GENETIC ABERRATIONS IN ADULT CD10 POSITIVE/ CYTOPLASMIC IGM NEGATIVE B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Summary. In the recent years progress in the basic laboratory science has allowed for implementation of many advanced methods in the clinical practice. Flow cytometry is a method, which is most commonly used for clinical diagnosis of 'de novo' acute leukemias, and in combination with molecular-cytogenetic studies provides more opportunities for determination of patients' risk factors and disease prognosis. We report on high percentage of adult CD10(+)/cyIgM(-) B-cell acute lymphoblastic leukemia (56% of all studied B-ALL) and unusual for this group chromosome findings – (8q24)/C-MYC, trizomy 8, t(1;19), no ploidy changes and low t(9;22) frequency.

Key words: *cytogenetics, adult acute lymphoblastic leukemia, CD10(+) acute leukemia*

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant (clonal) disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the bone marrow. ALL may be distinguished from other malignant lymphoid disorders by the immunophenotype of the cells, immunochemistry, cytochemistry and cytogenetic markers.

The malignant cells of ALL are lymphoid precursor cells (i.e., lymphoblasts) that are arrested in an early stage of development. This arrest is caused by an abnormal expression of genes, often as a result of chromosomal translocations. Chromosomal abnormalities detection in leukemic patients is very helpful in estab-

lishing the correct diagnosis and very often provides information concerning the disease prognosis.

Advances in immunophenotyping, cytogenetics, and molecular genetics and their combined application to characterize leukemic blasts have not only improved our knowledge of the pathobiology of ALL, but have also contributed to a correct identification of distinct clinicopathologic entities [1, 4, 6]. CD10, initially known as CALLA, was identified as one of the earliest markers expressed by leukemic cells of the lymphoblastic lineage [3]. In B-lineage leukemia, CD10 expression is involved in the definition of EGIL B-I versus B-II stages: it must be absent from B-I blasts and present on B-II blasts [2]. According to EGIL the combination of CD10 (+) expression and the absence of cytoplasm/surface IgM synthesis (cyIgM/sIgM-) characterizes common B-cell ALL [2].

Positive CD10 expression was associated with favorable clinical outcome in children [15, 18]. In addition, lower white blood cell count (WBC), younger age, and hyperdiploidy were associated with higher expression of CD10 [16]. In contrast to childhood ALL, the cell-biological features and genetic aberrations of CD10+/ cyIgM-B ALL have not yet been determined in larger series of adult patients.

AIM OF THE STUDY

The aim of the present study was to analyze the frequency and cytogenetic features of adult CD10+/ cyIgM (-) B-ALL and to compare our data with data available in the literature.

PATIENTS AND METHODS

Patients: Bone marrow aspirates from 25 consecutive adult patients (over 18 years) with B-cell acute lymphoblastic leukemia were analyzed for a 3-year period. B-cell ALL was selected upon immunophenotyping and further subdivided, based on the maturation stage of leukemic blasts. Clinical information including age, sex and mode of presentation was determined from data available to the laboratory.

Immunophenotyping: Leukemic cells were analyzed from fresh bone marrow samples collected in EDTA-containing tubes. Surface, cytoplasmic and nuclear antigens were detected by a standard two-color direct immunofluorescent assay, with the use of a broad panel of lymphoid- and myeloid-associated, commercially available monoclonal antibodies.

According to the European Group for Immunophenotyping of Leukemia (EGIL) [9], B-lineage acute leukemias were separated into the following 4 groups: *pro-B-ALL (BI)*: CD19+, CD22+, cyCD79a+, CD10- cyIg-, and sIg-; *common B-ALL (BII)*: CD10+ (CALLA+), cyIg-, sIg-; *pre-B-ALL (BIII)*: CD10+/-, cyIg+, and sIg-; sIg+. T-lineage ALL was characterized based on CD1a, CD2, CD3, CD4, CD5, CD7, and CD8 cell marker expression. Myeloid markers (CD13, CD33, CD14, and CD15)

were tested in most patients. Results were considered positive if > 20% of the cells expressed a particular antigen.

Conventional cytogenetics: We have used the routine bone marrow cytogenetic investigation including both direct and indirect methods (bone marrow samples, bone marrow cell cultivation, obtaining metaphase spread, G – banding, karyotyping by using the IKAROS Metasystems software program). G-banded chromosomes were classified according to the International System for Human Cytogenetic Nomenclature [9]. A minimum of twenty G-banded metaphases were required to consider the case evaluable.

Fluorescent in situ hibridization (FISH): 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.

RESULTS

Clinical and laboratory characteristics of CD10(+)/cyIgM(-) B-ALL patients. Clinical and laboratory characteristics of patients are presented in Table1. Median age of disease presentation was 47 years with equal sex distribution. Median WBC count and median bone marrow blast fluctuated in wide ranges: from 1.7 to 69.6 x109/l and from 36% to 95%, respectively.

Table 1. Clinical and laboratory characteristics of CD10(+) /cyIgM(-) B-ALL patients

Parameters	Median (range)
Age (years)	47 (31-69)
Sex (male/female)	7/7
WBC count (x10^9/l)	24.6 (1.7-69.6)
Bone marrow blasts (%)	56 (36-95)

Immunophenotyping data. The B-cell lineage of acute leukemia cells was accepted when at least two of the B-lineage markers were positive (EGIL, 1995). The expressions of CD10 (CALLA) without cytoplasmic synthesis of Ig were characteristic features of common B-ALL (BII), which was the most frequent subtype of B-ALL. However, CD10 antigen can also be found in pre-B-ALL, as well as in mature ALL. For these reasons our finding of 14/25 (56%) CD10+ cases among all B-ALL, is not striking. All patients, enrolled in our study, were found to have common B-ALL with CD10(+)/ cyIgM (-) expressions. Immunophenotyping data of all B-ALL patients is presented in Table 2.

Table 2. Immunophenotyping data of all B-ALL patients

EGIL group	N	%	Aberrant myeloid expression
pro-B-ALL (BI)	5	20.0	2/5 cases
common B-ALL (BII)	14	56.0	7/14 cases
pre-B-ALL (BIII)	2	8.0	2/2 cases
mature B-ALL (BIV)	4	16.0	2/4 cases
Total	25	100.0	13/25 cases

Cytogenetic and molecular analyses. Cytogenetic and/or molecular abnormalities were detected in 8/14 (57%) CD10(+)/cyIgM (-)ALLs (Table 3). In case 4 chromosome studies exhibited poor morphology and cytogenetic assay was unsuccessful.

Numerical aberrations: Ploidy changes were not established in this patient group. The only numerical aberration was trizomy 8 (case 6, Table 3). Leukemic blasts in this case showed co-expression of myeloid-lineage markers CD33(+) and CD13(+).

Table 3. Conventional cytogenetic and FISH results of CD10(+)/cyIg(-) B-ALL patients

N	Conventional cytogenetics	FISH
1	46, XY	Bcr/abl (-), MLL(-)
2	46, XY	Bcr/abl (-), MLL(-)
3	46,XY, t(1;19)(q23;p13)	PBX1/E2A (+)*
4	unsuccessful	C-MYC (+), Bcr/abl (-), MLL(-)
5	46, XX, t(9;22)(q34;q11)	Bcr/abl (+), MLL(-)
6	47, XY, +8, del4p	Bcr/abl (-), MLL(-)
7	46, XY	Bcr/abl (-), MLL(-)
8	46, XX	Bcr/abl (-), MLL(-)
9	46, XX	Bcr/abl (-), MLL(-)
10	46, XX, t(9;22)(q34;q11)	Bcr/abl (+), MLL(-)
11	46, XY	Bcr/abl (-), MLL(-)
12	46,XX,i(7q)	Bcr/abl (-), MLL(-)
13	46, XX, t(9;22)(q34;q11)	Bcr/abl (+), MLL(-)
14	46,XX,del 4p-,del17p-,mar14,inv(9)	Bcr/abl (-), MLL(-)

*By RT-PCR method

Structural aberrations: structural abnormalities were found in 7/14 (50%) cases by conventional cytogenetic method and, in addition, in 1 case by FISH. Five of 7 patients (71%) had structural changes as a sole chromosome abnormality and in 2 of 7 (29%) they were as a part of multiple chromosomal aberrations. The most frequent chromosomal aberration or its molecular equivalent was the Philadelphia

chromosome t(9;22)(q34;q11) – in 3 of 14 (21.4%) CD10(+) ALLs. C-MYC rearrangement (14q32 chromosome region) was detected in case 4 by FISH.

T(1;19)(q23;p13) and fusion gene PBX1/ E2A were detected in 1 case. Clinical features included young age (31 years), hepato – and splenomegaly and high WBC count 70 x109/l; the leukemic blasts had aberrant expression of myeloid markers CD13(+) and CD33(+).

DISCUSSION

Acute lymphoblastic leukemia is characterized with clonal proliferation, accumulation and tissue infiltration by malignant lymphoblasts. ALL has high incidence in childhood (80% of childhood leukemias) [16], but in adults ALLs have lower frequency (about 20%-30% of all adult leukemias) [8, 10].

We reported a relatively high proportion of CD10+ ALLs (56%) in a small adult ALL group. CD10+ (CALLA+)B-ALL is the typical acute leukemia in childhood ALL [17]. Its frequency being about 90% in children under 10 years of age and less than 50% in adolescents, is even lower in adults.

It is known, that CD10+ (CALLA+) B-ALL is frequently associated with hyperdiploidy, t(12;21) and t(9;22) [3, 7, 11, 14, 19]. We have not found ploidy changes in the leukemic cells. The only numerical aberration was gain of chromosome 8 (trizomy 8), combined with a structural rearrangement - deletion of the short arm of chromosome 4 (del4p). According to published data, 90% of trisomy 8 cases are found in acute myeloid leukemia (AML) and are associated with an intermediate disease prognosis. This is an unusual anomaly in ALL (1-2%), especially as a sole aberration and its prognostic value is unknown yet [5].

The most frequent cytogenetic aberration in adult ALL (25-30%) is Ph chromosome- t(9;22)/bcr-abl, related to shorter remission duration and overall survival [12, 19]. According to the Groupe Francias de Cytogenetique Hematology (GFCH) [6], 87% of adult Ph+ ALLs were CD10+ (CALLA+). Hoelzer et al (2002) reported that Ph(+) is found in about 40-50% of CD10+B-ALL [8]. In this study we recorded lower percent of Ph (+) CD10+ALL-21.4%, compared to other published studies.

Translocations (8q24)/C-MYC are the molecular-cytogenetic “label” of Burkitt’s leukemia/lymphoma. Blast cells usually have typical mature B-cell phenotype (CD19, CD20, CD10, surface IgM and CD 79a) [2]. We detected C-MYC rearrangement in one of our CD10+ patients (case 4, Table 3). This is not the first time when the t(8;14) has been reported outside the context of mature-B ALL phenotype [11]. The presence of t(8q24)/C-MYC is associated with poor prognosis and low rate of remissions [17].

Translocation t(1;19)(q23;p13) is a rare cytogenetic aberration in adult ALL and occurs preferentially in young patients [13]. The translocation is associated with the most immature B-lineage cell phenotypes [8]. Leukemic blasts of our patient presented with untypical for t(1;19) more mature immu-

nophenotype with expression of CD10 (CALLA) antigen. We established that leukemic lymphoblasts with t(1;19) and trizomy 8 have aberrant expression of the myeloid markers CD33+ and CD13+, not fulfilling the criteria for biphenotypic leukemia.

CONCLUSION

CD10(+)/cyIg (-) B-ALL has principally favorable prognosis but the presence of such anomalies as trisomy 8, t(1;19) and t(9;22) may indicate a poorer answer to therapy and shorter remission.

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