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IMMUNOCYTOCHEMICAL MARKERS IN ACUTE LEUKAEMIAS DIAGNOSIS

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The study included 1742 patients with acute myeloblastic leukaemias (AML) and acute lymphoblastic leukaemias (ALL), Kyiv city residents and patients from 20 regions of Ukraine. Bone marrow and blood smears were sent at diagnosis to Reference Center. The analysis was based on May-Grünvald-Giemza (MGG) stain and cytochemical reactions (MPO, acNSE, CAE, AP, PAS). Immunocytochemical techniques (APAAP, LSAB) and broad panel of monoclonal antibodies (MoAbs) against lineage specific and differentiation antigens of leukocytes were employed for immunophenotyping of leukemic blast cells directly in blood and bone marrow smears. Different types of AML were defined by the expression of the cell surface and cytoplasmic antigens. Immunocytochemical study was required especially in diagnosing of AML with minimal differentiation, acute megakaryoblastic leukaemia, acute erythroid leukaemia and acute leukaemias of ambiguous lineage. Acute lymphoblastic leukaemias was broadly classified into B-lineage and T-lineage ALL. According to the degree of B-lymphoid differentiation of the blast cells four subtypes of B-lineage ALL were established. T-lineage ALL observed in patients were also divided into four subtypes. Immunocytochemical examination was required to diagnose AL of ambiguous lineage with no clear evidence of lineage differentiation (acute undifferentiated leukaemia) or those with blasts that express markers of more than one lineage (mixed phenotype acute leukaemias).
***Key Words*: acute leukaemias, diagnosis, immunophenotyping, classification.**

To date cytomorphology has remained the cornerstone of diagnosis and provided the basis for the first widely accepted classification of acute leukemias (AL) that was developed by the efforts of French-American-British (FAB) group of hematologists [1]. Subsequent modification of the FAB classification system [2] has also included cytochemistry and immunophenotypic analyses of blood and bone marrow blast cells. In recent years, cytogenetic and molecular techniques have provided deeper insights into the biology of specific subtypes of AL [3, 4].

According to the new WHO classification the diagnosis of leukaemia should be based on the complex of clinical, morphological and immunophenotypical findings supplemented by the data of molecular biology and genetics of blast cells [5, 6]. In our opinion this classification, must be considered as an up-to-date reflection of the current, still incomplete understanding of biology of different forms of acute myeloid (AML) and acute lymphoblastic (ALL) leukaemia.

AL as well as other tumors of haematopoietic and lymphoid tissues represents the clonal processes. The blast cells infiltrating the bone marrow, which are detected in the peripheral blood and in the tissues, are the descendents of the single hematopoetic stem cell (HSC) or committed progenitor cells (CPC) undergoing the malignant transformation. The recent data suggest that several forms of AML as well as CML develop as a result of mutations accumulated in HSC.

The immunophenotype of these leukaemic stem cells (LSC) in different types of leukaemia was delineated [7, 8]. The suggested immunophenotype of LSC in AML is $CD34^+$ $CD38^-$ $CD90^-$ $CD123^+$ $CD117^-$ $CD71^-$ $HLD\text{-}DR^-$, while in ALL the suggested immunophenotype is $CD34^+$ $CD38^-$ Lin^- $CD10^-$ $CD19^-$. Moreover, a universal phenotype of LSC may not exist and patient-to-patient variations in cell surface antigen expression may be the rule.

Malignant changes rarely occur in HSC prior to its commitment to either the myeloid or lymphoid lineage. ALL is a malignant disorder of B- or T- lymphoid progenitor cells with peak of prevalence between the ages of 2 and 5 years. AML is the most common form of AL in adults, accounting for over 80% of all AL. AML is a heterogeneus group of leukaemias that arise in precursors of myeloid, erythroid, megakaryocytic and monocytic cell lineages. AML and ALL are divided into a number of different subtypes and the correct diagnosis is based upon a wide range of clinical, morphologic methods as well as novel technique of immunophenotyping, cytogenetics and molecular biology.

We believe that the standard panel for classification of AL [5, 6] should be supplemented with several new markers allowing us to identify more precisely the different forms of the disease.

Unfortunately, up to the present, only cytomorphology and several cytochemical techniques were routinely used for the diagnostic purposes in oncohematology in the vast majority of the hematological clinics in Ukraine. In 1993, the Reference Center was set up as a public service at R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine with the aim

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Abbreviations used: AL – acute leukaemias; ALL – acute lymphoblastic leukaemias; AML – acute myeloblastic leukaemias; AP – acid phosphatase; APAAP – alkaline phosphatase-anti-alkaline phosphatase (complex, methods); acNSE – acid non-specific esterase; CAE – naphthol-ASD-chloracetate esterase; CPC – committed progenitor cells; FAB – French-American-British; HSC – hematopoetic stem cell; LSC – leukaemic stem cells; MGG – May-Grünvald-Giemza stain; MoAbs – monoclonal antibodies; MPO – myeloperoxidase; PAS – periodic acid-Schiff reaction; WHO – World Health Organization.

of precise diagnosis of the haematopoietic malignancies based on cytomorphology, enzyme cytochemistry and immunophenotyping in accordance with FAB, EGIL (European group for the immunological characterization of leukaemias) and WHO classifications.

This study included 1742 patients with AML and ALL, residents of Kyiv city and 20 regions of Ukraine. The bone marrow and blood smears were sent at diagnosis to Reference Center from February 12, 1996 to June 1, 2010.

The analysis was based on May-Grünwald-Giemsa (MGG) stain, myeloperoxidase reaction (MPO), acid non-specific esterase using alpha-naphtyl-acetate (acNSE, sodium fluoride-sensitive), chloracetate-esterase (CAE) and acid phosphatase (AP) stain. Glycogen was assayed cytochemically by PAS-reaction. All stainings were performed routinelly according to standard procedures [3, 9–11]. Immunocytochemical techniques (APAAP, LSAB) and broad panel of monoclonal antibodies (MoAbs) against lineage specific and differentiation antigens of leukocytes were employed for immunophenotyping leukaemic blast cells directly in blood and bone marrow smears.

Acute myeloid leukaemias. The defining criterion for AML was 20% and more myeloblasts in peripheral blood or bone marrow. For the sub-classification of AML the primary basis were the morphological and enzyme-cytochemical/ immunocytochemical features of leukaemic cells [12].

Different types of AML were defined immunologically by the expression of the following cell surface and cytoplasmic antigens: markers of HSC and progenitor cells (CD34, CD38, CD117, HLA-DR), granulocytic markers (CD13, CD15, CD33, cytoplasmic MPO), monocytic markers (CD11c, CD14, CD4, CD11b), megakaryocytic markers (CD41, CD61), erythroid marker (glycophorin A).

The series of 1271 consecutive patients with AML were classified by the WHO system into such entities: AML with minimal differentiation; AML without maturation; AML with maturation; acute monoblastic and monocytic leukaemia; acute promyelocytic leukaemia; acute erytroid leukaemia; acute megakaryocytic leukaemia; blastic plasmacytoid dendritic cell neoplasm (BPDC).

Immunocytochemical study was required especially to establish the diagnosis of AML with minimal differentiation, acute megakaryoblastic leukaemia, acute erythroid leukaemia and AL of ambiguous lineage.

AML with minimal differentiation is an AML without morphologic and cytochemical evidence of differentiation. Blast cell in most cases (19 patients) expressed CD34, CD38, CD13 and CD117 antigens. Expression of CD7 has been seen in 13 cases. MPO was negative in blast cells by cytochemistry, but the result of detection of intracytoplasmatic MPO antigens in blast cells was positive by immunocytochemictry with anti-MPO MoAbs.

AML without maturation (28 patients) was characterized by MPO-positive blasts (Fig. 1) and expression CD13, CD33 and CD117 antigens. CD34 and HLA-DR were positive in 19 patients. Expression of CD7 was registred in 8 and CD56 in 3 of cases.

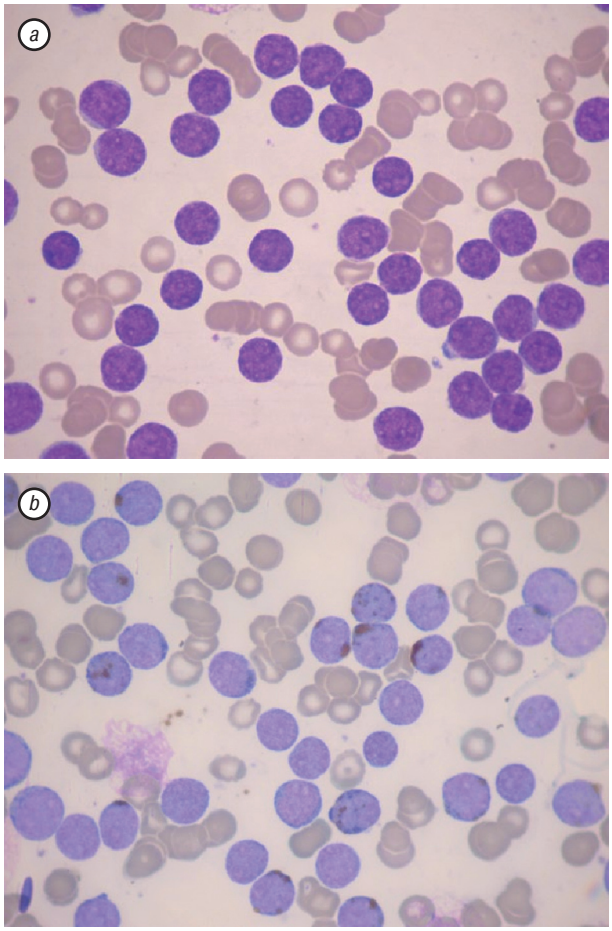

Fig. 1. Acute myeloid leukaemia without maturation, bone marrow smear: *a*, the cells predominantly myeloblasts; *b*, myeloblasts with myeloperoxidase activity.

AML with maturation (126 patients). The blast cells in our study expressed such myeloid-associated antigens: CD13 (114 patients), CD33 (108), CD15 (109). In a fraction of blasts expression of HLA-DR and CD34 was revealed.

Acute myelomonocytic leukaemia (448 patients) (Fig. 2) showed population of blast expressing myeloid antigens CD13, CD33, CD15 and population of leukaemic cells with markers of monocytic differentiation: CD14, CD4, CD11b, CD11c. In most cases blast cells were positive for HLA-DR and in 287 patients for CD7.

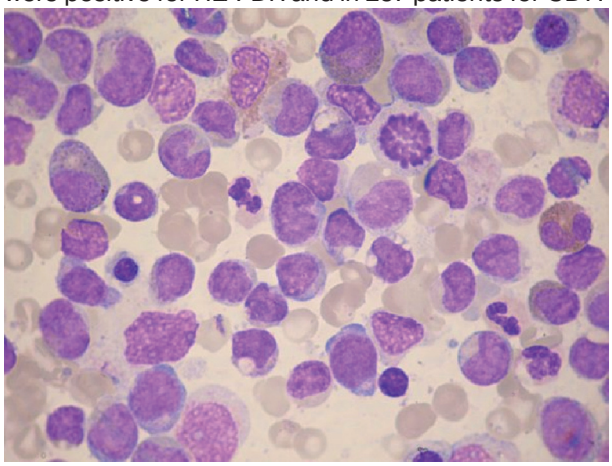

Fig 2. Acute myelomonocytic leukaemia associated with inv(16)(p13.1;q22) and abnormal eosinophils.

Acute monoblastic and monocytic leukaemia (498 patients) (Fig. 3). Blast cells were acNSE positive, expressed HLA-DR, myeloid antigens CD13, CD15 and markers of monocytic differentiation CD14, CD4, CD11b, CD11c, CD68. In 104 cases expression was found of CD7 and CD56. MPO was expressed more often in acute monocytic leukaemia.

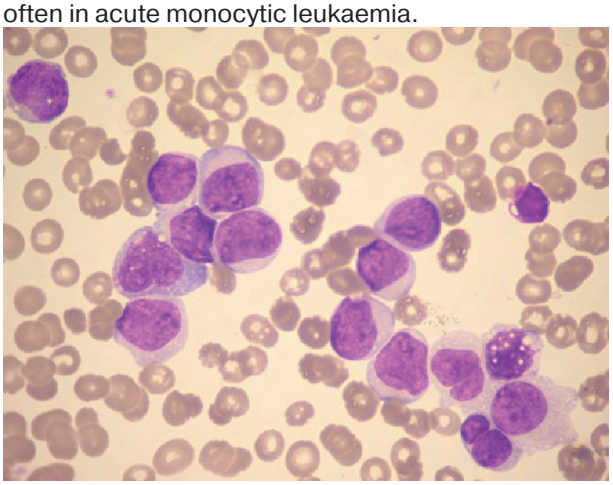

Fig. 3. Acute monoblastic leukaemia.

Acute promyelocytic leukaemia (116 patients) (Fig. 4) was characterized by absence of HLA-DR, CD34 and CD15 expression, weak positive reaction with MoAbs to CD117.

Acute erythroid leukaemia. The blast cells were negative for HLA-DR, CD34 and MPO. Expression of glycophorin A was detected in 16 cases with more differentiated leukaemic cells and antigen CD36 that may be expressed also by monocytes and megakaryocytes in 26 patients. The need of an early marker specific for the erythroid lineage would be stressed as it is likely that cases resulting from leukaemic transformation of primitive erythroid cells were underdiagnosed.

Acute megakaryoblastic leukaemia was diagnosed in 17 patients.

Megakaryoblasts typically expressed CD41 (glycoprotein IIb/IIIa), CD61 (glycoprotein IIIa) antigens and CD36. Reactions for CD34 and HLA-DR were negative.

Blastic plasmacytoid dendritic cell neoplasm. The blast cells in BPDC were negative with myeloperoxidase and weak positive with acNSE stains. Neoplastic cells expressed CD4 and CD56 antigens. In 2 of 3 cases on the cell surface membrane had heen demonstrated expression of CD7, CD38 and CD71. Cells were CD34 and CD117 negative.

Future studies will be addressed to investigate whether an immunological classification of AML is possible in relation to the various AML groups defined by cytogenetics and molecular features. Thus data will be collected to disclose the most common immunological phenotypes, if any, in cytogenetically defined AML subgroups with t(8;21), inv(16), t(15;17) or t(9;11) [12].

Acute lymphoblastic leukaemias. Acute lymphoblastic leukaemias (ALL) (463 patients) was broadly classified into B-lineage and T-lineage ALL. In total 387 patients with B-ALL and 76 with T-ALL in our Reference Center were examined.

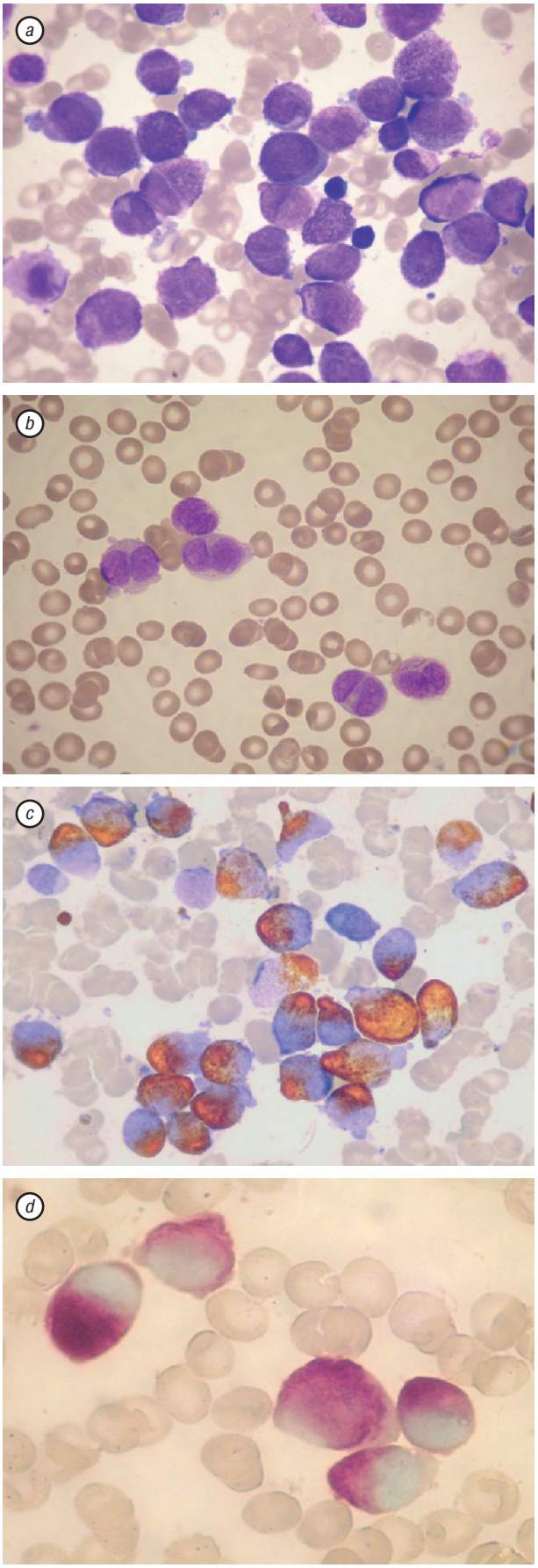

Fig. 4. Acute promyelocytic leukaemia with t(15;17)(q22;q12); PML-RARA, bone marrow: *a*, hypergranular type; *b,* microgranular variant; c, myeloperoxidase reaction showing intensely positive abnormal promyelocytes; *d*, acid non-specific esterase stain. The promyelocytes are intensely reactive.

B-lineage ALL was defined by the expression of the following three early B-cell markers: CD19, cytoplasmic CD79a and cytoplasmic CD22. According to the degree of B-lymphoid differentiation of the blast cells, four subtypes of B-lineage ALL designated from B-I to B-IV were established, namely B-I (pro-B ALL): $CD34^{+/-}$, $HLA\text{-}DR^{+}$, $CD19^{+}$, $cyCD22^{+}$, $cyCD79a^{+}$; B-II (common ALL): $CD34^{+/-}$, $HLA\text{-}DR^{+}$, $CD19^{+}$, $cy/sCD22^{+}$, $CD79a^{+}$, $CD10^{+}$; B-III (pre-B ALL): $HLA\text{-}DR^{+}$, $CD19^{+}$, $CD22^{+}$, $CD79a^{+}$, $CD10^{+/-}$,$CD20^{+}$, $cyIgM^{+}$; B-IV (B-cell ALL): $HLA\text{-}DR^{+}$, $CD19^{+}$, $CD22^{+}$, $CD79a^{+}$, $CD10^{+/-}$, $CD20^{+}$, $sIgM^{+}$, $cy\kappa^{+}$ or $cy\lambda^{+}$ (Fig. 5, 6).

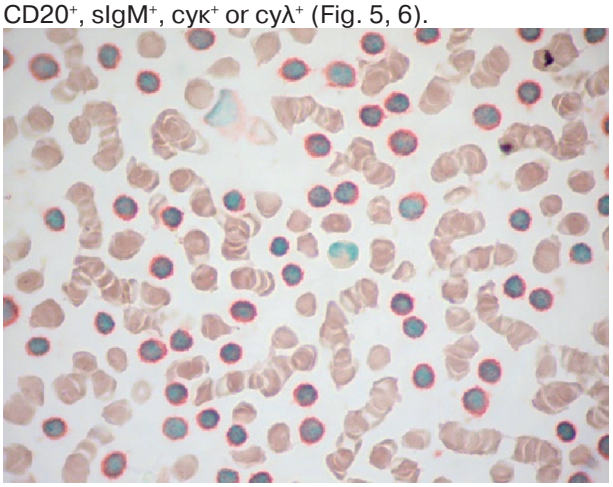

Fig. 5. The lymphoblasts in pro-B-ALL (B-I) are positive for the antigen CD34. Bone marrow smears.

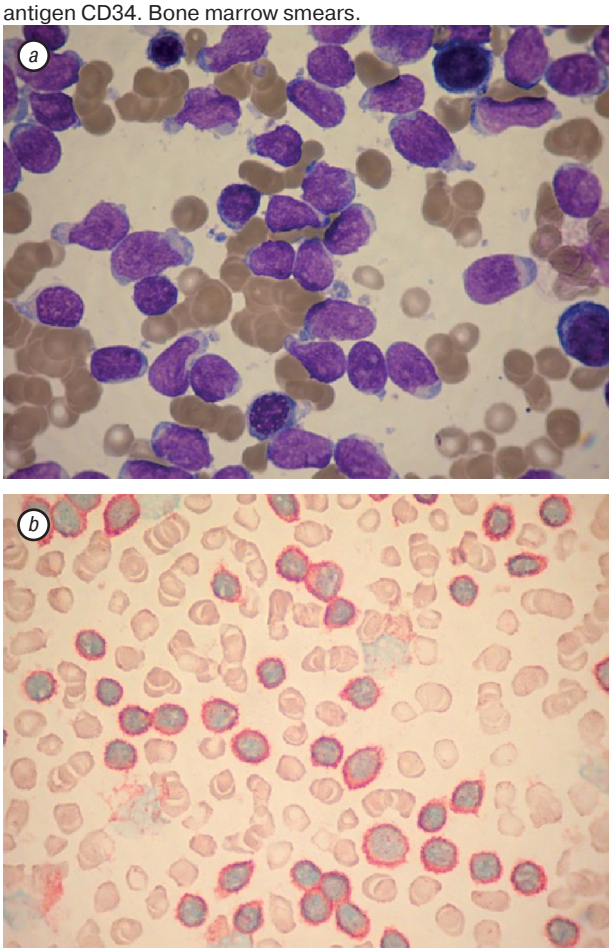

Fig. 6. Common acute lymphoblastic leukaemia (B-II), bone marrow: *a*, lymphoblasts with a high nuclear/cytoplasmic ration; *b*, the lymphoblasts are positive for CD19 antigen.

The modern concept of LSC allows one to analyze in more depth the mechanisms of leukaemic transformation of target cells in AL of the closely related origin, namely pro-B ALL *vs.* acute monoblastic leukaemia, acute erytroid leukaemia vs. acute megakaryoblastic leukaemia. The similarity of immunophenotype and cytogenetic abnormalites in blast cells in pro-B ALL and acute monoblastic leukaemia seem to be the hint explaining the cases of acute monoblastic leukaemia as a recurrence of leukaemia in children with originally diagnosed pro-B ALL.

T-lineage ALL observed in our patients were also divided into four variants, namely T-I (pro-T ALL): $HLA\text{-}DR^{+/-}$, $CD34^{+/-}$, $CD7^{+}$, $cyCD3^{+}$, $CD5^{+/-}$; T-II (pre-T ALL): $CD7^{+}$, $cy/sCD3^{+}$, $CD5^{+}$, $CD2^{+}$,$CD8^{+}$; T-III (with cortical thymocytes phenotype): $CD7^{+}$, $CD3^{+}$, $CD5^{+}$, $CD2^{+}$, $CD1a^{+}$, $CD4/8^{+}$; T-IV (mature T ALL): $CD7^{+}$, $CD3^{+}$,$CD5^{+}$, $CD2^{+}$, $CD4^{+}$ or $CD8^{+}$.

The majority of acute leukaemias parameters (the ratio of ALL to AML; frequency of B- and T-cell ALL; age structure of ALL; the percentage of different variants of of B-cell origin; the frequency of aberrant expression of myeloid antigens on blast cells from AML) are comparable with corresponding data from USA and most European countries [13, 14].

Immunocytochemical studying is required to diagnose acute leukaemias of ambiguous lineage that show no clear evidence of lineage differentiation (acute undifferentiated leukaemia) or those with blasts that express markers of more than one lineage (mixed phenotype acute leukaemias).

Today we continue the attempts for precise characterization of this rare group of acute leukaemias.

REFERENCES

1. **Bennet JM, Catovsky D, Daniel MT, *et al*.** Proposals for the classification of the acute leukemias. French-American-British (FAB) Cooperative Group. Br J Hematol 1976; **33**: 451–8.

2. **Bennet JM, Catovsky D, Daniel MT, *et al*.** Proposed revised criteria for the classification of acute myeloid leukaemia: A report of the French-American-British Cooperative Group. Ann Intern Med 1985; **103**: 620–5.

3. **Bain BJ.** Leukaemia diagnosis. Oxford: Blackwell Seience Ltd 1999. 200 p.

4. **Jaffe ES, Harris NL, Stein H, Vardiman JW, *eds*.** World Health Organization classification of tumours. Pathology and genetics of tumours of haematopoetic and lymphoid tissues. Lyon: IARC 2001. 351 p.

5. **Swerdlow SH, Campo E, Harris NL, *et al, eds*.** WHO classification of tumours of haematopoetic and lymphoid tissues. Lyon: IARC 2008. 439 p.

6. **Vardiman JW, Thiel J, Arber DA, *et al*.** The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukaemia: rationale and important changes. Blood 2009; **114**: 937–51.

7. **Passegue E, Jamieson CHM, Ailles LE, *et al*.** Normal and leukaemic hematopoesis: are leukaemias a stem cell disorder or a reacquisition of stem cell characteristics? Proc Natl Acad Sci USA 2003; **100**: 11842–9.

8. **Wang JCY, Dick JE.** Cancer stem cells. In: Principles and practice of oncology, 8th ed. Lippincot Williams a Wilkins, 2008: 136–45.

9. **Gluzman DF, Abramenko IV, Sklyarenko LM, *et al.*** Leukaemia diagnosis. Atlas and practical manual. Kyiv: Morion 2000. 224 p. (In Russian).

10. **Gluzman DF, Imamura N, Sklyarenko LM, *et al.*** Malignant diseases of hematopoetic and lymphoid tissues in Chernobyl clean-up workers. The Hematol J 2005; **5**: 565–71.

11. **Gluzman DF, Nadgornaya VA, Sklyarenko LM, *et al.*** Study of morphocytochemical and immunophenotypic features of acute leukaemia stem cells. Exp Oncol 2008; **30**: 102–5.

12. **Döhner H, Estey EH, Amadori S, *et al.*** Diagnosis and management of acute myeloid leukaemia in adults: recommendation from an international expert panel, on behalf of the European Leukemia/Net. Blood 2010; **115**: 453–74.

13. **Yamamoto JF, Goodman MT.** Patterns of leukemia incidence in the United States by subtype and demographic characteristics, 1997-2002. Cancer Causes Control 2008; **19**: 379–90.

14. **Haferlach T, Schoch C, Löffler H, *et al.*** Morphologic dysplasia in de novo acute myeloid leukemia (AML) is related to unfavorable cytogenetics but has no independent prognostic relevance under the conditions of intensive induction therapy: results of a multiparameter analysis from the German AML Cooperative Group studies. J Clin Oncology 2003; **21**: 256–65.