Leukemic cells and aberrant phenotypes in acute leukemia patients: a flow cytometry analysis

D. Vošková, Ľ. Váleková, J. Fedorová, J. Hudeček, P. Kubisz*

Clinic of Hematology and Transfusiology, Faculty Hospital of Martin and Jessenius Medical Faculty, Comenius University, Martin, Slovak Republic, e-mail: Peter.Kubisz@jfmed.uniba.sk

Received April 8, 2003

The aim of this study was to evaluate the heterogeneity of immunophenotype features in acute leukemia patients and to detect the presence of leukemia-associated immunophenotypes.

We prospectively investigated the phenotype of blast cells from 44 adult acute leukemia patients using a large panel of monoclonal antibodies by multiparametric flow cytometry. Thirty-three patients were classified as AML according to the FAB classification. Eleven patients were diagnosed as ALL (10 cases B-ALL, 1 case T-ALL) according to both FAB and immunnophenotyping. We found leukemia-associated phenotypes in 28 of 33 AML patients (84.8%) and in 8 of 11 ALL patients (72.7%). In 61.1% of patients more than one aberrant phenotype was observed. Linear infidelity was the most frequent aberrancy in both AML (64.3%) and ALL (37.5%) subgroups.

The present study shows that MFC is a helpful method for sufficient identification of leukemic cells and for determination of blast cells immunophenotype heterogeneity. The double stain flow cytometry in our study revealed aberrant phenotypes in up to 81.8% patients.

Key words: Flow cytometry, acute leukemia, leukemia-associated immunophenotype, minimal residual disease.

The acute myeloid leukemias (AML) are a heterogeneous group of diseases with diverse pattern of cell surface markers expression. Recent studies using multidimensional flow cytometric analysis have clearly shown that during normal myeloid lineage differentiation cells exhibit a highly reproducible pattern of cell-surface antigen expression and some membrane-associated differentiation antigens are initially expressed in the cytoplasm before their insertion into the cell membrane [24, 29–31]. More than 95% of AML cases can be distinguished from acute lymphoblastic leukemia (ALL) types using surface marker analysis and M0 subtype of AML can be distinguished from ALL with help of myeloperoxidase (MPO) detection in cell cytoplasm [28]. In contrast to AML blasts, which can be reliably identified in most cases by the presence of Auer rods, MPO, or monocyte-associated esterases, leukemic lymphoblasts lack specific morphologic or cytochemical features, so that diagnosis of ALL depends of immunophenotyping [4]. The conventional value of immunophenotyping in acute lymphoblastic leukemia is to differentiate B-lineage ALL from T-lineage ALL and to establish a solid basis for precise and biologically originated classification of the disease [3, 13, 26, 29]. Currently used extensive panels of antibodies by multiparameter flow cytometry (MFC) allow the lineage assessment of AL blasts with accuracy more than 99% [7, 14].

The MFC is an attractive method also for investigation of minimal residual disease (MRD). The detection of MRD becomes increasingly important for the management of the acute leukemia patients and is incorporated into all modern treatment protocols [6, 27, 32]. MFC together with the polymerase chain reaction (PCR) is highly sensitive technique for the detection of MRD in acute leukemia patients. For MRD follow-up, PCR uses clonal markers as the T-cell receptor gene, immunoglobulin gene rearrangements (in ALL cases) as well as the fusion transcripts (in AML cases); MFC is based on the detection of leukemia-associated immunophenotypes (LAIP), that do not normally occur in

*Author to whom correspondence should be sent.

cells of the peripheral blood and bone marrow [1, 8, 10, 21, 34]. Several studies have shown that leukemic cells frequently display phenotypic aberrancies [1, 2, 5, 6, 8, 9, 11, 12, 14–18, 22, 25, 28, 32, 33].

There are six mainly accepted subgroups of LAIP: a) lineage infidelity: co-expression of non-lineage antigens (myeloid and lymphoid antigens or T- and B-lymphoid antigens simultaneously); b) asynchronous antigen expression: expression of markers in a combination that is not found in the normal myeloid and lymphoid differentiation; c) antigen overexpression: abnormally high antigen expression of blast cells; d) absence of lineage specific antigens: absence of antigens expression CD13, CD33 (myeloid blasts), CD19 or CD7 (lymphoblasts) and CD45; e) ectopic phenotype: expression of markers on T-cells that are not found outside the thymus and f) abnormal light-scatter pattern: abnormal FSC/SSC distribution of leukemic blasts according to their phenotype [1, 9, 14, 17, 18].

The aim of the study was to characterize the phenotype of leukemic cells and to analyze the presence of LAIP in acute leukemia patients.

Material and methods

Forty-four adult patients (25 males, 19 females) were *de novo* diagnosed as AL. The mean age of patients was 49 years (18 to 83 years). The AML was diagnosed with help of light microscopy cytochemical reaction with myeloperoxidase, PAS and esterase according to the criteria of French-American-British (FAB) classification.

The ALL diagnosis was based on both the FAB classification criteria and immunophenotyping. The immunophenotype findings assignment to the B- or T-cell lineages based on the criteria proposed by European Group for the Immunological Classification of Leukemias (EGIL) [3, 4].

The bone marrow (BM) samples were collected in heparin anticoagulant and peripheral blood (PB) samples in EDTA anticoagulant and immediately diluted in phosphate-buffered saline (PBS), with final cell concentration of $1x10^7$/ml. For the immunophenotype analysis the direct immunofluorescence method as well as the stain and lysis technique was used. For the differentiation of antigens with low antigen density on the cell surface PE conjugates were utilized.

Briefly, 100 μl of PBS-diluted BM or PB cells were incubated for 15 minutes with 5 μl of the fluorochrome-conjugated monoclonal antibody reagents listed later. In the next step, 2 mL of fluorescence-activated cell sorter (FACS) lysing solution (Becton Dickinson, Heidelberg, Germany) in distilled water were added to each tube and another incubation for maximum 12 minutes in the dark (room temperature) was performed followed by centrifugation (5 min, 1700 rpm) and cell pellet washing (5 min, 1700 rpm) in 2 mL of PBS. Finally, the cells were resuspended in 0.5 mL of PBS. Stainings for MPO, CD22, CD3 and TdT (terminal deoxynucleotidyl-transferase) were performed at the cytoplasmic and nucleus level, respectively, using the Intra Prep reagent (Immunotech, Marseille, France). The limit of surface and cytoplasmic markers cut off was considered 20% and 10%, respectively.

The panel of monoclonal antibodies. To increase the accuracy of lineage assessment, maturation state and detection of the maximum number of phenotypic aberrancies present at the time of diagnosis, antigen expression was analyzed in all cases using double combinations of the monoclonal antibody conjugated with fluorescein isothiocyanate (FITC) and phycoerythrin (PE): IgG2a-FITC/IgG1-PE; CD45-FITC/CD14-PE; CD33-FITC/CD7*-PE; CD2-FITC/CD13-PE; CD34-FITC/CD56-PE; CD15-FITC/CD117-PE; CD15-FITC/CD11b-PE; CD34-FITC/CD14-PE; CD33-FITC/CD34-PE; HLA-DR*-FITC/CD13-PE; CD71*-FITC/CD13-PE; CD33-FITC/CD22-PE; CD45-FITC/GlykoA-PE; CD61-FITC/CD13-PE; CD10-FITC/CD19-PE; CD24-FITC/CD13-PE;TdT*-FITC/CD10-PE; CD38-FITC/CD20-PE; Kappa*-FITC/CD19-PE; Lambda*-FITC/CD19-PE; CD33-FITC/CD19-PE; CD71-FITC/CD19-PE; CD2-FITC/CD22-PE; TCR$\alpha\beta$-FITC/TCR$\gamma\delta$-PE; CD4-FITC/CD8-PE; CD34-FITC/CD5-PE.

If needed, the cytoplasmatic detection of MPO*(FITC), CD22(FITC), CD3(FITC) and IgM** was used. All antibodies were purchased from Becton Dickinson, Heidelberg, Germany, except for: *Immunotech, Marseille, France; **Dako, Glostrup, Denmark.

In all cases, isotype-matched immunoglobulins with no reactivity to BM and PB cells were used as negative controls.

Measurements were performed on a FACSCalibur flow cytometer (Becton Dickinson, Heidelberg, Germany) equipped with an argon ion laser tuned at 488 nm and 15 mV. Calibration of the instrument was performed before data acquisition using bead standards. For the data acquisition and analysis, the CellQuest software (Becton Dickinson, Heidelberg, Germany) was used. In samples from patients at diagnosis, 20.000 cells pro tube was measured. The colour gating function of CellQuest software was used to analyze the double stain data. We used a CD45 intensity expression with right-angle light scatter to detect leukemic cells and separate them from the other BM and PB populations.

Results

Immunophenotype in AL patients. AML was diagnosed in 33 AL patients with distribution as follows: M0 in two

Table 1. Distribution of AML and B and T lineage ALL cases according to immunophenotype

AML	No cases	CD34	CD117	HLA-DR	CD13	CD33	CD15	CD11b		CD4	C D14	CD45	MPO
M0	2	2	2	1	2	1	0	0		0	0	2	2
M1	16	4	16	16	16	15	5	4		0	0	15	5
M2	5	3	5	5	5	5	5	4		0	0	5	–
M3	2	0	1	0	2	2	1	1		0	0	2	–
M4	7	1	3	6	6	7	6	7		7	7	7	–
M5	1	0	0	1	1	1	0	1		1	1	1	–
B-lineage		**CD34**	**TdT**	**HLA-DR**	**CD10**	**CD19**	**CD20**	**cyCD22**	**sCD22**	**CD24**	**CD45**	**cyIgM**	**sIgM**
pre-pre-B	1	1	1	1	0	1	0	1	0	1	0	0	0
common B	6	6	6	6	6	6	1	6	0	6	6	0	0
pre-B	2	0	2	2	2	2	0	2	0	2	2	2	0
mature B	1	0	0	1	0	1	1	0	1	0	1	0	1
T-lineage		**CD34**	**TdT**	**HLA-DR**	**CD5**	**CD2**	**CD7**	**sCD3**		**TCR$\alpha\beta$**	TCR$\gamma\delta$	CD45	
mature T	1	1	1	0	1	1	1	1		1	0	1	

s – surface, cy – cytoplasmic.

patients (6.1%), M1 in sixteen patients (48.5%), M2 in five patients (15.2%), M3 in two patients (6.1%), M4 in seven patients (21.2%) and M5 in one patient (3.0%). The ALL was diagnosed in 11 of studied cases. (ALL=11: L1, n=0; L2, n=7, L3, n=3, L2-L3, n=2, according to FAB classification). Based on EGIL classification, pro-B-ALL was diagnosed in one patient, common B-ALL in 6 patients, pre B-ALL in 2 patients and mature B-ALL in one patient. In one patient, we diagnosed the T-ALL with the expression sCD3 and TCR$\alpha\beta$. Table 1 shows the incidence of expression of antigens in AML and ALL subclasses.

LAIP. Thirty-six patients from all acute leukemia patients (81.8%) showed the presence of at least one LAIP. The total detected number of LAIP was 79 (mean = 2.2 LAIP/patient). As shown in Figure 1, two or more aberrant phenotypes co-existed in 22 patients as follows: 2 LAIP in 15 cases (12 AML; 3 ALL), 3 LAIP in 6 cases (5 AML; 1 ALL) and 4 LAIP in one case (AML).

In the 28 of 33 AML patients (84.8%) 67 LAIP were detected (mean = 2.4 LAIP/patient). The most of LAIP were found in subclass M1 (61.2%). The most frequent aberrant phenotype (35.7%) the CD33/CD7 was found.

In the 8 of 11ALL patients (72.7%) we detected total of 12 LAIP (mean=1.5 LAIP/patient). The most of LAIP were found in common B-ALL cases (50.0%).

We found total of 29 lineage infidelity LAIP in 18 (64.3%) of AML patients and in 3 (37.5%) of ALL patients. In most AML cases (94.4%) isolated co-expression of one T-or B-lymphoid antigens (CD2, CD5, CD7, CD19, CD22, CD24) was observed and only in one patient simultaneous reactivity for CD2 and CD7 on myeloid blasts was detected. In two ALL cases the isolated coexpression of myeloid antigens (CD13 or CD33) was found and in one of B-ALL patient the simultaneous coexpression of T-lymphoid and myeloid antigens was detected (Tab. 2, 3).

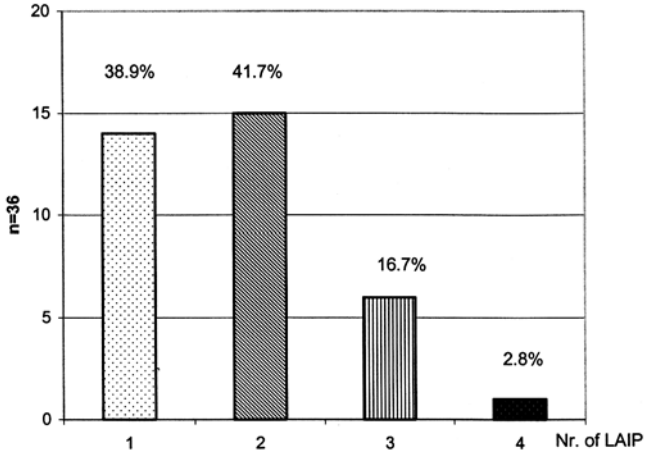

Figure 1. Incidence of aberrant phenotypes in 36 acute leukemia patients.

Asynchronous expression was seen in 14 (50%) of AML and in 3 (37.5%) of ALL patients (Tab. 2, 3).

Over-expression was detected in 4 (14.3%) of AML and in 3 (37.5%) of ALL patients (Tab. 2, 3). The absence of lineage specific antigens was observed in 10 (27.8%) cases (9 AML, 1 B-ALL) (Tab. 2, 3). Abnormal FSC/SSC pattern we observed in 14 (50%) of AML cases and in 1 (12.5%) B-ALL case. In most of them, it was due to expression of lymphoid markers in cells displaying high light-scatter properties. Thus, most of these cases were already identified as phenotypically aberrant based on the presence of lineage infidelity.

Table 2. Aberrant phenotypes in AML patients (n=28)

	No. of cases (%)
Lineal infidelity	
CD13+/CD2+	4 (14.3)
CD33+/CD7+	10 (35.7)
CD13+/CD5+	1 (3.6)
CD13+/CD7+	2 (7.1)
CD33+/CD22+	2 (7.1)
CD13/CD19+	1 (3.6)
CD13/CD24+	1 (3.6)
CD33/CD24+	3 (10.7)
CD13/+CD22+	1 (3.6)
total	25 (89.2)
Asynchrous antigen expression	
CD15+/117+	5 (17.9)
CD34+/56+	2 (7.1)
CD33+/CD34-	5 (17.9)
C13+/HLA-Dr-	2 (7.1)
CD34+/CD117-	1 (3.6)
total	15 (53.6)
Overexpression	
CD34++	1 (3.6)
CD33++	2 (7.1)
HLA-DR++	1 (3.6)
total	4 (14.3)
Lack of lineage specific antigen	
CD33+/D13-*	1 (3.6)
CD13+/CD33-*	1 (3.6)
CD13+/ CD33dim**	2 (7.1)
CD33+/ CD13dim**	3 (10.7)
CD33+/CD45-*	2 (7.1)
total	9 (32.1)
Aberrant light-scatter patterns	
CD2/high FSC/SSC	2 (7.2)
CD7/high FSC/SSC	9 (32.1)
CD19/high FSC/SSC	1 (3.6)
CD33/low FSC/SSC	1 (3.6)
CD13/low FSC/SSC	1 (3.6)
total	14 (50.0)

*antigen positivity <10%; **antigen positivity >21%, but more than 50% higher than fluorescent intensity of the other lineage specific antigen.

Table 3. Aberrant phenotypes in ALL patients (n=8)

	No. of cases (%)
Lineal infidelity	
CD19+/CD13+	1 (12.5)
CD19+/CD2+	1 (12.5)
CD24+/CD33+	1 (12.5)
CD19+/CD33+	1 (12.5)
total	4 (50.0)
Asynchronous antigen expression	
CD34+/CD3+	1 (12.5)
CD19+/CD24-*	2 (25.0)
total	3 (37.5)
Over-expression	
CD19++	1 (12.5)
CD34++	2 (25.0)
total	3 (37.5)
Lack of lineage specific antigen	
CD19+/CD45-*	1 (12.5)
Aberrant light-scatter patterns	
CD33/high FSC/SSC	1 (12.5)

*antigen positivity <10%

Discussion

The heterogeneity of the leukemic blasts in AML is one of the reasons why the immunophenotyping of AML is more difficult than that of the relatively homogenous acute lymphoblastic leukemia [24, 29].

In our 33 AML patients we analysed the phenotype of leukemic cells according to subclasses of cytomorphological FAB classification. We used specific monoclonal antibodies for definition of myeloid lineage (CD13, CD33, MPO), cell immaturity (CD34, CD117, HLA-DR) and degree of cell differentiation (CD15, CD11b, CD4, CD14). These markers are reported to be sufficient for AML diagnosis [7, 13, 28, 31].

For the subclassification of ALL cases, we used the criteria of the European Group of Immunophenotyping Leukemia (EGIL). Athough this classification recognises the subclasses of ALL according of normal B- and T-cell maturation, the only distinctions with therapeutic importance are those between the precursor B-cell immunophenotype and the T-cell or mature-B-cell immunophenotype [26].

The sensitivity of MFC for LAIP detection depends on selection of monoclonal antibodies combinations and aberrant leukemic cell antigens incidence [9, 12, 17, 33].

In the present study with 44 patients (33 AML cases and 11 ALL cases), using a large panel of monoclonal antibodies in double staining combinations and considering five different types of aberrancies most patients (81.8%) expressed total of 79 LAIP (mean 2.2 LAIP/patient).

In AML patients group we detected LAIP in 28 of cases (84.8%). In ALL patients group we found LAIP in 8 of 11 (72.7%) cases.

The lineage infidelity was the most frequent phenotypic aberration in our AL cases and its overall incidence was 58.3%.

The asynchronous antigen expression was found in 6 AML (21.4%) cases and in 1 ALL (12.5%) case. In other MFC studies [9, 14, 18], this aberrancy was reported to be the most frequent which was based on the fact, that the lost or low expression of lineage specific antigens was considered to be the asynchronous antigen expression. In the

study of KERN where LAIP in 626 AML patients were analyzed, asynchronous antigen expression in 20.7% and absence of lineage specific antigens in 26.9% of cases were detected [17]. Only 7% of the AML cases analyzed coexpressed the CD56 and CD34 antigens, this incidence is relatively low compared to study by COUSTAN-SMITH et al [10]. However, it should be mentioned that most of the patients included in their series were children, while all cases analyzed in our study correspond to adult AML, this probably explains the discrepancy. To detect antigen overexpression, appropriate calibration and standardization of both immunofluorescence and flow cytometry techniques is important. We found these aberrancy in 14% of AML cases and CD33 overexpression was detected most frequently. In 37% ALL cases the overexpression of CD34 was the most frequently detected. In contrast to lineage infidelity and the asynchronous expression, the overexpression of more that one antigen was exceptional. The antigen overexpression in AML study groups were found from 9% to 30% cases [17, 19]. Overexpression of CD33, CD34 and HLA-DR is reported most frequently. In ALL studies reported by CIDAUD et al [9] the frequency of antigen overexpression in 38% and GARCIA-VELA et al [14] in 23% of patients, in both of these studies the CD10 overexpression was the most frequent. The flow cytometric characterization of leukemic cells revealed also the aberrant lack of lineage specific antigens of cell populations. Such cell population usually displays the antigenic and the light scattering properties characteristic of their counterpart in normal bone marrow, except that a typical antigen is missing [29, 31].

In the light of these facts, results of the presented study are comparable also with studies, where three colors MFC and threshold value for LAIP positivity at level of 10% were used [1, 2, 5, 6, 8, 9, 11, 12, 14–18, 22, 25, 28, 32].

The determination of LAIP is essential for the MFC detection of MRD [6, 9, 10, 32, 34]. To make the MFC enough sensitive for MRD detection in all AL patients, it would be helpful if three- or more colors MFC would be used and threshold value for LAIP positivity would be set lower than 10%.

In conclusion, the present study evaluated the phenotype of leukemic cells in AML and ALL patients. The present study shows that MFC is a helpful method for sufficient identification of leukemic cells and for determination of blast cells immunophenotype heterogeneity. Using a large panel of monoclonal antibodies we characterized precisely LAIP in AML and ALL which has a great importance for MRD detection.

References

[1] BABUŠÍKOVÁ O, GLASOVÁ M, KONÍKOVÁ E, KUSENDA J. Leukemia-associated phenotypes: their characteristics and incidence in acute leukemia. Neoplasma 1996; 43: 367–372.

[2] BABUŠÍKOVÁ O, KONÍKOVÁ E KUSENDA J, KOUBEK K. Aberrant marker expression in T- and B-lymphoid and myeloid leukaemia cells of different differentiation stages. Neoplasma 1999; 46: 277–282.

[3] BENE MC, CASTOLDI G, KNAPP W, LUDWIG WD, MATUTES E, ORFAO A, VAN'T VEER MB. Proposals for the immunological classification of acute leukaemias. The European Group for the Immunological Characterisation of Leukaemias (EGIL). Leukemia 1995; 9: 1783–1786.

[4] BENNETT JM, CATOVSKY D, DANIEL MT, FLANDRIN G, GALTON DAG, GRALNICK HR, SULTAN C. Proposals for the classification of acute leukemias. A report of the French-American-British Cooperative Group. Br J Haematol 1976; 33: 451–458.

[5] BRADSTOCK KF, KIRK J, GRIMSLEY PG, KABRAL A, HUGHES WG. Unusual immunophenotypes in acut leukaemias: incidence and clinical correlations. Br J Haematol 1989; 72: 512–518.

[6] CAMPANA D, COUSTAN-SMITH E. Detection of minimal residual disease in acute leukemia by flow cytometry. Cytometry 1999; 38: 139–152.

[7] CAMPANA D, BEHM FG. Immunophenotyping of leukaemia. Journal of Immunological Methods 2000; 243: 59–75.

[8] CAMPANA D, YOKOTA S, COUSTAN-SMITH E, HANSEN-HAGGE TE, JANOSSY G, BARTRAM CR. The detection of residual acute lymphoblastic leukemia cells with immunologic methods and polymerase chain reaction: a comparative study. Leukemia 1990; 4: 609–614.

[9] CIDAUD J, SANMIGUEL JF, LOPEZ-BERGES MC, VIDRIALES B, VALVERDE B, OCQUETEAU M, MATEOS G, CABALLERO MD, HERNANDEZ J, MORO MJ, MATEOS MV, ORFAO A. Prognostic value of immunophenotypic detection of minimal residual disease in acute lymphoblastic leukemia. J Clin Oncol 1998; 16: 3774–3781.

[10] COUSTAN-SMITH E, BEHM FG, HURWITZ CA, RIVERA GK, CAMPANA D. N-CAM (CD56) expression by CD34+ malignant myeloblasts has implications for minimal residual disease detection in acute myeloid leukemia. Leukemia 1993; 7: 853–858.

[11] DRACH J, DRACH D, GLASSL H, GATTRINGER C, HUBER H. Flow cytometric determination of atypical antigen expression in acute leukemia for the study of minimal residual disease. Cytometry 1992; 13: 893–901.

[12] DWORZAK MN. Immunological detection of minimal residual disease in acute lymphoblastic leukemia. Onkologie 2001; 24: 442–448.

[13] European Working Group on Clinical Cell Analysis (EWGCCA). Consensus document on leukemia immunophenotyping. Leukemia 1996; 10: 877–895.

[14] GARCIA VELA JA, MONTESERIN MC, DELGADO I, BENITO L, ONA F. Aberrant immunophenotypes detected by flow cytometry in acute lymphoblastic leukemia. Leuk Lymphoma 2000; 36: 275–284.

[15] GRIESINGER F, PIRÓ-NOACK M, KAIB N, FALK M, RENZIENHAUSEN A, TROFF C, GROVE D, SCHNITTGER S, BÜCHNER T, RITTER J, HIDDEMANN W, WÖRMANN B. Leukaemia-associated immunophenotypes (LAIP) are observed in 90% of adult and childhood acute lymphoblastic leukaemia: detection in remission marrow predicts outcome. Br J Haematol 1999; 105: 241-255.

[16] Gore SD, Kastan MB, Goodman SN, Civin CI. Detection of minimal residual T cells acute lymphoblastic leukemia by flow cytometry. J Immunol Methods 1990; 132: 275–286.

[17] Kern W. Einsatz der multiparametrischen Durchflusszytometrie zur Identifizierung von Prognoseparametern bei der akuten myeloischen Leukämie: Erfassung von Antigenexpressionsmustern bei Diagnosestellung und Quantifizierung minimaler Resterkrankung im Behandlungsverlauf. Habilitationschrift 2003: 54–59.

[18] Macedo A, Orfáo A, Vidriales MB, López-Berges MC, Valverde B, González MD, Caballero MD, Ramos F, Martínez M, Fernández-Calvo J, Martínez A, San Miguel JF. Characterisation of aberrant phenotypes in acute myeloblastic leukemia. Ann Hematol 1995; 70: 189–194.

[19] Macedo A, Orfáo A, Gonzalez M, Vidriales MB, López-Berges MC, Martínez A, San Miguel JF. Immunological detection of blast cell subpopulations in acute myeloblastic leukemia at diagnosis: implications for minimal residual disease studies. Leukemia 1995; 9: 993–998.

[20] Nakamura K, Ogata K, An E, Dan K. Flow cytometric assessment of CD15+CD117+ cells for the detection of minimal residual disease in adult acute myeloid leukaemia. Br J Haematol 2000; 108: 710–716.

[21] Neale GA, Coustan-Smith E, Pan Q, Chen X, Gruhn B, Stow P, Behm FG, Pui CH, Campana D. Tandem application of flow cytometry and polymerase chain reaction for comprehensive detection of minimal residual disease in childhood acute lymphoblastic leukemia. Leukemia 1999; 13: 1221–1226.

[22] Orfao A, Cidaud J, Lopez-Berges MC, Lopez A, Vidriales B, Caballero MD, Valverde B, Gonzales M, San Miguel JF. Acute lymphoblastic leukemia (ALL): detection of minimal residual disease (MRD) at flow cytometry. Leuk Lymphoma 1994; 13: 87–90.

[23] Paietta E, Andersen J, Gallagher R, Bennett J, Yunis J, Cassileth P, Rowe J, Wiernik PH. The immunophenotype of acute promyelotic leukemia (APL): an ECOG study. Leukemia 1994; 8: 1108–1112.

[24] Paredes-Aquilera R, Romero-Guzman L, Lopez-Santiago N, Burbano-Ceron L, Camacho-del Monte O, Nieto-Martinez S. Flow cytometric analysis of cell-surface and intracellular antigens in the diagnosis of acute leukemia. Am J Hematol 2001; 68: 69–74.

[25] Plata E, Choremi-Papadopolou H, Viglis V. Flow-cytometric detection of minimal residual disease with atypical antigen combinations in patients with de novo acute myeloid leukemia. Ann Hematol 2000; 79: 543–546.

[26] Pui CH, Evans WE. Acute lymphoblastic leukemia. N Engl J Med 1998; 339: 605–615.

[27] Radich JP. Clinical applicability of the evaluation of minimal residual disease in acute leukemia. Curr Opin Oncol 2000; 12: 36–42.

[28] Reading CL, Estey EH, Huh YO, Claxton DF, Sánchez G, Terstappen LWMM, O, Brien MC, Baron S, Deisseroth AB. Expression of unusual immunophenotype combinations in acute myelogenous leukemia. Blood 1993; 81: 3083–3090.

[29] Terstappen LWMM, Koenemann S, Safford M, Loken MR, Zurlutter K, Buechner T, Hiddemann W, Woermann B. Flow cytometric characterisation of acute myeloid leukemia. Part II. Phenotypic heterogenity at diagnosis. Leukemia 1991; 5: 757–767.

[30] Terstappen LWMM, Koenemann S, Safford M, Loken MR, Zurlutter K, Büchner T, Hiddemann W, Wörmann B. Flow cytometric characterisation of acute myeloid leukemia. Part I. Significance of light scattering properties. Leukemia 1991; 5: 315–321.

[31] Terstappen LWMM, Loken MR. Myeloid cell differentiation in normal bone marrow and acute myeloid leukemia assessed by multi-dimensional flow cytometry. Analytical Cellular Pathology 1990; 2: 229–240.

[32] van Dongen JJ, Szczepanski T, de Bruijn MA, van den Beemd MW, De Bruin-Versteeg S, Wijkhuijs JM, Tibbe GJ, van Gastel-Mol EJ, Groeneveld K, Hooijkaas H. Detection of minimal residual disease in acute leukemia patients. Cytokines Mol Ther 1996; 2: 121–133.

[33] Venditti A, Buccisano F, del Poeta G, Maurillo L, Tamburini A, Cox C, Battaglia A, Catalano G, del Moro B, Cudillo L, Postorino M, Masi M, Amadori S. Level of minimal residual disease after consolitation therapy predicts outcome in acute myeloid leukemia. Blood 2000; 96: 3948–3952.

[34] Vošková D, Váleková Ľ, Kubisz P. Recent findings in the diagnosis of minimal residual disease in acute leukemias, focused on flow cytometry (in Slovak). Vnitřní lékařství 1997; 43: 332–336.