

# Molecular-cytogenetic aberrations in B-cell adult acute lymphoblastic leukemia (B-ALL) - frequency and correlation with immunophenotype

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## ABSTRACT

B-cell acute lymphoblastic leukemia (B-ALL) accounts for 20-30% of acute leukemias in adults. Combined application of data from immunophenotyping, karyotyping and molecular analyses allows a better understanding of this heterogeneous disease. We studied 30 adult patients with newly diagnosed B-ALL by conventional cytogenetics, fluorescent in situ hybridization (FISH) and immunophenotyping analyses. We report statistically significant prevalence of structural aberrations (43%) over numerical changes (17%) ( $p=0.02$ ). The most frequent structural changes were  $t(9;22)(q34;q11)/bcr-abl$ -17%,  $t(8q24)/C-MYC$ -10%,  $t(11q23)/MLL$ -6%,  $del\ 4p$ -6%,  $del\ 12p$ -3%, and  $t(1;19)$ -3%. Complex karyotype was found in 17% and normal karyotype in 30%.

The most frequent immunophenotype was of common B-ALL (43%), and cytogenetic and/or molecular abnormalities were found in 78% of them. We distinguished a relatively high incidence (17%) of mature B-ALL and 60% of them were associated with  $t(8;14)/C-MYC$ . We established association of cytogenetic aberrations with immunophenotype only in mature B-ALL. The other immunophenotypes are characterized by genetic heterogeneity and the presence of cytogenetic abnormalities unusual for adult B-ALL - trisomy 8 and  $t(1;19)(q23;p13)$ .

**Key Words:** Acute adult leukemia, B-acute lymphoblastic leukemia, cytogenetics, fluorescent in situ hybridization.

## INTRODUCTION

B-cell acute lymphoblastic leukemia (B-ALL) is biologically and clinically a heterogeneous group of diseases. In adults, B-ALL accounts for 20-30% of acute leukemias<sup>[1-3]</sup>.

Combined application of data from immunophenotyping, karyotyping and molecular analyses allows a better understanding of this complex disease entity and application of risk-adapted therapy<sup>[4-6]</sup>. In B-lineage ALL, it has become evident that immunophenotypic subgroups mirror a high degree of genotypic diversity and that multiple, distinct molecular pathways are involved in ALL pathogenesis<sup>[2]</sup>. This information has been useful to achieve a more precise distinction of biologically and clinically relevant subgroups, including immature CD10 (-) B-cell precursor ALL, often associated with 11q23 translocations<sup>[7]</sup>, common or pre-B-ALL with fusion transcripts of BCR/ABL due to a t(9;22), and mature B-ALL with Burkitt's type leukemia and t(8;14)<sup>[8,9]</sup>.

The correlation between genotyping and phenotyping features of leukemic cells can, on the one hand, offer a better insight into their biology. On the other hand, the study of this correlation might be of great value in therapeutic approach and prognosis of disease<sup>[6,10]</sup>. The majority of cases of B-ALL demonstrate an abnormal karyotype, either in chromosome number (ploidy) or in structural changes such as translocations, inversions, or deletions<sup>[11,12]</sup>. Sometimes chromosome studies in ALL exhibit poor morphology, tend to spread poorly, and appear fuzzy with indistinct margins, making banding studies challenging or even impossible<sup>[12]</sup>. In these cases, fluorescent in situ hybridization (FISH) technique is of enormous significance to detect the genetic changes.

The aim of our study was to determine the type and frequency of molecular-cytogenetic aberrations in adult B-cell ALL and their correlation with immunophenotyping subgroups of the disease.

## MATERIALS and METHODS

### Patients:

From January 2002 to January 2005, 33 patients over 18 years of age with newly diagnosed B-cell ALL were studied. Successful immunophenotyping and cytogenetic analyses were performed in 30 of them. Patients' clinical and biological characteristics are shown in Table 1.

**Table 1.** Clinical and biological characteristics of patients

Parameters	
B-ALL patients	30
Age (years)	
Mean	38.6
Range	18 - 72
Age groups	
18-30 years	12
31-40	5
41-50	5
51-60	4
Over 60	4
Sex	
Male / female	19/11
WBC count (x10 <sup>9</sup> /L)	
Mean	33.4
Range	1.7 - 300.0
WBC over 30x10 <sup>9</sup> /L	5 cases
Malignant blasts in bone marrow (%)	
Mean	58%
Range	36 - 96%

### Immunophenotyping:

Leukemic cells were analyzed from fresh bone marrow or peripheral blood 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 (MoAbs). These included: MoAb to the pan-leukocyte antigen CD45; B-cell associated MoAbs (CD19, CD20, CD21, CD22, CD24, surface immunoglobulins (sIg), cytoplasmic - cyCD79a, cyIgM); T-cell associated MoAbs (CD1a, CD2, CD3, CD4, CD7, CD8); myeloid-associated MoAbs (MPO, cyPOX, CD11b, CD11c, CD13, CD14, CD15, CD33, CD64, CD117); platelet-associated MoAbs (CD42a, CD9); erythrocyte-associated MoAbs (Glycophorin A, CD71) and non-lineage specific MoAbs (CD10, CD34, CD38, TdT, HLA-DR). For cytoplasmic and nuclear staining, cells were first permeabilized by means of permeabilizing solution (Becton-Dickinson, San Jose CA). Fluorescent labeling was evaluated by flow cytometry using a FACScan (Becton Dickinson Immunocytometry Systems, Mountain View, CA). Data acquisition and anal-

**Table 2.** Frequency of molecular-cytogenetic aberrations, some clinical characteristics and immunophenotype in B-cell ALL

Aberrations	Frequency (in 30 cases)	Age * (years)	WBC * (x10 <sup>9</sup> /L)	Phenotype
Hyperdiploidy	5 (17%)	37.5 (33-49)	12.9 (3.6-98)	BI, BII, BIV
t(9;22)/bcr-abl	5 (17%)	43.4 (33-58)	13 (3.6-28)	BI, BII, BIII, BIV
t(8q24)/C-MYC	3 (10%)	44 (25-62)	16.8 (7.7-24)	BIV
t(11q23)/MLL	2 (6%)	25 (25-25)	154 (7.9-300)	BI, BIII
del (4p)	2 (6%)	52 (49-56)	24.8 (20.7-28.6)	BII
del (12p)	1 (3%)	31	23	BI
t(1;19)	1 (3%)	31	69.9	BII
Complex karyotype	5 (17%)	38.8 (25-56)	75.2 (3.6-300)	BI, BII, BIV,
Normal karyotype	9 (30%)	40 (19-72)	20.7 (1.7-87.3)	BI, BII, BIII, BIV
Unsuccessful karyotyping	5 (17%)	27.2 (18-54)	29.5 (6.2-96.6)	BI, BII

\*Values and range (in parentheses) are shown .

ysis were performed with CELLQuest software (Becton Dickinson). Isotype-matched non-reactive mouse monoclonal antibodies with the same protein concentrations were used as negative controls in all experiments. Coexpression of lymphoid and myeloid antigens was confirmed by standard two-color flow cytometric analysis using appropriate MoAbs conjugated to fluorescein isothiocyanate and phycoerythrin.

A sample was considered positive for the antigen under investigation if more than 20% of the leukemic cells expressed it. The B-cell lineage of acute leukemia cells was accepted when at least two of the B-cell lineage markers were positive<sup>[13]</sup>.

Leukemias of B-cell lineage were separated according to the European Group for Immunophenotyping of Leukemias (EGIL)<sup>[13]</sup> into the following four groups:

*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+

#### **Conventional cytogenetics:**

The bone marrow was treated by direct (without cultivation) and indirect methods (after 48 hours of cultivation with RPMI and 15% fetal bovine serum at 37°C) to obtain metaphases. The chromosomes were stained by G-banding method and were analyzed by light microscopy

and the software programs Icarus Metasystem. Karyotypes were determined according to ISHC (International System for Human Cytogenetic) nomenclature<sup>[14]</sup>: Clonal aberration was accepted in the presence of at least two metaphases with the same structural changes or the same chromosome gain, or at least three metaphases with deletion in the same chromosomes. For better accuracy, the analysis of 20 metaphases of each patient was carried out.

#### **Fluorescence in situ hybridization (FISH):**

FISH analyses were performed with direct labeling locus specific probes (Vysis) for MLL gene, bcr/abl double color/double fusion and C-MYC gene. Fluorescent signals were detected by fluorescent microscopy and the software programs ISIS Metasystem. The part of genetically abnormal clones was determined upon analysis of at least 100 successfully hybridized cells. We had selected patients suitable for FISH according to clinical data and the results from conventional cytogenetics.

#### **Statistical methods:**

We used Student-Fisher's and Chi-square tests for statistical calculations and determination of statistical significance of the results.

#### **RESULTS**

Numerical chromosomal abnormalities were found in 5 patients (17%) with B-ALL (Table 2, Figure 1). All of them were with hyperdiploidy

(over 46-chromosome mode): 2 cases with pro-B, 2 with common-B and 1 with mature B-cell ALL. Hyperdiploid karyotypes were mainly characterized by trisomies of chromosomes 8, 12, 16, 20, and 22. Trisomy 8 was found as an anomaly that was part of a complex karyotype with deletion of the short arm of chromosome 4 (del 4p-). The leukemic blasts in this case showed coexpression of the myeloid markers CD33 and CD13.

Sole numerical aberrations were less frequent (1 hyperdiploid case) than the numerical aberrations in combination with a structural change (4 cases). We found additional presence of Philadelphia chromosome (t (9; 22), Ph+) in 2 out of 5 patients with hyperdiploid karyotypes: del 12p- (1 case) and del 4p- (1 case). All patients with combined hyperdiploidy and a structural aberration were under 50 years (mean 37.5 years), had a mean WBC count of  $12.9 \times 10^9/L$  and expression of myeloid antigens CD13 and/or CD33.

Structural chromosomal and/or molecular aberrations were found in 13 out of 30 (43%) B-ALL patients. Translocations constituted the most common changes (77%). In 8 cases (62%), structural changes were found as a sole chromosome abnormality, and in 5 (38%), they were part of a complex karyotype.

The most frequent structural changes (5 out of 30 B-ALL cases - 17%) were translocations between 9 and 22 chromosomes- t(9;22)(q34;q11) (Table 2, Figure 1), which led to formation of a fusion gene bcr/abl. Immunophenotyping showed that Ph+ ALLs had different immunophenotypes - 1 case of pro-B, 2 cases of common-B, 1 of pre-B and 1 of mature B-cell ALL. Aberrant expression of myelo-monocyte antigens was found in 4 out of 5 cases (80%) of Ph+ B-ALL: CD13 and/or CD33 were expressed in 3 cases (60%) and CD15 in the other (20%).

In one patient with common B-ALL, there was a normal karyotype by conventional analysis, but the FISH method proved a fusion gene bcr/abl.

The mean age of Ph+ B-ALL patients was 43.4 years (range 33-58 years) and the mean WBC count was  $13 \times 10^9/L$  (range  $3.6-28 \times 10^9/L$ ).

Translocation t(8;14)(q24;q32) and/or a molecular rearrangement of C-MYC (8q24) protooncogene were detected in 3 out of 30 (10%) cases (Table 2, Figure 1). All of them expressed cyto-

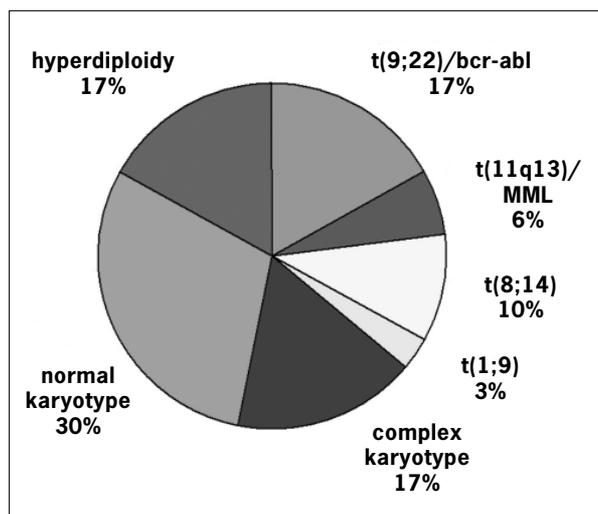


Figure 1. Frequency of molecular-cytogenetic aberrations in B-ALL patients.

plasmic and surface IgM immunoglobulins and we classified them as mature B-ALL.

The mean age of C-MYC+ B-ALL patients was 44 years (range 25-62 years), and the mean WBC count was  $16.8 \times 10^9/L$  (range  $7.7-24 \times 10^9/L$ ).

Translocation t(4;11)(q22;q23) and rearrangement of MLL gene were found in 2 patients (6%) (Table 2, Figure 1). Both were young men (25 years) -one with a pro-B-cell phenotype and normal WBC count of  $7.9 \times 10^9/L$  and one with pre-B phenotype, hyperleukocytosis ( $300 \times 10^9/L$ ) and an additional chromosomal aberration i(7q). In both cases with t(4;11)(q22;q23), leukemic blasts showed expression of TdT, HLA-DR, CD19, variably CD10 and aberrant myeloid antigens CD13 and CD33.

Deletion of the short arm of chromosome 4- del (4p) was cytogenetically detected in 2 out of 30 patients (6%) (Table 2). Deletions of 4p were combined in complex karyotypes - in 1 case together with del(17p), mar(14) and inv(9) and in another case with a gain of chromosome 8 - trisomy 8. Both cases expressed CD20+, CD22+, and CD10+ and were characterized by a common-B-cell phenotype. The mean age of the patients with del (4p) was 52 years (range 49-56), and the mean WBC count was  $24.8 \times 10^9/L$  (range  $20.7-28.6 \times 10^9/L$ ).

Abnormalities of the short arm of chromosome 12 (12p) were found in 1 out of 30 cases (3%). This was a young man (31 years) with cytogenetic data of 2 leukemic clones and 1 clone

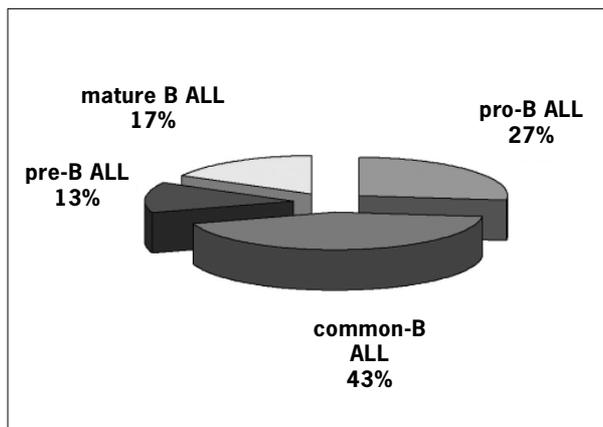


Figure 2. Distribution of immunological subgroups of B-ALL.

with a normal karyotype - 50% of the blasts had a karyotype 46,XY, del (12)(p11.2pter), 37.5% - 48,XY, +6, +12,del (12)(p11.2pter) and 12.5% - 46,XY. Immunophenotypic data demonstrated positive CD19, CD24, CD71 and absence of CD10 (pro-B-cell ALL) and aberrant myeloid markers CD13 and CD33.

The translocation t(1;19)(q23;p13) was found in 1 out of 30 B-ALL cases (3%) in our study (Table 2, Figure 1), in a 31-year-old man with hyperleukocytosis ( $69.6 \times 10^9/L$ ), common B-ALL and overexpression of the myeloid marker CD33 and cytoplasm CD13. The progenitor stem cell marker CD34 was positive and TdT-negative.

Complex karyotypes were detected in 5 patients (17%). The main abnormalities were t(9;22), t(4;11), trisomy 8, del 12p, and del 4p, which were combined with additional structural aberrations (40%) or numerical changes (60%). Three of the cases with a complex karyotype (60%) had phenotyping features of common B-ALL (CALLA+), 1 case (20%) - pro-B and 1 case (20%) -mature B-ALL. The coexpressions of two myeloid markers - CD13 and CD33 - were found in leukemic blasts of 2 patients (40%).

The mean age of the patients with complex aberrations was 38.8 years (range 25-56) and the mean WBC count was  $75.2 \times 10^9/L$  (range  $3.6-300 \times 10^9/L$ ).

Nine out of 30 (30%) B-ALL patients were with normal karyotype with a non-systematic loss of random chromosomes (Table 2, Figure 1). The FISH study showed in all of them an absence of bcr/abl fusion, MLL and C-MYC rearrangements. Four out of 9 cases (44%) with normal

karyotypes had immunophenotyping features of pro-B ALL, 2 patients (22%) - common B-ALL, 2 patients (22%) - pre-B ALL and 1 patient - mature B-ALL. There was aberrant antigen expression of CD13 in only 2 cases - in 1 common B-ALL and in 1 mature B-ALL.

The mean age of the patients with a normal karyotype was 40 years (range 19-72) and the mean WBC count was  $20.7 \times 10^9/L$  (range  $1.7-87.3 \times 10^9/L$ ).

In this study we had unsuccessful cytogenetic analyses in 5 out of 30 cases (17%) - 4 with common-B and 1 with pro-B ALL. All of them showed negative results by FISH for bcr/abl, MLL and C-MYC. The mean age of these patients was 27.2 years (range 18-54) and the mean WBC was  $29.5 \times 10^9/L$  ( $6.2-96.6 \times 10^9/L$ ).

## DISCUSSION

In our study, we found higher frequency of B-ALL in the age group between 18 and 30 years (12 out of 30 cases, or in 40% of B-ALL patients) and slight prevalence of male over female patients (1.73:1). There was a statistically significant difference in WBC counts in patients with t(1;19) and complex karyotypes ( $p < 0.0001$ ) compared with normal karyotypic cases.

Chromosomal and/or molecular aberrations were detected in half of the patients, and in 5 cases (17%) they were combined in a complex karyotype. We report statistically significant prevalence of structural aberrations (43%) in adult B-ALL over numerical changes (17%) ( $p = 0.02$ ).

B-ALL is also known for its association with Philadelphia chromosome and/or the presence of the fusion gene bcr/abl (25-30%), related to shorter remission [3,7,8,11]. The Groupe Francais de Cytogenetique Hematologique (GFCH)<sup>[15]</sup> found that in adult patients with Philadelphia chromosome positive ALL, 87% of them were CD10-positive. In our study, we found lower frequency of t(9;22)/bcr-abl (17%), and 80% of the cases were CD10-positive. There were no significant differences ( $p = 0.31$ ) in the expression of myeloid markers in Ph+ and Ph- B-ALL, as reported by Taberero et al. in 2001 [9].

The most frequent immunophenotype was of common B-ALL (43%) (Figure 2), exhibiting a significantly higher number of cases compared to all other groups. According to other studies [16],

this is an expected frequency. In 78% of patients with this phenotype, cytogenetic and/or molecular aberrations were found, most of them with poor prognostic value – t(9;22)/bcr-abl fusion, t(4;11)(q22;q23)/MLL rearrangements, t(1;19) and trisomy 8. According to data in the literature, common B-ALL was associated with hyperdiploidy and t(9;22)<sup>[8,17]</sup>. Translocations t(9;22) and/or fusion genes bcr/al were found in 15% of our common B-ALL cases and hyperdiploidy had a low frequency (11%) in our series.

But in this phenotyping group we found gain of chromosome 8 (trisomy 8) combined with structural rearrangements in a complex karyotype. Trisomy 8 is typical of acute myeloid leukemias and represents an unusual cytogenetic aberration in B-ALL<sup>[3,12,18]</sup>.

In 1 case with common B-ALL we established a t(1;19). The frequency of t(1;19) is high in childhood pre-B-ALL, but this is an unusual aberration in adult CD10+ B-ALL<sup>[12,19-21]</sup>.

Translocations involving 11q23 (MLL) are rare in adults at 3-6%<sup>[1,22,23]</sup> and our results (6%) supported this fact. According to Katevas<sup>[16]</sup> and Ludwig<sup>[24]</sup>, pro-B-ALL, accounting for approximately 10% of adult ALL cases, is associated with a prevalence of MLL rearrangement.

Our pro-B group was characterized with a higher case incidence – 27% (Figure 2). There was no t(11q23) nor rearrangements of MLL gene, and the normal karyotypes were higher (71%, p=0.03) than those with chromosomal changes (29%).

We distinguished a relatively high incidence (17%) of mature B-ALL phenotypes, and 60% of them (3 out of 5 cases) were associated with t(8;14)(q24;q32)/C-MYC (+). Our data proved the association between mature B-ALL and t(8;14)/C-MYC rearrangements<sup>[1,16,25]</sup>.

## CONCLUSION

In this study of adult B-ALLs, we found association of cytogenetic aberrations with immunophenotype only in mature B-cell leukemia. The other immunophenotypes are characterized with genetic heterogeneity and the presence of unusual cytogenetic abnormalities for adult ALL – trisomy 8 and t(1;19)(q23;p13).

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