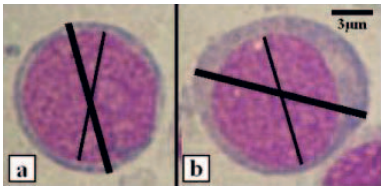


*Fig. 1*. M2 AML myeloblasts without azurophilic granules. (**a)** "Small" myeloblast characterized by a very large nuclear body surrounded by a very narrow cytoplasmic shell. MxCellDm – thick line (≈ 12.2 μm), MxNuDm – thin line (≈ 11.1 μm). The cell space estimate occupied by the nucleus = 90.9 %. (**b**) "Large" myeloblast with a larger proportion of the cytoplasm. MxCellDm – thick line (≈ 14.2 μm), MxNuDm – thin line (≈ 12.1 μm). The cell space estimate occupied by the cell nucleus = 85.2 %.

The first type of "agranular" myeloblasts without azurophilic granules (Fig. 1) was studied in bone marrow smears stained with current haematological May-Grünwald-Giemsa-Romanowsky panchromatic procedure (MGGR) using computer-assisted maximal nuclear (MxNuDm) and cell diameter (MxCellDm) measurements. In addition, these myeloblasts were also studied in the chronic phase of chronic myeloid leukaemia (CML). In comparison with myeloblasts of acute myeloid leukaemias, these cells are characterized by the full differentiation potential (Bessis, 1973; Cline, 1975; Cáteres-Cortés, 2013). According to results of the present study, the largest cell space occupied by the nucleus in agranular myeloblasts was noted in M0 and M1 AML. Agranular myeloblasts with nuclear bodies occupying more than 90 per cent of the cell space were frequent in M0 and M1 AML, less frequent in M2 AML or RAEB but very rare in CML. In the electron microscope, the narrow cytoplasmic shell surrounding the nucleus of such myeloblasts did not possess any structures specific for the granulocytic cell lineage.

## Material and Methods

Maximal nuclear and cell body diameters of agranular myeloblasts stained with the current haematological MGGR procedure (Undritz, 1972) were measured using the "Quick Computer Photoprogram" (Olympus, Tokyo, Japan) in bone marrow smears of two patients suffering from acute myeloblastic leukaemia with minimal differentiation (M0 AML), three patients suffering from acute myeloblastic leukaemia without maturation (M1 AML), four patients with acute myeloblastic leukaemia with maturation (M2 AML) and four patients with refractory anaemia of the myelodysplastic syndrome (MDS RAEB). For comparison, nuclear and cell body maximal diameters of myeloblasts were also measured in four patients suffering from the chronic phase of CML. The bone marrow samples of the studied patients were originally taken for diagnostic purposes, with the supervision and approval of the Institute authorities. The 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 roughly estimated the approximate proportion of the cell body space occupied by the nucleus (Smetana et al., 2021), presuming the nuclear and cell conformity in the smear preparations (Tocco et al., 2018). The diameter data for statistical evaluation were collected and summarized from measurements of myeloblasts that were not compressed and damaged by the smear preparation.

For the electron microscopy, bone marrow samples of acute myeloblastic leukaemias (M1 and M2 AML) were fixed in glutaraldehyde and/or osmium tetroxide, post-fixed in uranyl acetate during dehydration and embedded in the epoxy resin. Then ultrathin sections were stained with uranyl acetate and/or lead citrate (Smetana, 1970).

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 the Primer of Biostatistic Program, version 1 developed by S.A. Glantz (McGraw-Hill, Canada, 1968).

## Results

The rough estimate of the cell space occupied by the nucleus in M0 or M1 and M2 AML agranular myeloblasts was larger than in MDS RAEB, and especially in CML (Table 1). In M0 and M1 agranular myeloblasts, the cell space occupied by the nucleus appeared to be larger than in M2 AML despite the large variation. It should also be mentioned that the incidence of agranular myeloblasts with the decreased size of the nuclear body in the cell space was more frequent in M2 AML (64.8 ± 7.7 %), MDS RAEB (77.7 ± 1.6 %) and CML (95.3 ± 5.6 %).

*Table 1. The percentage of the cell space occupied by the nuclear body of myeloblasts (type 1) and the percentage of such myeloblasts with the nucleus occupying more than 90 % of the cell space*

| Patients | Cell space | Myeloblasts containing > 90 % of the cell space |
|---|---|---|
| | occupied by the nucleus | |
| M0 AML | 89.6 ± 0.8 (0.08) | 57.7 ± 3.2 (0.05) |
| M1 AML | 88.6 ± 2.3 (0.25) | 54.4 ± 14.4 (0.26) |
| M2 AML | 85.6 ± 1.7$^{\#}$ (0.01) | 34.5 ± 8.2$^{\#}$ (0.23) |
| RAEB MDS | 83.9 ± 1.4$^{\S}$ (0.01) | 22.5 ± 1.7$^{\S}$ (0.07) |
| CML | 82.3 ± 2.0$^{\S}$ (0.02) | 4.1 ± 4.9$^{\S\S}$ (> 1.1) |

* – Mean and standard deviation measured in more than 40 myeloblasts of two patients suffering from M0 AML, 60 myeloblasts of three patients with M1 AML, four patients with M2 AML or with RAEB MDS and in 50 myeloblasts of four patients with the chronic phase of $Ph^+$ CML. The measurement was carried out in cells without alteration due to the smearing. Numbers in brackets represent the variation coefficient. $^{\#}$ – significantly different from M0 and M1 AML, $^{\S}$ – significantly different from M0, M1, M2 AML, $^{\S\S}$ – significantly different from M1, M2 AML and RAEB MDS using *t*-test ($2\alpha = 0.05$).

The histograms based on the cell space occupied by the cell nucleus in agranular myeloblasts provided surprising information (Table 1). Nuclear bodies occupying more than 90 per cent of the cell space (Fig. 1a) were noted in agranular myeloblasts of all patients with M0, M1, M2 AML or MDS RAEB. The incidence of such myeloblasts appeared higher in patients with M0 or M1 AML in comparison with patients suffering from M2 AML and MDS RAEB. In patients suffering from CML, the incidence of agranular myeloblasts was very rare (~ 4 %) and variable, as indicated by a very high standard deviation and variation coefficient (Table 1). It seems to be also important that both light and electron microscopy demonstrated that the narrow cytoplasmic shell around the nucleus of such myeloblasts did not possess any structures characteristic of the granulocytic lineage such as azurophilic or specific granules (Figs. 1a and 2).

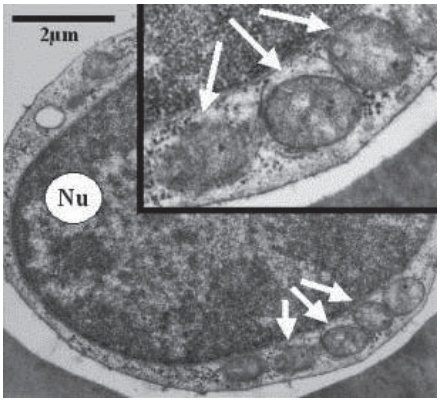


*Fig. 2*. Selected electron micrograph of a sectioned myeloblast that was characterized by a very narrow cytoplasmic shell surrounding the nucleus occupying more than 90 % of the cell body space. The narrow cytoplasmic shell does not contain any specific structural components characteristic of the granulocytic cell lineage. Nu – nucleus, mitochondrial bodies – white arrows. Magnified cytoplasmic rim with mitochondrial bodies – insert. Fixation with osmium tetroxide, post-fixation with uranyl acetate during dehydration in ethanol, staining with uranyl acetate followed by lead citrate.

## Discussion

According to the results of the present measurements, the cell space occupied by the nuclear body in agranular myeloblasts in M0 and M1 AML was larger than in M2 AML or RAEB MDS, and particularly in CML. On this occasion, it should be mentioned that myeloblasts especially in M0 and M1 AML carry stem cell markers in addition to myeloid antigens (Kawthalkar, 2006). On the other hand, agranular myeloblasts with nuclear bodies occupying more than 90 per cent of the cell space were very frequent in patients suffering from M0 or M1 AML (> 50 %) and less frequent (> 20 %) in patients with M2 AML or MDS RAEB. The presence of such myeloblasts in MDS RAEB patients was not surprising because this haematological disorder might precede the M2 AML (Malcovati et al., 2006). The very rare incidence of these myeloblasts in CML patients might be related to the potential transformation of the chronic to the acute "blastic" phase (Bornstein et al., 1972, Calabretta and Perrotti, 2004). Actually, the incidence of myeloblasts with the high nuclear cytoplasmic ratio and scanty agranular cytoplasm seems to be characteristic of the "blastic" phase of CML (Invernizzi, 2020).

It seems to be likely that the very small and narrow cytoplasmic shell surrounding the nuclear body in agranular myeloblasts with the nuclear bodies occupying more than 90 per cent of the cell space does not provide a satisfactory space for the cytoplasmic components characteristic of cell differentiation into the granulocytic cell lineage. Thus, such cells might be considered as less differentiated committed stem cells. Electron micrographs of ultrathin sections of agranular myeloblasts with the small cytoplasmic shell in the present study (Fig. 2) were in harmony with such interpretation of the light microscopy (Anderson, 1966; Marmont et al., 1981; Laszlo and Rundles, 1983). The cytoplasmic basophilia, due to the presence of RNA (light microscopy) or ribosomes (electron microscopy), just reflected a low differentiation state and immaturity of such myeloblasts, similarly as in stem cells including "micro-myeloblasts" described in the classical haematological literature (Naegeli, 1931; Anderson, 1966; Undritz, 1972; Bessis, 1973; Marmont et al., 1981). Actually, myeloblasts and Ferrata's haemocytoblasts with a narrow cytoplasmic shell surrounding the nucleus without any specific structure represented the precursor cells for granulocytes (Naegeli, 1931). In addition, the low microscopic magnification of some "pro-neutrophil progenitors" (Kwok et al., 2020) also resembled agranular myeloblasts with the very narrow cytoplasmic rim in the present study.

Additional interpretation of the presence of agranular myeloblasts (type 1) with a narrow cytoplasmic shell around the large nucleus in M0, M1 or M2 AML and MDS RAEB would be difficult. However, there is a possibility that such myeloblasts might be in the postmitotic phase or in the state of altered and immature terminal differentiation. The large nuclear size and/or small surrounding cytoplasmic shell characteristic of the postmitotic state would be in harmony with such supposition (Saunders et al., 1967; Nagl, 1976; de Robertis and de Robertis, 1987). Myeloblasts with large nuclei and a minimal cytoplasmic shell might also represent a nonproliferative cell pool (Cline, 1975). The incidence of resting dominant ring-shaped nucleoli in some of AML myeloblasts and altered differentiation of AML myeloblasts would be in harmony with such speculations (Smetana et al., 1998; Cáteres-Cortés, 2013).

Agranular myeloblasts with a smaller proportion of nuclear bodies in the larger cytoplasmic space in myeloblastic AML or RAEB might be in the S and G2 phases (Fig. 1b). Such myeloblasts might represent cycling cells

with the differentiation potential. They resembled those in CML with the mitotic or differentiation potential and were also characterized by a smaller heterochromatin condensation state (Cline, 1975; Smetana et al., 2015; Smetana, 2020). The mitotic potential with a looser heterochromatin condensation state in some AML myeloblasts was also reported previously (Saunders et al., 1967; Cline, 1975; Minden et al., 1978; Smetana et al., 2015; Smetana, 2020).

Our retrospective analysis highlights the complexities inherent in the diagnostic process of myeloid neoplasms, where distinguishing between the various stages of myeloblast differentiation can be particularly challenging (Orazi, 2007; Xiao et al., 2024).

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### *Competing interests*

The authors declare that no competing interests exist.

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