Aberrant phenotypes in acute myeloid leukemia: a high frequency and clinical significance

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Background and Objectives. Immunophenotyping is an essential method for diagnosis and classification of acute myeloid leukemias (AML), and its extensive use could identify blast cell subpopulations with aberrant phenotypes rarely seen in normal myelopoiesis. The aberrant phenotypes have been correlated with clinical, morphologic and prognostic features but their occurrence in AML differs in the various studies.

Design and Methods. In this study we analyzed 35 cases of AML, examining them for aberrant phenotypes by multiparametric flow cytometry. Co-expression of lymphoid-associated markers in myeloblasts and asynchronous antigen expression were correlated with clinical features.

Results. Aberrant phenotypes were found in 88.6% of the cases studied. In this group, cross-lineage antigen expression was present in 34.3% and asynchronous expression in 82.4% of the cases. CD7 was the most frequent lymphoid-associated antigen. Among the cases of asynchronous antigen expression, the most frequent phenotype was CD117$^+$ and/or CD34$^+$ in association with CD11c$^+$, followed by CD15$^+$ and CD65$^+$, corresponding to 67.6%, 61.7% and 50.0% of the cases, respectively. Twenty out of 33 patients were available for complete remission assessment. The CD117$^+$ CD15$^+$ phenotype correlated significantly with complete remission achievement and with the lack of unfavorable chromosome associations.

Interpretation and Conclusions. We conclude that aberrant phenotypes, as they are described here, are present in the great majority of cases of AML, asynchronous antigen expression being the most frequent example, and that CD117$^+$ CD15$^+$ phenotype shows a relevant association with clinical prognosis.

Key words: aberrant phenotype, flow cytometry, AML, prognosis.

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Acute myeloid leukemia (AML) is a heterogeneous disease, presenting with a high diversity of phenotypes. Immunophenotyping is essential for diagnosis and for definition of particular AML subtypes such as M0, M7 and M3 variants. In addition to contributing to the diagnosis, flow cytometry immunophenotyping can also evidence blast cell heterogeneity as reflected by the existence of a high variety of phenotypes, and detect antigen associations rarely seen in normal bone marrow cells. Early reports trying to correlate the phenotypes of leukemic blast cells with normal myeloid differentiating subsets did not observe equivalence between leukemic and normal cells of early myelopoiesis, and identified the presence of antigens not associated with normal myeloid differentiation.[1,2] However, some of the leukemic-associated phenotypes, despite being considered aberrant, are documented in normal bone marrow cells, albeit in very low numbers.[3,4] Currently, the aberrant phenotypes are classified into different types: co-expression of lymphoid-associated antigens or lineage infidelity; asynchronous antigen expression, in which early antigens are co-expressed with more mature ones; or antigen over-expression and existence of abnormal light scatter patterns.

The incidence of the aberrant phenotypes in AML is still controversial and divergent results have been found by different groups, probably because of the use of a large variety of monoclonal antibody (MoAbs) panels; incidences as high as 88 % have been reported.[4,5]

In order to evaluate the occurrence of aberrant phenotypes and to correlate their presence with the various morphologic subtypes, we analyzed 35 cases of *de novo* AML. Correlations between the aberrant phenotype and clinical response, patients' age, leukocyte count, and chromosomal abnormalities were also examined.

Design and Methods

Patients

Among 54 *de novo* AML adult patients consecutively diagnosed at our laboratory, 35 were investigated for aberrant phenotype expression. All cases were analyzed according to the French-American-British (FAB) Cooperative Group morphologic criteria, and classified as follows: M0 (1 case), M1 (7 cases), M2 (8 cases), M3 (4 cases), M4 (6 cases), M5 (6 cases), M6 (1 case) and M7 (2 cases).

Immunophenotyping studies

Erythrocyte-lysed whole bone marrow samples were analyzed by flow cytometry using a large panel of MoAbs in triple stainings [phycoerythrin (PE)/fluorescein isothiocyanate (FITC)/Peridin chlorophyll (PerCP)]: IgG1-FITC; IgG2a-PE; CD10-FITC; CD11c-PE; CD13-PE; CD14-PE; CD19-PE; CD33-PE; CD34-FITC; CD45-FITC/PerCP; HLA-DR-FITC (Becton Dickinson, San José, CA, USA); CD2-PE; CD4-PE; CD15-FITC; CD65-FITC; CD117-PE (DAKO, Glostrup, Denmark); CD7-FITC; CD38-PE; CD41-PE; CD56-PE (Immunotech, Marseille, France).

Data acquisition and analysis were performed on a FACScalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA) using Cell-Quest software. Calibration and fluorescence compensation were carried out using Calibrite beads (Becton Dickinson, San José, CA, USA) and immunoglobulin isotype-matched negative controls. Identification of blast cells was performed using forward scatter (FSC) versus side scatter (SSC) parameters and/or CD45 intensity versus SSC dot plots. Antigen expression was considered to be positive when the percentage of positive blast cells was equal or greater than 20%. Similarly, aberrant phenotypes were defined when at least 20% of the blast cells expressed that particular phenotype.

The studied aberrant phenotypes correspond to: co-expression of lymphoid-associated antigens (CD2, CD7, CD10, CD19) on myeloid blast cells, and asynchronous antigen expression based on reactivity on the blast cells for both mature and early myeloid cell associated antigens [$CD117^+$ $CD34^+$ and $CD15^+$ (or $CD65^+$ or $CD11c^+$ or $CD14^+$); $CD117^+$ $CD34^-$ and $CD15^+$ (or $CD65^+$ or $CD11c^+$ or $CD14^+$); $CD117^-$ $CD34^+$ and $CD15^+$ (or $CD65^+$ or $CD11c^+$ or $CD14^+$); $CD34^+$ $CD56^+$].

Clinical characteristics

Twenty out of 33 patients could be evaluated for complete remission achievement after one cycle of chemotherapy. The induction protocol consisted of cytarabine 200 mg/m^2/day on days 1-7 plus daunorubicin 45 mg/m^2/day on days 1-3 or idarubicin 12 mg/m^2/day on days 1-3. All AML-M3 cases also received all trans retinoic acid (ATRA) 45 mg/m^2/day until complete remission. Complete remission was defined according to criteria reported by Cheson *et al.* (<5% bone marrow blast cells and recovery of hematologic parameters).[6] Correlations between the presence of leukemia-associated phenotypes and the patients' age, initial leukocyte count, response to chemotherapy and karyotype features were evaluated. The karyotype was studied in bone marrow samples submitted to short term culture without mitogens. At least 15-20 G-banded metaphases were analyzed and abnormalities described following ISCN 1995. According to the literature, karyotypes presenting t(3;3)(q21;q26), t(9;22)(q34.1;q11.2), +8 and +11 were considered unfavorable.[7] The unusual abnormalities found [del(18q), t(1;2) and t(10;13)] were not considered unfavorable since reliable prognostic information could not be extracted.

Statistical analysis

The exact Fisher´s test was used to correlate the complete remission and karyotype findings with aberrant phenotype expression. Other clinical features such as age and leukocyte count were evaluated using the Mann-Whitney test. The statistical significance value was chosen to be 0.05.

Results

Out of the 35 cases, 31 (88.6%) showed aberrant phenotypes. More than one phenotypic aberration could be detected in 74.2% of the cases. Interestingly, a higher incidence of multiple (3 to 7) aberrant phenotypes was observed among M4 and M5 AML cases (80%) (Table 1).

Co-expression of lymphoid associated antigens and correlation with FAB subtypes

Co-expression of lymphoid-associated antigens was observed in 12 out of 35 cases (34.2%). The most frequent lymphoid antigen was CD7 (25.7%) followed by CD2 (11.4%), CD19 (8.6%), and CD10 in one case (Table 1). CD7 was observed in all AML subtypes except M3 and M6, and all three CD19 positive cases were diagnosed as AML-M2.

Asynchronous expression and correlation with FAB subtypes

Asynchronous expression, the most frequent aberration, was present in 82.4% (28/34) of the studied cases (Table 1). The mature antigens were more frequently seen in association with $CD117^+$ $CD34^+$ phenotype (17/28 cases), than with $CD117^+$ $CD34^-$ (9/28) or $CD117^-$ $CD34^+$ phenotype (2/28). The most frequent phenotype was $CD117^+$ and/or $CD34^+$ in association with CD11c (67.6% of the

Table 1. Correlation between FAB subtypes and aberrant phenotypes / AML case.

	FAB classification								
	M0	*M1*	*M2*	*M3*	*M4*	*M5*	*M6*	*M7*	*Total*
	(n=1)	*(n=7)*	*(n=8)*	*(n=4)*	*(n=6)*	*(n=6)*	*(n=1)*	*(n=2)*	*(n=35)*
Aberrant phenotypes	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N (%)*
CD2	0	1	0	0	1	2	0	0	4 (11.4)
CD7	1	2	1	0	2	2	0	1	9 (25.7)
CD10	0	1	0	0	0	0	0	0	1 (2.8)
CD19	0	0	3	0	0	0	0	0	3 (8.6)
$CD117^+$ $CD34^+$ $CD15^+$	0	4	3	0	4	2	0	0	13 (38.2)
$CD117^+$ $CD34^+$ $CD65^+$	0	2	2	0	4	2	0	0	10 (29.4)
$CD117^+$ $CD34^+$ $CD11c^+$	0	3	3	0	4	2	1	2	15 (44.1)
$CD117^+$ $CD34^+$ $CD14^+$	0	0	0	0	1	2	0	0	3 (8.8)
$CD117^+$ $CD34^-$ $CD15^+$	0	0	1	3	1	1	0	0	6 (17.6)
$CD117^+$ $CD34^-$ $CD65^+$	0	0	2	3	0	0	0	0	5 (14.7)
$CD117^+$ $CD34^-$ $CD11c^+$	0	2	2	0	1	1	0	0	6 (17.6)
$CD117^+$ $CD34^-$ CD14+	0	0	1	0	1	1	0	0	3 (8.8)
$CD117^-$ $CD34^+$ $CD15^+$	0	0	0	0	0	2	0	0	2 (5.9)
$CD117^-$ $CD34^+$ $CD65^+$	0	0	0	0	0	2	0	0	2 (5.9)
$CD117^-$ $CD34^+$ $CD11c^+$	0	0	0	0	0	2	0	0	2 (5.9)
$CD117^-$ $CD34^+$ $CD14^+$	0	0	0	0	0	2	0	0	2 (5.9)
$CD34^+$ $CD56^+$	0	0	2	0	0	2	1	0	5 (14.7)
N	1	7	7	3	5	5	1	2	31 (88.6)
>1 phenotype*	0	4	6	3	5	5	1	1	25 (80.6)
>3 phenotypes*	0	0	3	0	4	4	0	0	11 (35.5)

*N=number of cases with aberrant phenotype; *number of aberrant phenotypes/case; co-expression of lymphoid -associated antigens: 12/35 cases (34.3%). Asynchronous expression: 28/34 cases (82.4%)(not investigated in one patient).*

cases), followed by CD15 (61.7%) and CD65 (50.0%). All AML-M3 cases expressing the aberrant antigens showed only the following phenotypes: $CD117^+$ $CD34^-$ $CD15^+$ and $CD117^+$ $CD34^-$ $CD65^+$. Co-expression of CD56 and CD34 was found in five cases (M2, M5a and M6).

Aberrant phenotypes and their correlation with clinical features

The presence of $CD117^+$ $CD15^+$ asynchronous expression (either $CD34^+$ or $CD34^-$) showed the most relevant association between aberrant phenotype and response to treatment. Nine out of 11 (81.2%) patients expressing this phenotype achieved remission, in contrast to only 2 of 9 (22.9%) patients who did not show this aberration (p=0.0216) (Table 2). Other phenotypes ($CD117^+$ $CD11c^+$, $CD117^+$ $CD65^+$ or $CD7^+$) did not show a correlation with achievement of complete remission or any other analyzed clinical features. Analysis of karyotype demonstrated the presence of more unfavorable chromosome abnormalities among patients without $CD117^+$ $CD15^+$ phenotype (p=0.0230), while those usually associated with better prognosis were present among the $CD117^+$ $CD15^+$ positive group. Other clinical characteristics such as patients' age or leukocyte count did not show any correlation with this phenotype (Table 3).

Discussion

The high frequency of aberrant phenotypes observed in the present study is in accordance with more recent literature data. The large panel of MoAbs employed in our study certainly contributed to this finding. It should be emphasized that, in contrast to other reports,[4] a relatively high percentage cut-off value (≥20% of blast cells) was used in this study to characterize the presence of aberrant antigen expression in order to be certain of the association. It is interesting to note the finding of more than three aberrant phenotypes more frequently co-existing among AML cases with a monocytic component (M4 or M5).

Asynchronous expression was the most frequent type of aberration and the presence of early antigens in association with CD11c was slightly more frequent than in association with CD15 even when the $CD117^+$ $CD34^-$ or $CD117^-$ $CD34^+$ phenotypes were considered together, in contrast to other reports in the literature.[5] This fact may be due to the higher expression cut-off levels used in our study.

Co-expression of lymphoid-associated markers occurred in about 34% of the patients in agree-

Table 2. Correlation of aberrant phenotypes with complete remission achievement.

Aberrant phenotypes N (total=20)	*Complete remission N=11*	*Not complete remission N=9*
CD117+CD15+*		
(+)	9	2
(-)	2	7
CD117+CD11c+		
(+)	5	6
(-)	6	3
CD117+CD65+		
(+)	5	3
(-)	6	6
CD117+CD14+		
(+)	1	1
(-)	10	8
CD34+CD56+		
(+)	2	1
(-)	9	8
CD2+		
(+)	2	1
(-)	9	8
CD7+		
(+)	1	3
(-)	10	6
CD10+		
(+)	0	1
(-)	11	8
CD19+		
(+)	1	0
(-)	10	9

**p = 0.0216.*

ment with other reports which showed incidences of 40% in all AML cases.[4,5] Despite the fact that CD4 is not considered as an aberrant phenotype, it was expressed in 51.4% of the cases among different FAB subtypes (M1, M2, M4, M5 and even M7) (data not shown).

Detection of aberrant phenotypes is of clinical importance not only for accurate diagnosis of AML, but potentially also for AML sub-classification and comparison with prognosis. Associations of some antigens with special FAB subtypes of AML are well documented in the literature, e.g. the presence of CD19 in AML-M2 with the t(8;21) translocation. This type of AML is associated with a good prognosis.[4,8-11] In our study, all three CD19 positive cases were diagnosed as having AML-M2, and the t(8;21) translocation was detected in two of them.

Table 3. Characteristics of AML patients in relation to CD117+ CD15+ expression.

Clinical features	*CD117+CD15+ Positive*	*CD117+CD15+ Negative*
Complete remission*	9/11	2/9
Age (mean ± SD)	36.5±16.5	45.9±19.8
Leukocytes (×10⁹/L) (mean± SD)	44.5±51.6	30.2±28.3
Cytogenetic findings	t(3;3), 1 case t (1;2), 1 case t(8;21), 2 cases t(15;17)-2 cases t(10;13), 1 case 46,XY, 1 case del(18), 1 case	t(9;22), 2 cases 46,XY, 1 case trisomy 8, 1 case trisomy 11, 1 case
Unfavorable chromosome°	1/9	4/5

**p=0.0216 ; °p=0.0230.*

Recent reports have addressed the ability of immunophenotyping to predict the detection of AML-M3 with t(15;17). This information is of great utility to clinicians for the therapeutic decision-making process, since, although M3 with t(15;17) usually shows an initially dramatic clinical course with severe bleeding complications, it rapidly responds to treatment with ATRA. The M3 leukemic cells display a specific pattern of the following antigens: absence of CD34, low expression of CD15 and heterogeneous pattern of CD13, in a single blast cell population.[11] This pattern was also observed in 3 of 4 cases of promyelocytic leukemia [t(15;17)] studied. Interestingly, it should also be highlighted that CD117+ CD34- CD15+ and CD117+ CD34- CD65+ asynchronous antigen expression was present in 3 of the 4 AML-M3 cases, and CD15 expression never acquired high levels.

Prognostic factors which correlate with aberrant phenotypes have been reported, including the presence of CD56 in both M2 and M3 AML subtypes in association with a worse clinical course.[12-15] Another antigen that has been related to a poor prognosis in AML is the CD7 antigen. The co-expression of this antigen with early ones is described in AML with an aggressive clinical course.[16] In our study, 7 out of 9 CD7 positive cases also expressed CD34 which has been described in association with the multiple drug resistant protein (MDR) and a worse prognosis. CD7 expression occurred more among non-responders, but this association did not reach the level of statistical significance probably because of the small sample size.

The co-expression of lymphoid-associated anti-

gens has been better evaluated in terms of prognostic significance, although the value of different forms of asynchronous expression has not been well defined. Nakamura *et al.*[17] were the first to study the correlation between CD117$^+$ CD15$^+$ expression and achievement of clinical remission. This phenotype was more frequent among younger patients, and correlation with karyotype abnormalities was not seen. In our study, the mean age was also lower in this group, although not statistically significant (again, perhaps due to the small number of cases). However, the significant correlation of CD117$^+$ CD15$^+$ phenotype with complete remission reinforces its value as a good prognostic factor in AML. Additionally, it should be highlighted that unfavorable chromosome abnormalities were seen only among CD117$^+$ CD15$^+$ negative patients and, surprisingly, well defined good prognosis chromosomal abnormalities were seen only in the CD117$^+$ CD15$^+$ positive group (Table 3). Although correlations between other forms of asynchronous expression and achievement of complete remission did not reach the level of statistical significance, we observed that the presence of the CD117$^+$ CD65$^+$ phenotype was higher among the group achieving complete remission than among the group that did not. Considering our small sample size, additional studies should address this question.

In summary, we conclude that in our series the great majority of AML cases showed aberrant phenotypes. These phenotypes might be associated with different leukemia subtypes that should be studied for better understanding of their biological significance. The aberrant phenotypes can be useful for the diagnostic process and for therapeutic decision making, especially in some particular AML subtypes such as M2 t(8;21) and M3 t(15;17). The CD117$^+$ CD34$^+$ CD15$^+$ or CD117$^+$ CD34$^+$ CD15$^+$ is widely expressed in AML cases adding important information for prognosis and, at the same time, could be of help when looking for minimal residual disease during morphologic remission.[18-21] For all these purposes, the most useful markers in our experience include CD117, CD34, CD15, CD65, CD7 and CD19, and therefore we strongly recommend their analysis in all AML diagnostic samples.

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DMMB: conception and design, literature search, analysis and interpretation of data; MY: conception, design, interpretation of data, drafting the article and revision; EYSK: technical support, analysis of data; MdLLFC: cytogenetic studies; JOB: financial support and comments on drafts; MACF: technical support; JK: final approval of the version to be submitted.

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Disclosures

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Potential implications for clinical practice

Immunophenotyping provides an accurate diagnosis and differential diagnosis of AML, leading to appropriate management. Aberrant phenotypes may be useful for the detection of minimal residual disease.[22]

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