# The value of dot plot patterns and leukemia-associated phenotypes in AML diagnosis by multiparameter flow cytometry*

T. ŽELEZNÍKOVÁ, O. BABUŠÍKOVÁ

*Cancer Research Institute, e-mail: exontzel@savba.sk, Slovak Academy of Science, 833 91 Bratislava, Slovak Republic*

**Received May 9, 2005**

The immunophenotypic features in patients with acute myeloid leukemia (AML) were investigated at diagnosis using a wide antibody panel including progenitor-associated, myeloid and lymphoid markers in quadruple combinations. Analyzed were bone marrow samples from 37 adult and pediatric patients for exact identification of AML blasts according their localization on CD45/SSC dot plots and aberrant immunophenotypes in various subtype of AML.

We found the localization of AML blasts on CD45/SSC dot plots, which in combination with immunophenotype profile of blasts allow discrimination of several AML subtypes (M0-M2, M3, M4/M5 and other types). In 27/37 AML patients (73%) at least one leukemia-associated phenotype (LAP) was found, two or more aberrancies coexisted in more than a half of them (78%). Asynchronous expression was the most frequent type of LAP (77.8%, 21/27) followed by coexpression of lymphoid-associated antigens, which occurred in 18/27 (66.7%) patients.

Presented study showed that leukemic cells of each AML patient had a unique antigenic profile and could be discriminated from their normal counterparts based on typical light scatter profiles and aberrant antigen expression that could further be used for detection of minimal residual disease.

*Key words: AML, multiparameter flow cytometry, leukemia-associated aberrant immunophenotypes*

Multiparameter flow cytometric analysis of acute leukemia has become a powerful tool, combining the patterns and intensity of antigen expression, to characterize the myeloid or lymphoid origin of blast cell population and reach a final reliable diagnosis [6].

The bone marrow is a complex tissue containing cells of multiple hematopoietic lineages in various hematopoietic differentiation stages. Knowledge of the expression of different markers in normal hematopoietic development provides a frame of reference for identification of abnormal differentiation patterns [20]. At present, the association of side scatter (SSC) with an immunologic marker is the preferred method for the discriminating between normal and malignant cells. Many studies have shown that one of the best markers suitable for this purpose is pan-leukocyte marker CD45, which is differentially expressed at each stage of differentiation [2, 3, 9, 10, 14, 20].

Moreover, immunophenotyping has proved to be a reliable approach for minimal residual disease (MRD) investigation. Monitoring of MRD becomes increasingly important for the more accurate stratification of the therapy in acute leukemia [19]. Particularly it is known that in AML, the immunophenotype of leukemic cells may be heterogeneous and several leukemic subpopulations with different phenotypic patterns may be present at diagnosis. These subpopulations often correspond to different stages of maturation of the pathological clone and one of them may be resistant clone that may be responsible for the relapse of the disease [11, 15]. Recently, the accuracy of heterogeneous AML immunophenotyping was markedly improved by application of the multiparameter flow cytometry, the availability of great spectrum of monoclonal antibodies and by the improvement of the gating strategies [2, 8]. Precise identification of leukemic population is based on the identification of leukemia-associated immunophenotypes (LAPs) that allow the distinction of blast cells from normal cells because LAPs are absent or extremely infrequent in healthy peripheral blood and bone marrow samples. These LAPs can be identified in up to 90% AML patients by using multiparameter flow cytometry [2,

*This work was partly supported by Grant No.2/4021/5 from Slovak Grant Agency and grant of project GENOMICS, Subproject No. 9.

16]. These aberrant phenotypes generally result from 1 lineage infidelity: cross-lineage antigen expression (i.e. expression of lymphoid antigens in myeloid blasts and vice versa), 2) asynchronous antigen expression (expression of antigens and their combinations which are not found simultaneously in normal differentiation), 3) overexpression of antigens (i.e. presence of antigen in leukemic cells at abnormally high amount) and 4) the existence of abnormal light-scatter patterns (abnormal distribution of leukemic blasts on FSC/SSC dot plots according to their phenotype).

Presented study reports the diagnostic usefulness of multiparameter flow cytometric immunophenotyping of acute myeloid leukemias. The aim was to evaluate the effectiveness of localization AML blasts on CD45/SSC dot plots in reaching a definitive diagnosis. The next aim was to characterize the phenotype of leukemic populations in various AML subtypes and to identify and evaluate the occurrence of leukemia-associated phenotype in individual patients with AML for further investigation of minimal residual disease.

## Patients, material and methods

*Patients and samples*. Bone marrow (BM) samples from thirty-seven patients with newly diagnosed and untreated AML (4 children and 33 adult) were analyzed. The median age of patients at diagnosis was 47 years (range 3–80), and 17 were men and 20 were women. All cases included in this study were classified according to French-American-British (FAB) Cooperative Group criteria, immunological analysis and were submitted for cytogenetic analysis at the time of initial diagnosis.

The bone marrow samples of patients with AML were collected in heparine anticoagulant at the initial diagnosis and during disease duration. Normal BM samples, obtained from healthy donors, served as controls were analyzed by flow cytometry as described in detail below.

Briefly, combinations of antibodies (volume 10–20 µl) were added to $1x10^6$ BM cells (volume 100 µl) and incubated for 15 minutes in the dark, at room temperature. Isotype-matched antibodies were used as negative controls. After addition 100 µl of commercially available red cell lysing solution (Optilyse B, Immunotech, France), the samples were incubated for 10 minutes. Then 2 ml distilled water was added, incubated (in the dark) for another 10 minutes and followed by centrifugation (10 minutes, 1500 rpm). Finally, cell pellet was resuspended in 500 µl of phosphate-buffered saline (PBS).

*Flow cytometric analysis*. Monoclonal antibodies targeting membrane antigens directly conjugated by fluorochromes (fluorescein isothiocyanate [FITC], phycoerythrin [PE], R-phycoerythrin-texas red [ECD], phycoerythrin-cyanin 5 [PC5]) were used in the following combinations designed for the detection of myeloid markers and LAPs:
HLADR-FITC/CD13-PE/CD45-ECD/CD33-PC5;
CD11b-FITC/CD38(CD117)-PE/CD45-ECD/CD34-PC5, CD33-FITC/CD34-PE/CD45-ECD/CD7-PC5, CD4(CD2)-FITC/CD56-PE/CD45-ECD/CD34(CD3)-PC5,
CD14-FITC/CD13-PE/CD 45-ECD/CD19-PC5. For more particular analysis, some other monoclonal antibodies were used (Tab. 1). All antibodies were purchased from Immunotech (Marseille, France) except CD133 (clone 170411, R&D system, Deutschland). The list of all monoclonal antibodies used and their specificities are given in Table 1.

**Table 1. Monoclonal antibodies used for the immunostaining of AML patients**

| | |
|---|---|
| FITC (clone) | CD33(D3HL60-251), CD38(T16), CD14(RMO52), CD4(13B8.2), CD11b(BEAR1), CD65(88H7), CD2(39C1.5), CD16(3G8-FITC), CD36(FA6.152), CD41(P2),CD42b(SZ2), HLADR(Immu-357), GlycophorinA(D2.10) |
| PE (clone) | CD13(SJ1D1), CD117(104D2D1), CD34(581), CD5(BL1a), CD15(80H5), CD56(N901), CD61(SZ21), CD71(YDJ1.2.2) |
| PC5 (clone) | CD33(D3HL60-251), CD34(581), CD19(J4.119), CD7(8H8.1) |
| ECD (clone) | CD45(J33) |

Antigen expression on blast cells was systematically analyzed by multiparameter flow cytometry (EPICS ALTRA flow cytometer equipped by Expo 32 program for analysis). In samples at diagnosis at least 30.000 cells per tube were measured. LAPs were defined by gating of population that displayed an aberrant expression of antigens. In addition, CD45 intensity versus side scatter (SSC) was used to detect leukemic cells and separate them from the normal bone marrow populations. A case was considered as positive for specific antigen if the antigen was expressed at in least 20% of the leukemic cells of individual pathological population in sample (20% cut off level) and when the fluorescence intensity was clearly separated from negative controls (isotypic controls or negative cell population for the examined antigen).

## Results

The characteristics of 37 AML patients successfully immunophenotyped at diagnosis are detailed in Table 2. The distribution according to FAB classification and immunophenotype was as follows: M0 (5 patients), M1 (10), M2 (12 patients), M3 (3 patients), M4 (2 patients), M5 (3 patients), M6 (1 patient) and M7 (1 patient). The median age was 47 years and the median percentages of leukemic blasts in BM were 63%. In half of the patients, we were able to detect the presence of at least two subpopulations with different pathological phenotypic patterns.

In our study, we found combination of CD45 with side scatter (SSC) as very helpful not only for the discrimination between normal and malignant cells but also for diagnostic precision of their subtypes. The localization of blasts on

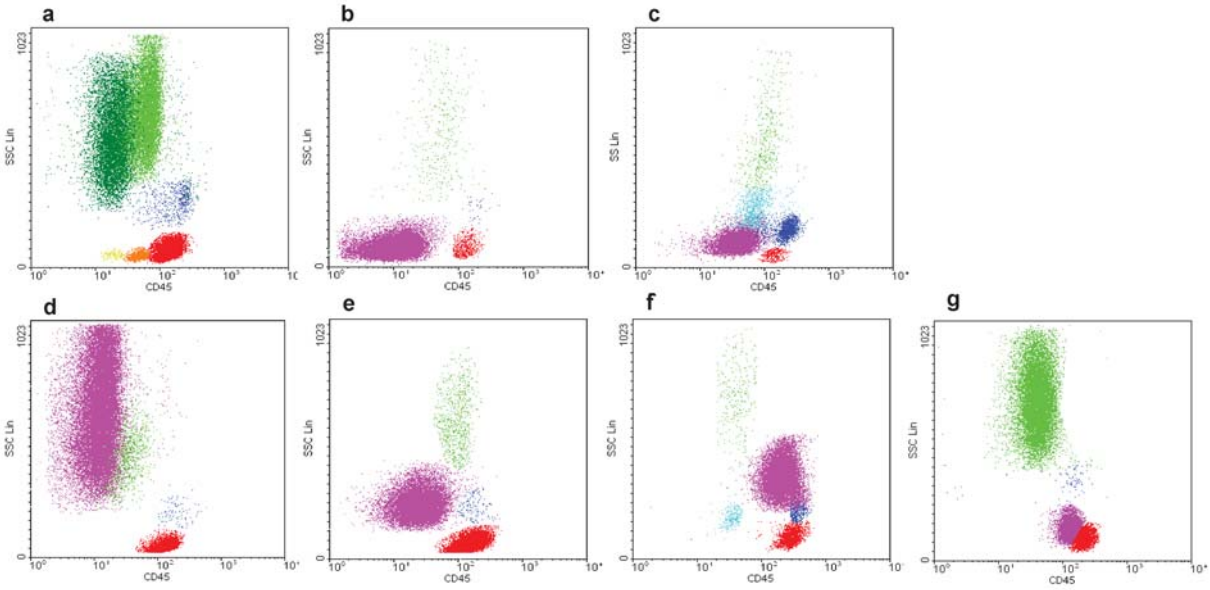


**Figure 1. The localization of several subtypes of AML blasts on CD45/SSC dot plots in comparison to populations of healthy bone marrow. In the dot plots, red dots depict lymphocyte, blue monocyte, green granulocyte and violet AML blasts. Plot (a) illustrates localization of population from healthy donor containing two maturation stages (hematogones, orange and yellow dots) of B-lymphocyte. Plot (b) shows AML M0 blasts with typical low SSC. Plot (c) illustrates two subpopulations of myeloblasts (violet and turquoise dots) in AML M2. Plot (d) exemplifies localization of hypergranular M3 blasts, plot (e) M4 blasts and (f) M5 blasts. Plot (g) shows atypical megakaryoblasts resembling lymphocyte.**

CD45/SSC dot plots allowed us to discriminate between several subtypes of AML (Fig. 1).

*Light scatter characteristic (CD45/SSC) of AML cells*. We observed that M0 blasts, which resembled lymphoblasts for the very small forward and side scatter characteristics, had typical localization on CD45/SSC dot plots (Fig. 1b). But M0 blasts could be clearly distinguished from lymphoblasts according to their immunophenotype.

M1 blasts due to the presence of a few azurophilic granules and occasional Auer rods expressing larger granularity were found to have slightly higher SSC and their localization on CD45/SSC dot plots was similar to M0 blasts. M2 blasts were seen to have higher SSC in comparison to M0/M1 blasts and on CD45/SSC dot plots in the majority of cases, several subpopulations of myeloblasts corresponding to different stages of maturation could be observed (Fig. 1c). For hypergranular variant of M3 blasts, a typical localization on CD45/SSC dot plots in region characteristic for mature granulocyte but with lower intensity of CD45 was found (Fig. 1d). The immunophenotype of M3 hypergranular blasts was found to be highly specific (Tab. 2) and in one case two subpopulations, which differ in two markers (CD2, CD34) have been observed (Fig. 2). The M4 and M5 blasts were seen to have higher FSC and SSC than M0/M1 blasts and their localization on CD45/SSC dot plots was found to merge into the monocytic region (Fig. 1e, f). In presented study one M7 case with atypical megakaryoblasts was observed, which resembled lymphocytes because of very low FSC and SSC values and also the localization on CD45/SSC was found to

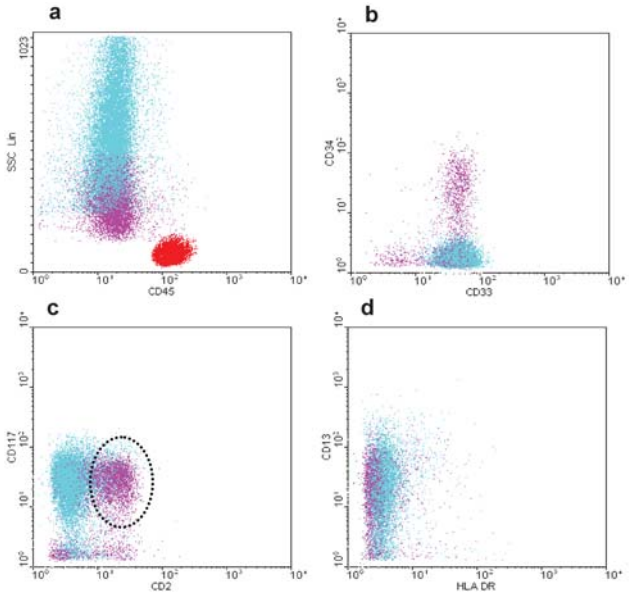


**Figure 2. The flow cytometric findings in acute promyelocytic leukemia (AML-M3). The leukemic populations are demonstrated as turquoise (typical hypergranular promyelocytes) and violet (CD34 positive blasts) dots and lymphocyte as red dots. Plot (a) illustrates typical high side scatter together with lower intensity of CD45 in both AML-M3 populations in comparison to mature granulocytes. Plots (b) and (c) illustrate the differences between these subpopulations in expression of CD34 and CD2. In the plot (c) blats (violet dots) coexpressing progenitor marker CD117 with T-lymphoid marker CD2 represent an example of LAP in AML (dashed circle highlights an empty space). Plot (d) illustrates characteristic lack of HLA-DR expression in both populations.**

**Table 2. Characteristics of AML patients and pattern of phenotypic expression according to FAB subtypes**

| | Age (y) | | WBC (x $10^9$/l) | | BM blasts (%) | | Sex (no.) | |
|---|---|---|---|---|---|---|---|---|
| Median | 47 (42M/50F) | | 58.8 | | 63 | | M | 17 |
| Range | 3-83 | | (0.8-369.0) | | (20-95) | | F | 20 |
| | FAB subtypes (No) and antigens expression and coexpression | | | | | | | |
| | M0 | M1 | M2 | M3 | M4 | M5 | M6 | M7 |
| No cases | 5 | 10 | 12 | 3 | 2 | 3 | 1 | 1 |
| Myeloid-lineage Ag | | | | | | | | |
| CD11b | **2** | 0 | **6** | 0 | 1 | 3 | 1 | 1 |
| CD13 | **5** | 10 | **10** | 3 | 1 | 2 | 1 | 1 |
| CD14 | **0** | 0 | **1** | 0 | 0 | 3 | 0 | 0 |
| CD15 | **0** | 0 | **3** | 0 | 2 | 2 | 1 | 0 |
| CD16 | **0** | 0 | **0** | 0 | 0 | 0 | 0 | 0 |
| CD33 | **3** | 9 | **11** | 3 | 2 | 3 | 1 | 1 |
| CD36 | **2** | 0 | **1** | 0 | 1 | 3 | 1 | 1 |
| CD41 | **0** | 0 | **0** | 0 | 0 | 0 | 0 | 1 |
| CD61 | **0** | 0 | **0** | 0 | 0 | 0 | 0 | 1 |
| CD65 | **0** | 0 | **3** | 3 | 2 | 1 | 0 | 0 |
| Non-lineage Ag | | | | | | | | |
| HLA DR | **5** | 10 | **9** | 0 | 2 | 3 | 1 | 0 |
| CD34 | **5** | 7 | **9** | 1* | 1* | 0 | 1 | 0 |
| CD117 | **5** | 10 | **9** | 3 | 1* | 1* | 1 | 0 |
| CD133 | **0** | 0 | **0** | 0 | 0 | 0 | 0 | 0 |
| CD71 | **4** | 0 | **2** | 0 | 1 | 0 | 1 | 0 |
| CD56 | **1** | 0 | **3** | 0 | 0 | 2 | 1 | 0 |
| CD38 | **5** | 10 | **12** | 3 | 2 | 3 | 1 | 1 |
| T,B-lineage Ag | | | | | | | | |
| CD7 | **4** | 6 | **6** | 0 | 0 | 0 | 1 | 0 |
| CD2 | **1** | 0 | **0** | 1* | 0 | 0 | 0 | 0 |
| CD19 | **0** | 0 | **2** | 1 | 0 | 0 | 0 | 0 |
| CD4 | **0** | 0 | **0** | 2 | 2 | 2 | 0 | 0 |

* expression on minor subpopulation

be very similar to the localization of lymphocytes (Fig. 1g). Their phenotypic lineage origin was made mainly on myeloid antigens detection.

*Identification of leukemia-associated immunophenotypes in AML*. All patients with AML were analyzed for exact myeloid-lineage determination and the expression of LAPs by multiparameter flow cytometry applying a broad panel of monoclonal antibodies described above. In 27 of 37 patients (72.97%), at least one LAP was defined at diagnosis. In 21 of these patients (77.78%) at least two LAPs were detected; two independent LAPs in 11 (52.4%), three LAPs in 5 (23.8%) and four or more LAPs in 5 (23.8%) of these cases. The percentage of AML blasts carrying the LAP was found to range from 13 to 100% and median LAP expression on blasts was 53.57%.

Total number of LAPs identified by application of various combinations of antibodies was 62 (mean = 2.3 LAIP/patient). The frequencies of LAPs are shown in Table 3. Most of the identified aberrant immunophenotypes were defined as asynchronous antigen expression (n=30) and cross-lineage antigen expression (n=22), while antigen over-expression was present in only two cases and aberrant light scatter pattern in eight cases.

Asynchronous expression was found in 21 (77.78%) of 27 AML patients and in majority of these cases at least one progenitor-associated marker (CD117, CD34) was included.

Lineage infidelity was present in 18 (66.67%) of 27 AML patients and the most frequent one was coexpression of CD7 (16 patients) followed by CD19 (four patients) and CD2 (two patients) (Fig. 2c). In one case, simultaneous expression of two T-lymphoid markers (CD7/CD2) in neoplastic myeloblasts were observed.

The overall incidence of overexpression was low (7.4%) and the only antigen involved was HLA-DR (Tab. 3). Abnormal light scatter patterns occurred in five patients (18.52%) and in all of them this aberrant expression involved antigen CD13 and in three of them also CD33.

In each LAP pan-leukocyte marker CD45 or a progenitor-associated antigens (CD34, CD117) were used for identifying AML blasts and as gating strategy. In our study, the most representative examples of these aberrancies are illustrated in Table 3.

## Discussion

A combined flow-cytometric evaluation of light-scattering properties and the immunophenotype pattern of acute myeloid leukemia (AML) cells from 37 patients was performed. Light-scatter characteristics of AML cells estimated by flow cytometry and multiple surface markers were analyzed and compared with FAB classification. A part of our study reports the diagnostic usefulness of localization of AML blasts on CD45/SSC dot plots, which in combination with immunophenotype profile of blasts allows to discriminate several AML subtypes. We were able clearly distinguish main AML subtypes: AML M0-M2, M3, M4/M5 and the other cases according to their physical properties and immunophenotypes. In some cases we were also able to discriminate M0/M1 from M2 subtypes. The most common FAB subtype was found to be AML-M2 (12 patients) and the most characteristic immunophenotype was found in patients with AML-M3. The immunophenotype of AML-M3 is highly specific [6] with the characteristic scatter features with the homogenous expression of CD33, CD117, CD38 and heterogeneous CD13 in absence of CD34 and HLA-DR. We also found similar phenotype in one M2 case; however the localization of blasts on CD45/SSC was characteristic for AML M2 subtype. This study demonstrates that multiparameter flow cytometry is a reliable technique in the diagnosis of AML, and some immunophenotypes correlate well with FAB subtype.

Simultaneous assessment of multiple surface markers at

**Table 3. Frequencies of aberrant phenotypes in AML patients (n=27)**

| Aberrant phenotypes (n=60) | No. of cases/% | % pos. AML blast (median) |
|---|---|---|
| Cross-lineage antigen expression (n=22) | | |
| CD7 | 16/59.23 | 20.2-98.6 (56.4) |
| CD19 | 4/15.4 | 20.1-45.3 (28.3) |
| CD2 | 2/7.7 | 19.6/90.2 (54.9) |
| Asynchronous antigen expression (n=30) | | |
| CD34+CD11b+CD117- | 3/11.5 | 35.1-80.5 (55.2) |
| CD34+CD11b+CD117+ | 3/11.5 | 13.2-67.4 (39.3) |
| CD34+CD15+ | 1/3.8 | 30 |
| CD117+CD65+ | 3/11.5 | 30-70.1 (37.7) |
| CD56+CD117+CD34- | 1/3.8 | 31.3 |
| CD56+CD34+CD117- | 1/3.8 | 83.2 |
| CD56+CD34+CD117+ | 3/11.5 | 33.7-99 (61.9) |
| CD33+CD34-CD56+ | 2/7.7 | 24/34 (29) |
| CD34+CD33+HLA-DR-CD13- | 2/7.7 | 84/90 (87) |
| CD34+CD33+HLA-DR+CD13- | 2/7.7 | 62.5/73.4 (67.9) |
| CD117+CD33+HLA-DR- | 6/23.1 | 33.6-80.1 (63) |
| CD117+CD13+CD33-HLADR- | 1/3.8 | 20 |
| CD33+CD34-HLADR-CD14-CD15- | 2/7.7 | 73.2/82.7 (77.9) |
| Antigen overexpression (n=2) | | |
| HLA-DR++ | 2/7.7 | 81/97 (89) |
| Abnormal light scatter (n=8) | | |
| Low FSC/SSC | | |
| CD13 | 5/18.5 | 38-84 (60.2) |
| CD33 | 3/11.5 | 73-96 (84.3) |

diagnosis helps both, to identify malignant cells (by their definition in the so-called 'empty spaces', where normal cells are absent) [13] and to determine the degree of immunophenotypic heterogeneity of the malignant cell populations. Based on our findings, in about half of the cases at least two subpopulations with different aberrant phenotypic patterns were detected and the heterogeneity of AML cases has been exactly confirmed.

In AML the expression of antigens is frequently heterogeneous and therefore it is not possible to include all leukemic cells into one LAP as a consequence of presence of several leukemic populations. Hence, the frequencies of LAP-positive cells are lower than described in acute lymphoblastic leukemia [4, 7].

At present, the incidence of aberrant phenotypes appropriate for MRD detection in AML ranges from 30–90%, depending on the criteria used for their definition and the panel of monoclonal antibodies employed [6, 16, 21, 22]. In this study, 73% of AML patients displayed LAPs, two or more aberrancies coexisted in more than a half of them (78%). The total 62 LAPs (mean = 2.3 LAIP/patient) were established at diagnosis in AML patients by applying a wide antibody panel including progenitor-associated, myeloid and lymphoid markers in quadruple combinations. There was no significant difference in age, leukocyte count or blast count between patients (n=27) with leukemia-associated immunophenotypes and those of patients (n=10) in which the study showed the lack of suitable aberrant immunophenotypes.

Asynchronous antigen expression (77.78%, 21/27) was the most frequent type of LAP in our study similarly to several studies that reported this aberrancy as the most frequent in AML [1, 13, 18]. In the other studies [7, 22] this aberrancy was reported to be less frequent what could be explained by the fact that, the lack of myeloid antigen expression was not considered as asynchronous expression. Many recent reports have analyzed the incidence of expression of lymphoid-associated antigens in AML blasts, the incidence ranged from 26% to 60%, with CD7 and CD2 being the most frequently found [1, 7, 12, 17, 23]. In this study the coexpression of lymphoid-associated antigens occurred in 67% of the patients with LAPs in agreement with studies of MACEDO et al [12] and VOSKOVA et al [23] who found incidence of 50% and 64% respectively in LAPs positive AML cases. It was shown in our study that the most frequent was the coexpression of CD7 (73%) which was found mainly in subtypes M0-M2. These findings are in accordance with findings of CASANOVAS et al [5] who found in 909 AML series decreasing of CD7 expression along with granulomonocytic AML maturation. In the study of SAXENA et al [19] the coexpression of CD7 antigen was associated with immature antigens (CD34, TdT), HLA-DR, and were found to be associated with worse clinical course. In this study, all CD7 positive cases also expressed HLA DR and either CD34 or CD117.

The overall incidence of antigen overexpression in AML is found up to 20–30% of AML patients and the overexpression of HLA-DR, CD34, CD33 and CD13 is reported most frequently [7]. In our study the incidence was relatively low (7%) and the only antigen involved was HLA-DR. The higher incidence than the overexpression in AML patients and abnormal light scatter pattern was found (18%), which involved myeloid markers CD13 and CD33 expression on myeloid blasts. These blasts resembled lymphoblasts due to their very low FSC/SSC. These findings are in accordance with results presented by SAN MIGUEL et al [17] who analyzed 126 adults suffering from AML for the presence of LAP and found 17% frequency of abnormal light scatter properties. The incidence of aberrant phenotypes in AML is still discutable and different results have been found which are probably based on the large variety of monoclonal antibodies used by different investigators.

In summary, we conclude that CD45/SSC pattern distribution of AML blast cells provides an additional objective and reproducible system for the exact classification of AML subtypes and in our study, the great majority of AML patients showed aberrant phenotypes appropriate for further detection of minimal residual disease.

The authors acknowledge the physicians from the National Cancer Institute and from the Department of Pediatric Oncology of University Children's Hospital, Clinic of Hematology and Transfusion of Medical Faculty, University Hospital, Bratislava, Slovakia for submitting patient samples and referrals. The authors thank Mrs. A. KOVARÍKOVÁ and Mrs. L. ŠTEVULOVÁ for their excellent technical support.

## References

[1] BAHIA DM, YAMAMOTO M, CHAUFFAILLE ML, KIMURA E, BORDIN JO et al. Aberrant phenotypes in acute myeloid leukemia: a high frequency and its clinical significance. Haematologica 2001; 86(8): 801–806.

[2] BASSO G, BULDINI B, DE ZEN L, ORFAO A. New methodologic approaches for immunophenotyping acute leukemias. Haematologica 2001; 86(7): 675–692.

[3] BOROWITZ MJ, GUENTHER KL, SHULTS KE, STELZER GT. Immunophenotyping of acute leukemia by flow cytometric analysis. Use of CD45 and right-angle light scatter to gate on leukemic blasts in three-color analysis. Am J Clin Pathol 1993; 100(5): 534–540.

[4] CAMPANA D, NEALE GA, COUSTAN-SMITH E, PUI CH. Detection of minimal residual disease in acute lymphoblastic leukemia: the St Jude experience. Leukemia 2001; 15(2): 278–279.

[5] CASASNOVAS RO, SLIMANE FK, GARAND R, FAURE GC, CAMPOS L et al. Immunological classification of acute myeloblastic leukemias: relevance to patient outcome. Leukemia 2003; 17(3): 515–527.

[6] KALEEM Z, CRAWFORD E, PATHAN MH, JASPER L, COVINSKY MA et al. Flow cytometric analysis of acute leukemias: Diagnostic utility and critical analysis of data. Arch Pathol Lab Med 2003; 127(1): 42–48.

[7] KERN W, DANHAUSER-RIEDL S, RATEI R, SCHNITTGER S, SCHOCH C et al. Detection of minimal residual disease in unselected patients with acute myeloid leukemia using multiparameter flow cytometry for definition of leukemia-associated immunophenotypes and determination of their frequencies in normal bone marrow. Haematologica 2003; 88(6): 646–653.

[8] KERN W, VOSKOVA D, SCHNITTGER S, SCHOCH C, HIDDEMANN W, HAFERLACH T. Four-fold staining including CD45 gating improves the sensitivity of multiparameter flow cytometric assessment of minimal residual disease in patients with acute myeloid leukemia. Hematol J 2004; 5(5): 410–418.

[9] LACOMBE F, DURRIEU F, BRIAIS A, DUMAIN P, BELLOC F et al. Flow cytometry CD45 gating for immunophenotyping of acute myeloid leukemia. Leukemia 1997; 11(11): 1878–1886.

[10] LI W, CHEN Z, LIU Z, ZOU P. Application of CD45/SSC gating multiparameter flow cytometry in the classification of acute leukemia-an analysis of 139 cases. J Tongji Med Univ 2001; 21(3): 209–211.

[11] MACEDO A, ORFAO A, GONZALEZ M, VIDRIALES MB, LOPEZ-BERGES MC et al. Immunological detection of blast cell subpopulations in acute myeloblastic leukemia at diagnosis: implications for minimal residual disease studies. Leukemia 1995; 9(6): 993–998.

[12] MACEDO A, ORFAO A, VIDRIALES MB, LOPEZ-BERGES MC, VALVERDE B et al. Characterization of aberrant phenotypes in acute myeloblastic leukemia. Ann Hematol 1995; 70(4): 189–194.

[13] MLCAKOVA A, BABUSIKOVA O. Multiparametric flow cytometry in detection of minimal residual disease in acute lymphoblastic leukemia of early B-cell phenotype. Neoplasma 2003; 50(6): 416–421.

[14] RAINER RO, HODGES L, SELTZER GT. CD 45 gating correlates with bone marrow differential. Cytometry 1995; 22(2): 139–145.

[15] SAN MIGUEL JF, CIUDAD J, VIDRIALES MB, ORFAO A, LUCIO P et al. Immunophenotypical detection of minimal residual disease in acute leukemia. Crit Rev Oncol Hematol 1999; 32(3): 175–185.

[16] SAN MIGUEL JF, MARTINEZ A, MACEDO A, VIDRIALES MB, LOPEZ-BERGES C et al. Immunophenotyping investigation of minimal residual disease is a useful approach for predicting relapse in acute myeloid leukemia patients. Blood 1997; 90(6): 2465–2470.

[17] SAN MIGUEL JF, VIDRIALES MB, LOPEZ-BERGES C, DIAZ-MEDIAVILLA J, GUTIERREZ N et al. Early immunophenotypical evaluation of minimal residual disease in acute myeloid leukemia identifies different patient risk groups and may contribute to post induction treatment stratification. Blood 2001; 98(6): 1746–1751.

[18] SAXENA A, SHERIDAN DP, CARD RT, MCPEEK AM, MEWDELL CC, SKINNIDER LF. Biologic and clinical significance of CD7 expression in acute myeloid leukemia. Am J Hematol 1998; 58(4): 278–284.

[19] VAN DER VELDEN VH, BOECKX N, VAN WERING ER, VAN DONGEN JJ. Detection of minimal residual disease in acute leukemia. J Biol Regul Homeost Agents 2004; 18(2): 146–154.

[20] VAN LOCHEM EG, VAN DER VELDEN VH, WIND HK, TE MARVELDE JG, WESTERDAAL NA, VAN DONGEN JJ. Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: reference patterns for age-related changes and disease-induced shifts. Cytometry 2004; 60B(1): 1–13.

[21] VIDRIALES MB, SAN-MIGUEL JF, ORFAO A, COUSTAN-SMITH E, CAMPANA D. Minimal residual disease monitoring by flow cytometry. Best Pract Res Clin Haematol 2003; 16(4): 599–612.

[22] VOSKOVA D, SCHOCH C, SCHNITTGER S, HIDDEMANN W, HAFERLACH T, KERN W. Stability of leukemia-associated aberrant immunophenotypes in patients with acute myeloid leukemia between diagnosis and relapse: comparison with cytomorphologic, cytogenetic, and molecular genetic findings. Cytometry 2004; 62B(1): 25–38.

[23] VOSKOVA D, VALEKOVA L, FEDOROVA J, HUDECEK J, KUBISZ P. Leukemic cells and aberrant phenotypes in acute leukemia patients: a flow cytometry analysis. Neoplasma 2003; 50(6): 422–427.