CAN AGNOR COUNTS AND CONFIGURATION PREDICT COMPLETE REMISSION IN ADULT ACUTE MYELOGENOUS LEUKEMIA PATIENTS?

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Abstract: The analysis of the AgNORs was performed in patients with acute myeloid leukemia (AML) to verify the role of AgNOR parameters in predicting complete remission (CR). Bone marrow aspirates from 24 patients with AML were stained with silver nitrate and underwent morphological, immuophenotypic, and genetic assessment. The mean AgNORs number, mean AgNORs area, and the mean AgNOR area-to-nucleus-area ratio were calculated for each case. After induction therapy, patients who achieved complete remission (CR) received intensive consolidation treatment. Fifteen patients underwent allogenic bone marrow transplantation. A higher mean AgNOR area-to-nucleus-area ratio was found in group with the CR status.

Key words: acute myeloid leukemia; argyrophilic nucleolar organizer regions; complete remission.

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Introduction

Proliferation and accumulation in the bone marrow of neoplastic blast cells leads to the development of acute myeloid leukemia [1]. The assessment of genetic alterations of a leukaemia clone using classical cytogenetics and molecular methods is a basic element of the diagnosis of acute leukemias. It is also the basis for their classification and provides information on the prognosis and possible therapeutic goals [1,2,3]. The prognostic factors in acute myeloid leukemias can be divided into those that are patient-dependent (which determine treatment tolerance and treatment-related early mortality) and factors that depend on the characteristics of the leukemic clone (which determine chemotherapy resistance). The prognostic factors dependent on the leukemic clone are cytogenetic and molecular abnormalities. The classification developed by the European LeukemiaNet (ELN) distinguished three prognostic groups. In clinical practice, the ELN is used to assess the risk [4]. In case of failure of cytogenetic analysis (lack of appropriate material for analysis or its insufficient amount, non-sterile material collection), prognostic assessment is impossible. The nucleolus organizing region (NOR) is the segment of the chromosome in which genes for the major ribosomal RNAs are found. A specific group of acidic, highly silver-absorbing, non-histone protein complexes located in the same places as the NOR allows for the precise and quick visualization of the nucleolus organizing regions by staining with silver nitrate [5,6,7]. Silver-stained NOR is referred to as AgNOR, and silver-staining NOR proteins as AgNOR proteins. Nikicz and Norback were the first to use the silver staining method to visualize NOR in bone marrow cells in healthy people. This study determined the applicability of AgNOR staining in the differential analysis of bone marrow hematopoietic cells [8]. Pich A. et all showed that AML patients who achieved the complete remission after induction course showed a greater amount of AgNOR in the blastic cells. The amount of AgNOR was correlated with the duration of remission: the higher amount of AgNOR – the longer duration of remission [9]. The above mentioned analysis of AgNOR in blasts cells is one of the last publications. In this study, we analyzed parameters of AgNOR in blast cells of 24 AML patients. The work was aimed to verify its role in predicting complete remission (CR).

Materials and Method

Group Characteristics

The study group consisted of 24 patients diagnosed, hospitalized and treated in the Department of Internal Medicine and Haematology between 2017 – 2021. Characteristics of the study group are presented in Table1 and Table 2.

Table 1. Characteristics of the study group.

	No (range)
Whole series	24
Age (range)	46,0 (20 – 64)
Sex (m/f) No [%]	10/14 (41/59)
WBC G/l (range)	49 (1,1 – 352)
Hgb g [%] (range)	9,4 (7,8 – 12,5)
PLT G/l (range)	68 (12 – 213)
Blasts in pb [%] (range)	36,8 (1 – 92)
Blasts in bm [%] (range)	56,7 (20 – 93)
FAB classification M0 M2 M4 M5	 2 13 7 2
Cytogenetic risk ELN2 ELN3	 16 8

Abbreviation: m – male, f – female, WBC – white blood cell count, hgb – hemoglobin, PLT – platelets, Bm – bone marrow, FAB – French-American-British, pb – peripheral blood, ELN – European LeukemiaNet.

All patients were treated according to the protocols of the Polish Adult Leukemia Group (PALG). The individual chemotherapy courses used in treatment are presented in Table2.

Table 2. Characteristics of the study group.

	CR(-)	CR(+)
ELN2	6	8
ELN3	5	2
Inductjon therapy (all patients)	daunorubicin 60mg/m2 i.v 1-3 days cytarabine 200mg/m2 i.v 1-7 days cladribine 5mg/m2 i.v 1-5 days (DAC)	
Re-induction therapy	cladribine 5mg/ m2 i.v 1-5 days, cytarabine 2000mg/ m2 i.v 1-5 days, mitoxantron 10mg/ m2 i.v 1-3 days	
Consolidation therapy (all patients)	cytarabine 2-3g/m2 i.v. on 1,3,5 day (HD ARaC) cytarabine 1.5g/m2 i.v. 1-3 days and mitoxantone 10mg/m2 on 3 and 4 day (HAM)	

Abbreviation: ELN – European LeukemiaNet, CR – complete remission.

AgNOR analysis

1. Preparation of bone marrow aspirate for analysis
   a) smearing marrow aspirate on a glass slide
   b) drying at room temperature
   c) immersion in ethyl alcohol for 10 minutes
   d) rising in distilled water

2. AgNOR staining
   a) preparing solution A 2% solution of gelatin dissolved in distilled water, to which formic acid was added until 1% concentration of final solution was obtained
   b) preparing solution B
      50% solution of silver nitrate in distilled water

The preparation was then stained for 20 minutes in a solution obtained by immediate, fast mixing of one volume of solution A with two volumes of solution B. The preparations were placed for 10 minutes in a 5% sodium thiosulfate solution, and rinsed in distilled water.

3. Analysis of AgNOR parameters
   a) Slides were evaluated under an optical microscope (Olympus BX51 microscope, MDOB3 model, Tokyo, Japan). The total magnification was x1000.
   b) 200 blast cells were analyzed using a computerized image analysis system called cell* Soft Imaging System (Germany) and by the Microsoft Excel program.
   c) The nucleus and each selected AgNOR structure were outlined for each selected blast cell.
   d) The AgNORs parameters were measured: the mean number of AgNORs in the nucleus, the mean surface of AgNORs, and the ratio of AgNOR surface to cell nucleus surface.
   e) The data were calculated using the author's program and Excel.
   f) The structure and patterns of AgNOR in bone marrow blastic cells were classified according to the system developed by Nikicz, with author's modification. The modification consisted in differentiation AgNOR depending on surface size.

Statistical Analysis

The Python 3.8 and Statistica 13.3 software (Statsoft, TIBCO Software Inc., Dell Inc.,Plo Alto, CA, USA) were conducted. The $p < 0.05$ was set at statistical significance level.

Results

AgNOR parameters were calculated in leukemic cells in all patients with AML.

Figure 1. AgNOR staining in bone marrow smears of a patient with acute myeloid leukemia M2 (ELN2) with complete remission of CR (+).

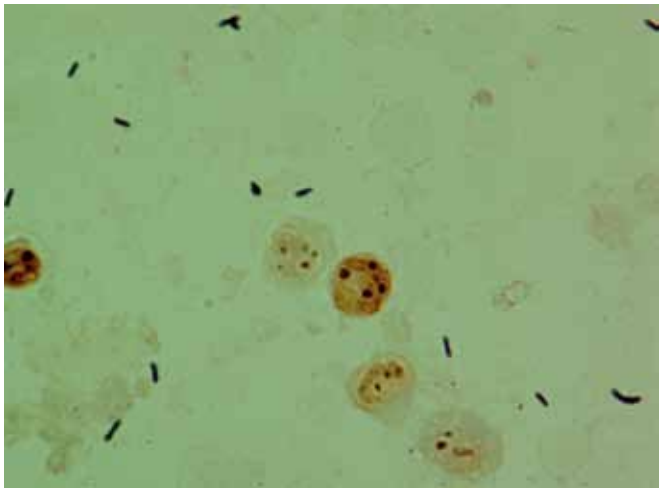
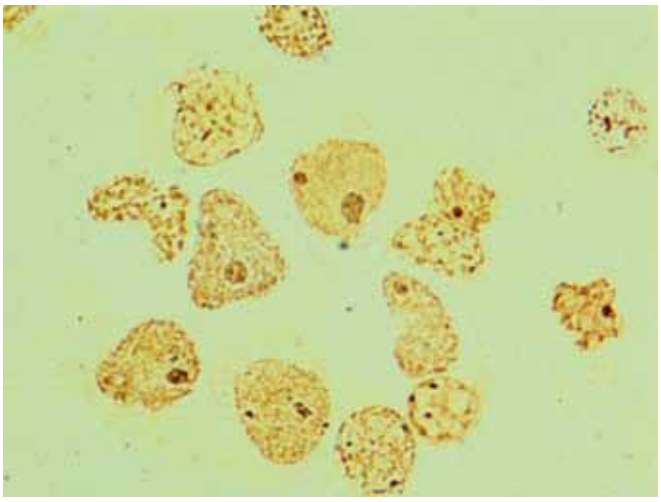


Figure 2. AgNOR staining in bone marrow smears of a patient with acute myeloid leukemia M4 (ELN3) without CR (-).



Significantly higher values of mean ratio of the AgNOR surface area to the nucleus surface area were found in patients who achieved CR compared to the group without this status (p= 0.02) (Table 3, Figure 3).

Table 3. AgNOR indexes in AML depending on remission status: the mean number, mean surface area, and mean ratio of AgNOR surface area to nucleus surface area, expressed as percentages.

AgNOR Cell Indexes	CR(+)	CR(-)	p
Mean number of AgNORs	(n=10)	(n=11)	
Mean surface of AgNORs [um2]	2.79+/-0.61	2.22+/-0.34	0.28
AgNOR surface/ nucleus surface[%]	14.44+/-3.31	13.77+/-3.74	0.06

Abbreviation: AgNOR – argyrophilic nucleolar organizer regions, CR – complete remission.

Figure 3. AgNOR indexes differences between patients who achieved CR and patients without CR: the mean number, mean surface area, and mean ratio of AgNOR surface area to the nucleus surface area.

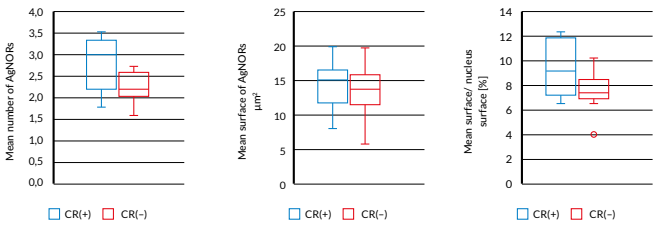
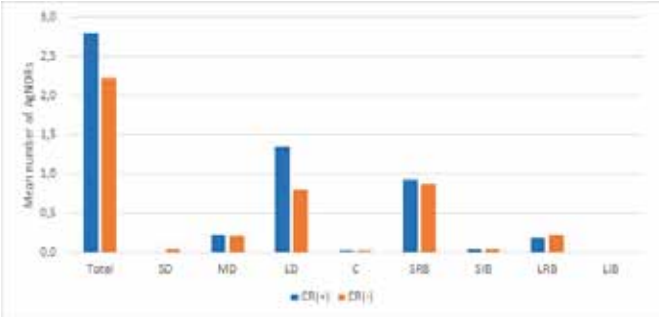
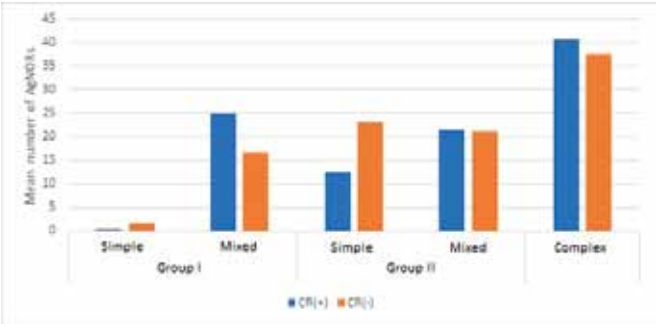


Figure 4. Presentation of particular structures dependent on remission status.



No statistically significant differences were found in the mean number of AgNOR in particular structures in AML depending on the remission status (Figure 4).

Figure 5. Presentation of the configuration of particular structures depending on remission status.



There were no statistically significant differences in group I among simple (p = 0.77) and mixed (p = 0.49) structures in the group of patients without remission in AML compared to patients who did not achieve this remission. Also in group II, no statistically significant differences were found among the simple (p = 0.54) and mixed (p = 0.33) structures in both groups (Figure 5).

Discussion

In our research the mean number of AgNORs, the mean surface area of AgNORs, and the ratio of AgNOR surface area to nucleus surface area were measured. Only in the case of the last parameter, our research showed a statistically significant difference between both groups. We observed a higher value of the mean number of AgNOR in the group of patients who achieved the CR status, but the difference was not statistically significant. Pich et all showed that in adult patients with acute myeloid leukemia who achieved disease remission, a higher content of AgNOR in the cell was obtained compared to patients without remission. The amount of AgNOR was correlated with the duration of remission: greater amount of AgNOR – longer duration of remission. These results suggested that patients with more AgNOR leukemia clones responded more easily to treatment. In contrast, in patients with low levels of AgNOR resistance to chemotherapy has been observed [9].

In the above-mentioned publication, an analysis was carried out in 40 patients with de novo AML. Cytogenetic analysis was done in 26 patients (chromosomal abnor-

malities were found in less than half of them). According to the literature available at that time, cytogenetic alterations were categorised in three prognostic groups: low (two patients), intermediate (17 patients), and high-risk group (seven patients) [10,11]. There was no relationship between AgNOR and cytogenetics. Patients in our study were classified according to ELN. Cytogenetic and molecular evaluation before the start of the treatment in patients with AML before starting treatment allows to plan it (including planning allo-hematopoietic steam cell transplant [allo-HSCT]). When comparing the two groups in terms of CR status depending on cytogenetic and molecular risk, it is difficult to make an ambiguous conclusion as to the chance of achieving remission depending on cytogenetic and molecular risk.

In our study the AgNOR classification proposed by Nikicicz and Norback for the analysis of normal bone marrow was used in our study [8]. Our analysis in relations to leukemic cells did not show any differences in all AgNOR structures and configuration. This may be due to the relatively small number of patients in groups compared in this study. We have no possibility of comparing our data with other studies.

The assessment of genetic disorders in the leukemic clone is an important element in the diagnosis of acute leukemias and is important in the assessment of patient's prognosis. The analysis of various AgNOR structures in acute leukemias can easily identify risk groups without the need for genetic tests.

References

1. Arber D. A, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia, Blood 2016; 127: 2391-2405
2. Kelly L. M, Gillilland D. G, Genetics of myeloid leukemias, Annu Rev. Genomics Hum. Genet, 2002; 3: 179-198
3. Cancer Genome Atlas Research Network: Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia, N. Engl, J.Med, 2013; 368: 2059-2074
4. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel, Blood, 2017; 129: 424-447
5. Derenzini M, The AgNORs, Micron, 2000; 31, 117-120
6. Egan M. J, Crocker J, Nucleolar organizer regions in pathology, Br J Cancer, 1992; 65, 1-7
7. Lee W, Kim Y, Lee KY i wsp. AgNOR of human interphase cells in relation to acrocentric chromosomes, Elsevier Science Ltd, 1999
8. Nikicz E. P, Norback D. H, Argyrophilic nucleolar organizer region (AgNOR) staining in normal bone marrow cells, J. Clin. Pathol, 1990; 43, 723-727
9. Pich A, Chiusa L, Audiso E, Marmont F, Nucleolar organizer region counts predict complete remission, remission duration, and survival in adult acute myelogenous leukemia patients, J. Clin. Oncol, 1998; 16, 1512-1518
10. Rowe J. M, Liesveld J. L, Treatment and prognostic factors in acute myeloid leukemia, Bailliere's Clinical Haematology, 1996; Vol 9, No 1, 87-105
11. Mrozek K, Heinonen K, de la Chapelle A, et al. Clinical significance ofcytogenetic in acute myeloid leukemia, Semin Oncol, 1997; 24: 17-31