EVALUATION OF MOLECULAR-CYTOGENETIC ABERRATIONS AND OVERALL SURVIVAL IN MYELOID ANTIGEN POSITIVE ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Summary. Leukemic cells from a significant number of adults with acute lymphoblastic leukemia (ALL) show aberrant co-expression of myeloid-associated markers. We studied the incidence and relations of myeloid-antigen (MyAg) expression to molecular-cytogenetic features of ALL and to outcome. Leukemic blasts from 33 newly diagnosed adults with untreated ALL were examined for myeloid surface antigen expression. The simultaneous expression of lymphoid-associated antigens and myeloid-associated antigen (CD33, CD13, CD15) on leukemic cells was detected by a standard two-color direct immunofluorescent assay. As a result, MyAg(+) ALL was established in 45% of the B-cell lineage. Immunologic subtyping of B-ALLs revealed an association between common B phenotype and coexpression of myeloid antigens – 53% of MyAg(+)ALL ($P < 0.05$). Various cytogenetic abnormalities, associated with MyAg(+) ALLs, were detected, including t(9;22), 11q23 abnormalities, del 4p, and del 12p. No differences in complete remission rate ($p = 0.51$) and overall survival ($p = 0.75$) were observed between MyAg(+) and MyAg(-) patients. In conclusion, a high incidence of poor prognostic chromosomal aberrations was recorded in MyAg (+) cases, but the aberrant myeloid antigen expression was not shown to have an impact on the outcome of B-ALL.

Key words: *cytogenetic aberrations, myeloid antigens, adult ALL, survival*

INTRODUCTION

Immunologic characteristics of adult acute lymphoblastic leukemia (ALL) show considerable differences in terms of presentation, molecular-cytogenetic patterns and clinical outcomes. After recent developments in molecular genetics and immunophenotyping of ALL, various subtypes were defined with different prognostic significance [1, 2, 3, 6, 7].

Aberrant co-expression of myeloid-associated markers on lymphoblasts is a well-known phenomenon and in ALL it has been reported in 10-47% of cases [1, 2, 4]. The prognostic value of aberrant myeloid antigens MyAg (+) expression is still controversial. Although the few early adult ALL studies had shown an inferior outcome for myeloid antigen positive patients [4], the recent data demonstrated no prognostic correlation using high-dose chemotherapies [2, 3, 13,14, 18].

The aim of this study was to correlate MyAg expression with clinical, hematologic and biological parameters, and to analyze their impact on the treatment response and prognosis in a small series of adult ALL.

PATIENTS AND METHODS

Patients

The present study included 33 patients with *de novo* adult acute lymphoblastic leukemia (ALL) for whom a complete set of clinical, immunophenotypic and molecular-cytogenetic information was available. The patients were treated according to the GET-LALA-94 (Groupe d'Etude et de Traitement de la Leucémie Aiguë Lymphoblastique de l'Adulte) [17] ALL protocol for adult. Diagnosis of ALL was based on the French-American-British (FAB) classification system's morphological and cytochemical criteria, and on lymphoid immunophenotype. A *complete remission* (CR) was defined as 5% or less blast cells in normocellular or hypercellular bone marrow, a normal peripheral and differential blood count and exclusion of extramedullary disease. *Remission time* was defined as time from diagnosis to remission. A *resistant disease* (RD) was accepted if CR was not achieved after three courses of induction therapy. *Overall survival* was measured from the time of treatment study to the time of death.

Immunophenotyping

For immunophenotyping, leukemic cells obtained from fresh bone marrow or peripheral blood samples, collected in EDTA-containing tubes, were analyzed. Surface, cytoplasmic, and nuclear antigens were detected via a standard 2-color direct immunofluorescence assay using a broad panel of commercially available lymphoid and myeloid-associated monoclonal anti-

bodies (MoAbs). According to the European Group for Immunophenotyping of Leukemia (EGIL) [1], the stage of B-lineage acute leukemias was defined as follows: pro-B-ALL (BI): CD19+ / CD22+ / cyCD79a+ / CD10- / cyIg- / sIg-; common B-ALL (BII): CD10+ (CALLA+) / cyIg- / sIg-; pre-B-ALL (BIII): CD10+/- / cyIg+ / sIg-; mature B-ALL (BIV): sIg+. T-lineage ALL was characterized based on CD1a, CD2, CD3, CD4, CD5, CD7 and CD8 cell marker expression. Lineage assignment was based on the positivity of at least two lineage-specific antigens. Myeloid markers (CD13, CD14, CD15, and CD33) were also tested. Markers were considered positive when present on more than 20% of the blast cells.

Cytogenetics and fluorescent in situ hybridization (FISH)

Metaphases from short-term (24 hours) and long-term (48 hours) bone marrow cultures were prepared according to standard methods. G-banded chromosomes were classified following the International System for Human Cytogenetic Nomenclature [10]. A minimum of twenty G-banded metaphases were required to consider the case evaluable.

FISH analysis was performed on cytogenetic preparations obtained from bone marrow cells. Direct labeling locus-specific probes (Vysis, Ltd.) were used for MLL gene rearrangements, bcr/abl gene fusion, and C-MYC rearrangements. The size of genetically abnormal clones was determined after analyzing at least 100 successfully hybridized cells.

Statistical analysis

Statistical analysis was performed taking into account gender, age, white blood cell count, hemoglobin level, platelet count, presence or absence of CD13, CD33 and CD15 antigens, cytogenetic and FISH data. Three- and 5-year survival was estimated using the life table's method. Kaplan-Meier [12] curves were constructed for CR time and survival; a Log rank test was used to compare these curves in both cytogenetic groups. Comparison of quantitative variables between patient groups was performed using one-way analysis of variance. Comparison of qualitative data was performed using the chi-square test and t-test. All statistical analyses were 2-sided. P values < 0.05 were considered statistically significant.

RESULTS

Clinical features

The clinical and biological characteristics of the 33 patients at presentation are shown in Table 1.

Table 1. Presenting biological and laboratory features of adult ALL patients, according to myeloid antigen expression

Parameters	All cases	MyAg(+)	MyAg (-)	P-value
Total number (n /%)	33/100	15/45	18/55	0.67
Gender-M/F	19/14	13/2	6/12	0.002
Age-years median (range)	40.8 (18-74)	39..5 (18.69)	42 (19-74)	0.78
WBC x10^9/l median (range)	16.5 (1.7-300)	42.2 (1,7-300)	21.6 (1.8-90)	0.009
Hb g/l median (range)	91.1 (49-161)	93.7 (49-161)	88.4 (58-149)	1
PLT x10^9/l median (range)	81 (7-214)	86 (7-196)	76 (5-214)	0.43
Bone marrow blasts median (range)	82 (30-100)	86 (34-100)	78 (30-100)	0.46
Immunophenotype B-lineage/T-lineage	28/5	15/0	13/5	0.03
Extramedulary involvement (n / %)	8/24.2	6/40	2/11	0.002
CR rate (n/%)	22/66.7	12/80	10/55.6	0.51
Resistant Disease (n / %)	14/42.2	7/46.7	7/39	0.38
Time to remission (median, months)	2.3	2.3	1.9	0.75
3-years OS (%)	45.2	26.7	18.5	0.57
5-years OS (%)	38.5	20.0	18.5	0.83
Overall survival (median, months)	12.33	11.1	12.4	0.75

Immunophenotyping

A total of 5 cases (15.2%) were diagnosed as pro-B ALL, 17 (52%) were common ALL, 2 (6.0 %) were pre-B ALL, 4 (12.0%) were mature-B ALL and 5 (15.2%) were T-ALL. Immunologic subtyping of B-ALLs revealed an association between common B phenotype and coexpression of myeloid antigens – 53% of MyAg(+) ALL ($P < 0.05$).

In 15 cases (45%), myeloid antigens (CD13, CD15 and CD33) were expressed. B-ALL expressing MyAg were found in all stages of cell maturation.

Cytogenetic and molecular analysis

A wide variety of cytogenetic abnormalities were observed in the patients with myeloid-antigen-positive ALL (Tabl. 2). Non-random and random cytogenetic aberrations were found in 11 of 15 (73.3%) of MyAg(+) ALL and in 6 of 18 (33.3%) of MyAg(-) ALL. Cytogenetic abnormalities that have been associated with MyAg(+) ALLs were common, including hyperdiploidy, t(9;22)/bcr-abl, 11q23/MLL abnormalities, t(8;14)/C-MYC, del 4p, and del 12p. All cases with complex karyotype (more than 5 aberrations) expressed aberrant myeloid antigens.

Table 2. Cytogenetic and molecular aberrations in adult ALL patients

	All cases N (%)	MyAg(+)ALL N (%)	MyAg (-) ALL N (%)	P-value
Abnormalities of cell ploidy				
Normal Diploid	16 (48.5)	4 (26.7)	12 (66.7)	0.02
Pseudodiploid (46, abnormal)	13 (49.4)	7 (46.7)	6 (33.3)	0.2
Hyperdiploid > 46	4 (12.1)	4 (26.7)	0	0.02
Structural abnormalities				
Non-random	12 (36.3)	8 (53.3)	4 (22.2)	0.002
Random	5 (15..2)	3 (20.0)	2 (11.1)	0.14
Normal diploidy	16 (48.5)	4 (26.7)	12 (66.7)	0.02
t(9;22)/bcr-abl	5 (15.2)	4 (26.7)	1(5.5)	0.001
t(11q23)/MLL	2 (6.1)	1(6.7)	1(5.5)	
t(8q24)/C-MYC	4 (12.1)	3 (20.0)	1 (5.5)	
t (1;19)/E2A-PBX1	1(3.0)	1 (6.7)	0	
complex karyotype	2 (6.1)	2 (11.1)	0	

Differences in CR rates and overall survival

CR was estimated in 22 of 33 ALL cases (66.7%) without significant difference between MyAg (+) and MyAg (-) patients (Tabl. 1). The estimated 3- and 5-years survival for patients with ALL was 18.5 % for those without myeloid-antigen expression and 26.7% and 20% respectively for those with myeloid-antigen expression (p = 0.57 for 3-years survival, p = 0.83 for 5-years survival). Overall survival showed minimal differences without clinical and statistical significance (fig. 1) – 11.1 months for MyAg(+) vs. 12.4 months for MyAg (-) cases, p = 0.75 (Tabl. 1, fig. 1).

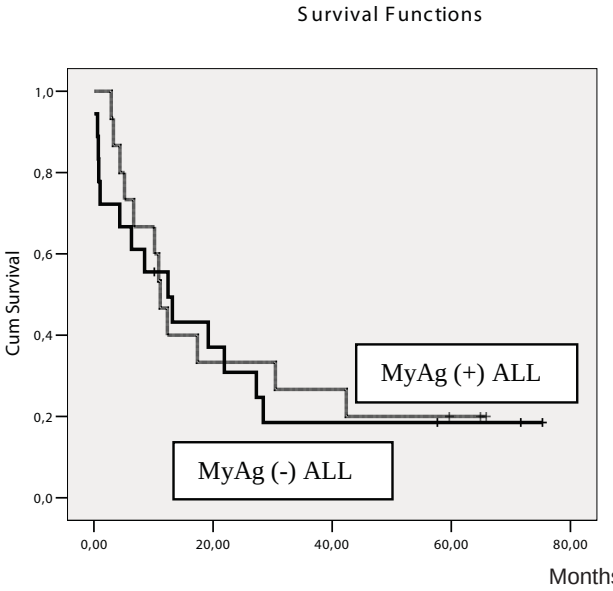

Fig. 1. Kaplan-Meier analysis of overall survival (months) in adult ALL patients with and without MyAg expression: MyAg(+) – 11,1months (95% CI: 8.39-13,86) and MyAg(-) – 12.4 months (95% CI: 3.59 – 21.27).

DISCUSSION

This study compared the biology and laboratory characteristics, molecular-cytogenetic data, the achieved complete remission rate and overall survival of 33 adults with newly diagnosed acute lymphoblastic leukemia according to myeloid antigen expression on leukemic cells.

The overall incidence of MyAg expression in our study (45%) is in line with the data reported in the literature [1, 2, 13]. The presence of MyAg was correlated with a number of clinical and biological data. The MyAg(+) cases were significantly more frequent in males than in females (87% vs. 33%, p = 0.002). MyAg (+) ALL is characterized with higher values of white blood cells (42.2 x 10^9/l vs. 21.6 x 10^9/l, p = 0.009) and higher incidence of extramedullary involvement (40% vs. 11%, p = 0.002), compared to MyAg (-) B-ALL.

For immunophenotypic data, there was a significant difference in the incidence of aberrant MyAg expression between B-lineage ALL and T-ALL subgroups (p = 0.03).

In this study we found a high incidence of structural chromosomal aberrations (73.3% vs. 33.3% in MyAg(-) cases, p = 0.02) and a lot of them were with poor prognostic significance- t(9;22)/bcr-abl, t(8q14)/C-MYC, t(1;19)/E2A-PBX1 and complex karyotype. Hyperdiploidy was found in 4 of 15 MyAg (+) ALL, all with structural changes. Leukemic blasts in all t(9;22)/bcr-abl (+) cases expressed B-lineage specific markers and aberrant myeloid markers in 4 of 5 cases. According to some authors [13, 15, 16] the frequency of myeloid antigen expression in bcr-abl (+) cases is higher but further studies will be required to determine the association of specific karyotype abnormalities with aberrant myeloid expressions.

A lot of published data demonstrated no prognostic correlation of aberrant myeloid expression using high-dose chemotherapies [2, 3, 11, 13, 14, 18]. In our study the presence of aberrant MyAg did not affect the achievement of CR and overall survival; no differences were found between MyAg(+) and MyAg(–) cases in OS at 3 and 5 years. The median time to achieve remission shows no significant difference for both groups – 2.3 months for MyAg(+) and 1.9 months for MyAg(–) patients (p = 0.75). No statistical differences in RD were recorded, but the percentage of resistant patients was higher in MyAg(+) ALL compared to MyAg(–) (46.7% vs. 39%, respectively).

Even though the myeloid-lineage antigen expression is associated with a high incidence of non-random genetic abnormalities, it lacks a prognostic value in adult ALL. This has clinical implications in terms of therapeutic decisions [8] (e.g. anti-CD33 treatment), but the expression of MyAg per se is not related to short or long-term prognostic significance.

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