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PEROXIDASE NEGATIVE ACUTE MYELOID LEUKEMIA WITH A DIFFUSE OR GRANULAR FORM OF GLYCOGEN IN BLAST CELLS. CASE REPORT

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We report a case of peroxidase negative acute myeloid leukemia (AML) with a diffuse or granular form of glycogen in leukemia cells by the periodic acid-Schiff (PAS) staining. A 21-year-old woman was admitted to our hospital because of a 4 week history of febrile episodes. Her blood counts revealed pancytopenia and bone marrow examination showed hypercellularity with 70% peroxidase negative blasts which were positive for CD45, CD34, CD7, CD13, CD33, CD117, and CD11b, but negative for cMPO, and lymphoid markers. Conventional cytogenetic analysis revealed 38, X, -X, -5, -7, -13, -16, -17, -19, -20, del(9)(p12p22) [20]. The leukemic cells did not have FLT3-ITD mutation. She was diagnosed AML-M0 according to FAB classification. However, PAS stain was strongly positive with diffuse and granular pattern in the leukemic cells. After administration of chemotherapy, severe infection developed leading to multi-organ failure and death on the 20th day.

Key words: *Acute myeloid leukemia, glycogen, peroxidase, PAS staining, immunophenotyping.*

Introduction

Acute myeloid leukemia (AML) is tumor disease of the hemopoietic system, characterized by uncontrolled proliferation of blast cells with myeloid lineage marker in peripheral blood and bone marrow. There are 2 classification systems for the diagnosis of AML that are commonly used in the world, the French-American-British (FAB) classification system which is based on morphology and cytochemistry [1], and the World Health Organization (WHO) classification which reviews chromosome translocations and specific immunotypes [2].

AML-M0 (AML with minimal differentiation), which accounts for approximately 5-10% of all AML patients, is relatively common in adult patients [3]. The diagnosis is made if less than 3% of the blasts are positive for peroxidase and if the blasts are positive for the myeloid-associated markers (CD13, 14, CD15 or CD33), and negative for B or T lineage marker (CD3, CD10, CD19 and CD5), (Bennette JM 1991). Almost no mature my-

eloid cells were seen. The blasts were small to medium-sized round cells with an eccentric nucleus. The nucleus often had a flattened shape and contained fine chromatin with several distinct nucleoli. The cytoplasm was lightly basophilic without granules. Auer rods were not found [3-5]. Prognosis in AML(M0) depends on the genetic research data and blast cells immunophenotype. According to literature data, median survival in AML (M0) is 9-11 months. (A. Okorokov).

Normally glycogen appears at the level of myeloblast, glycogen is diffusely distributed in cytoplasm and its amount increases with maturation of the cells. In mature neutrophils glycogen contains in the form of fine granules. Sometimes glycogen may be contained in a finely-granular form in myeloblasts and in granular form in lymphoblasts [6]. Pattern of PAS reaction was considered important in literatures to differentiate between ALL (with clear cytoplasm between the positive granules) and AML (with cytoplasmic smudge positivity between the posi-

tive granules) [7]. Although some authors (Gamal Abdul-Hamid (2011) note that the PAS diagnosis adds a minor support to diagnosis of ALL as all similar reaction can be seen, although less frequently in AML with maturation [5].

The peculiarity of this case is unusual reaction to glycogen disclosed in blast cells with minimal differentiation by staining PAS. Glycogen is found in large quantities in various forms in 98% of blast cells. We had a negative reaction to myeloperoxidase and Sudan black, which confirms that the blasts are minimally differentiated. The nature of such a severe PAS reaction or the association with AML (M0) is unclear.

Case description

A 21-year-old woman was admitted to our hospital because of a 4 week history of febrile episodes. HIV test was negative. The deterioration of health has been noted over the last month: weakness, dizziness, headache, nausea. Significant deterioration began a week before admission to our Center, where there was a painful formation in the submandibular area, pain in the mouth, fever up to 39°C. Although multiple hyperplastic lymph nodes up to 2,0 cm were found in the left submandibular area by ultrasound examination, the abscess formation was not obtained. There was no hepato-splenomegaly or skin rash. Bleeding tendency was not seen at the admission.

Laboratory findings of peripheral blood revealed pancytopenia, which showed WBC $0,8 \times 10^9/l$, RBC $2,1 \times 10^{12}/l$, HGB 65,2 g/l, PLT $75,4 \times 10^9/l$, BLAST 3%, BAND 2%, NEU 3%, MONO 6 % LYMPH 86 %. Bone marrow findings revealed hypercellular marrow with 70,1% of blast cells. Blast cells are predominantly medium sized with nuclear-cytoplasmic ratio from moderate to high, a mesh structure of the nuclear chromatin, nucleoli in the majority of blasts, the cytoplasm in moderate quantity, moderately basophilic. Sometimes, there has been marked vacuolization of the cytoplasm. Erythro-karyocytes were single. There was no dysplasia in each lineage.

Cytochemistry tests revealed that myelo-

peroxidase and Sudan black were negative in blast cells, glycogen was diffusive positive in 28 % of blast cells, diffusive-granular positive in 57 % of blast cells, and the granular form positive in 13 % of blast cells.

Immunophenotyping of bone marrow cells were performed by flow cytometry.

In the investigated sample of marrow there were defined transformed cells with phenotype: CD45dim, CD34++, CD7+, CD33+++, CD13++, CD117+++, CD11b+++, HLA-DR-, CD56-, cMPO-, cTdT-, cCD79a-, cCD3-, GPA-, CD36-, CD61-, CD42b-, CD71-, CD38-, CD15-, CD14-, CD64-, CD5-, CD4-, CD8-, CD2-CD22-, CD10-, CD19-, CD20-, which showed that blastic cells by phenotypic profile could correspond to myeloid stem cells.

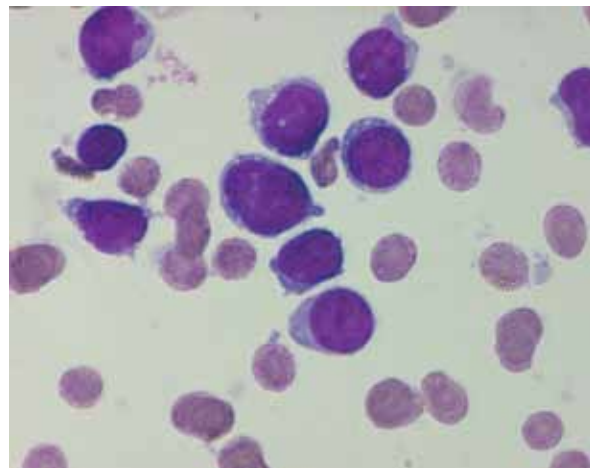


Figure 1 – A smear of bone marrow. The preparation is painted by Romanovsky-Giemsa

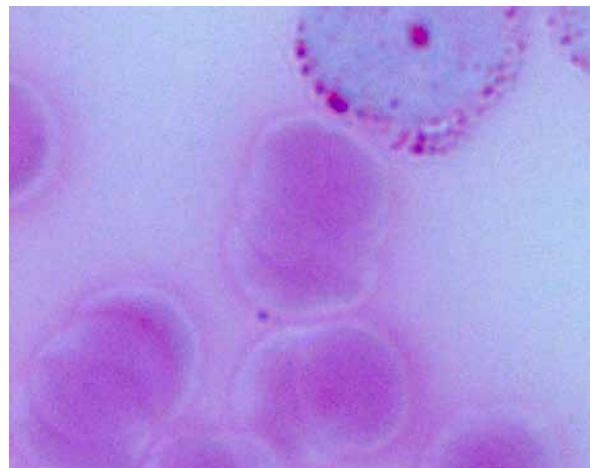


Figure 2 – Bone marrow. PAS-reaction in the granular form of blast cells

Conventional cytogenetic analysis of bone marrow revealed 38, X, -X, -5, -7, -13, -16, -17, -19, -20, del(9)(p12p22) [20]. FLT 3 ITD mutation was negative by molecular analysis.

Based on the above, a primary diagnosis has been set: AML-M0 by FAB-classification or AMLa with minimal differentiation by WHO classification. A course of chemotherapy including cytosine arabinoside for 7 days and daunorubicin for 3 days were initiated. In spite of intensive antibiotic therapy, a septic shock developed after chemotherapy leading to multi-organ failure and death on day 20 after the admission.

Discussion

Comprehensive diagnosis of tumors of hematogenous origin according to WHO classification includes: morphological, cytochemical, immunophenotypic, cytogenetic, molecular- biological research. All clinical information and biological characteristics to extract the specific variant of the disease are considered. It is considered that a multimodal approach provides an accurate diagnosis by increasing the overall specificity and sensitivity of individual diagnostic approaches for adequate diagnosis.

Our case of acute leukemia can be classified as AML with minimal differentiation according to WHO classification [2] and AML-

M0 subtype according to FAB classification [1]. Early stage of AML-M0 diagnosis is set on the basis of the negative results of all cytochemical stains of blast cells and / or on the basis of immunophenotyping [8].

Nature of malignantly transformed cells in certain forms of hemoblastosis in many cases can be set with a reasonable degree of accuracy based on the application of cytochemical methods, as shown by many authors, confirming the existence of the fundamental chemical and metabolic differences which were distinct in degrees of maturity of transformed cells of myeloid and lymphoid origin. [6, 7, 11] Cytochemical features of blasts underlying the differential diagnosis of acute leukemias are shown in Table 1.

Tests for Periodic acid-Schiff (PAS)-positivity (indicating mucopolysaccharides) in blasts have been performed using commercial diagnostic kits (Sigma, Australia). Under the influence of potassium periodate glycogen is oxidized with the formation of aldehydic compounds reacting easily with Schiff's reagent (fuchsin-sulfurous acid). In the areas of localization of glycogen a cherry-purple staining has been detected, according to the intensity of which it is possible to judge on the number of glycogen in the cells [9, 10, 11]. The received data on the cytochemical studies have been regarded as a defect in the accumu-

Table 1 – Cytochemical findings in acute leukemia

Form of acute leukemia	Peroxi- dase	Lipids	PAS-reaction	Non-specific esterase	Chloroacetate- esterase	Acid phosphatase
Blast embryonal leukemia	(-)	(-)	(-)	(-)	(-)	(-)
Lymphoblastic leukemia	(-)	(-)	(++) large granular	(±)not inhibited NaF	(-)	sometimes (+ +) diffuse
Myeloblastic leukemia	(++)	(++)	(++)diffuse	(+)not inhibited NaF	(++)	(++)
Myelomonoblastic leukemia	(+)	(+)	(++) fine granular	(+++) not inhibited NaF	(-)	(+++)
Monoblastic leukemia	(±)	(±)	(+)fine granular	(+++) inhibitedNaF	(-)	(+++)
Promyelocytic leukemia	(+++)	(+++)	(+++)	(+++) not inhibited NaF	(+++)	(+++)
Erythroleukemia (blast cells)	(++)	(++)	(++) diffuse	(+)	(++)	(++)
Erythroleukemia (erythro normoblasts)	(-)	(-)	(++) diffuse or large granular	(++)	(-)	(++)

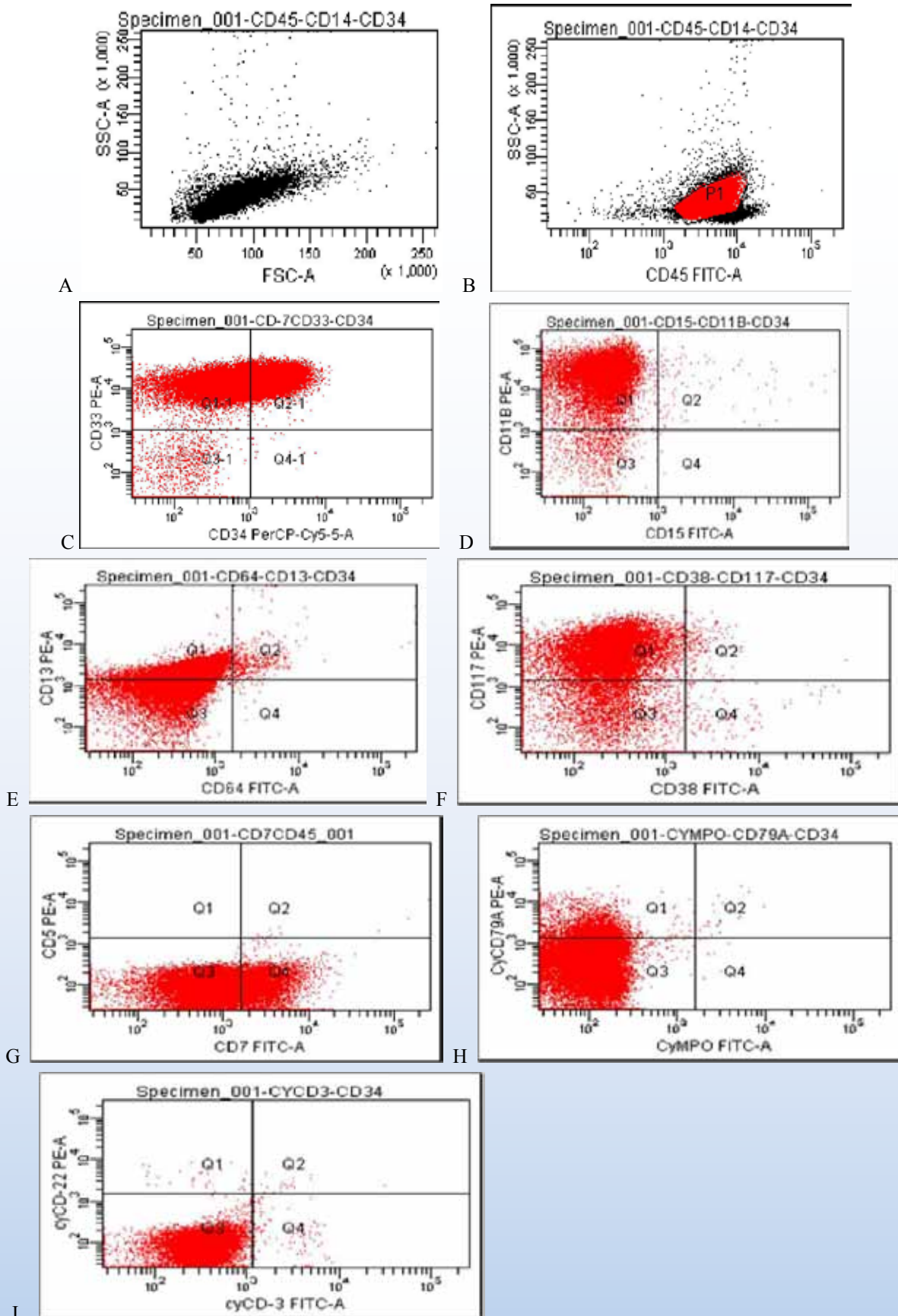


Figure 3 – Bone marrow immunophenotyping. Release of gate blast cells by expression of indicators CD45 and side light scattering SSC (B) and by parameters of channel scattering (A). Image analyzed markers of Dot plot (C-I)

lation of glycogen in the cells non-specific for AML. For a more accurate determination of nature of abnormal cells there has been used the method of flow cytometry. For immunophenotyping of transformed cells there has been applied a panel of monoclonal antibodies created by the recommendations of the members of the Euroflow consortium, covering all the hematopoietic lineages [12]. The antigenic profile identified on blast cells was more correspondent to the myeloid stem cell according to EGIL-95 (Bene M.C., et al) classification and literary sources on this theme. [8, 13, 14].

In summary, we report a case of peroxidase negative AML-M0 with a diffuse or granular form of glycogen in leukemia cells by PAS staining. Although the question whether atypical reaction to glycogen is a predictor of the risk of some complications or response against chemotherapy in acute myeloid leukemia remains open, this finding may be related to the dysregulation of a glycogen storage caused by a genetic abnormality accompanied with leukemogenesis.

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ПЕРОКСИДАЗА – НЕГАТИВНЫЙ ОСТРЫЙ МИЕЛОИДНЫЙ ЛЕЙКОЗ С ДИФфуЗНЫМ И ГРАНУЛЯРНЫМ ГЛИКОГЕНОМ В БЛАСТНЫХ КЛЕТКАХ

Мы сообщаем о случае отрицательного по пероксидазе острого миелоидного лейкоза (ОМЛ) с диффузным и гранулярным гликогеном в лейкозных клетках, окрашенных с использованием Шифф (PAS) реакции.

21-летняя женщина поступила в нашу больницу из-за 4-х недельного фебрилитета. В крови отмечалась панцитопения. В исследовании костного мозга – гиперцеллюлярность с 70% пероксидаза-негативных бластов, которые были положительны по CD45, CD34, CD7, CD13, CD33, CD117 и CD11b, но негативны по суMPO и лимфоидным маркерам. Цитогенетический анализ показал 38, X, -X, -5, -7, -13, -16, -17, -19, -20, del(9)(p12p22) [20]. В лейкозных клетках мутаций FLT3-ITD, NPH не выявлено. Был диагностирован ОМЛ (M0) согласно FAB-классификации. Однако PAS реакция была резко положительна в диффузной и гранулярной формах в бластных клетках. После проведения цитостатической химиотерапии, развившаяся тяжелая инфекция привела к полиорганной недостаточности и смерти пациента на 20-й день.

Ключевые слова: острый миелоидный лейкоз, гликоген, миелопероксидаза, PAS-реакция, иммунофенотипирование.

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