

The Prognostic Impact of the Karyotype in Patients with Acute Lymphoblastic Leukemia

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Background and Objective: Acute lymphoblastic leukemia (ALL), is characterized by uncontrolled precursor lymphocyte proliferation. Chromosomal abnormalities have been found in 60–85% of ALL patients. The aim of our work was to determine the chromosomal abnormalities and to evaluate the prognostic value of cytogenetic findings in a cohort of ALL patients.

Method: The study included 36 patients with ALL from Hematology Clinics Tg. Mures, Romania. Cytogenetic analyses were done on bone marrow cultures according to standard methods.

Results: We identified 22 cases (71%) with cytogenetic abnormalities. In our study, the frequency of chromosomal abnormalities was 50% in children and 85% in adults. The most common clonal karyotype aberration in ALL patients was numerical chromosomal abnormalities, detected in 62% of cases. Structural chromosomal abnormalities were found in 38% of our cases and were represented by translocations and deletions. We included our patients in different cytogenetic risk groups: 2 patients in low cytogenetic risk group, 23 in intermediate cytogenetic risk and 6 in severe cytogenetic risk group. We did not find a statistically significant difference in the median overall survival (OS) between the three cytogenetic risk groups ($p = 0.863$). There was a significantly better OS in patients who had a normal karyotype compared to those who had chromosomal abnormalities ($p = 0.008$).

Conclusion: Our study highlights the importance of cytogenetic analysis as an important prognostic factor in ALL.

Keywords: acute lymphoblastic leukemia, prognosis, cytogenetic

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Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disease characterized by uncontrolled precursor lymphocyte proliferation, accumulation, and tissue infiltration of neoplastic cells [1]. The associations between numerical or structural chromosome aberrations and hematological disorders are well known [2].

Cytogenetic aberrations have been found in 60–85% of ALL patients [3,4,5]. The age, white blood cell (WBC) count, immunophenotype, and chromosomal aberrations are the most useful prognostic factors in ALL.

Childhood and adult ALL vary significantly in the prevalence of different chromosomal aberrations. According to Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 study there are three cytogenetic risk categories. Cases with a normal karyotype and those with isolated 9p deletions have had a relatively favorable prognosis. Patients with 6q deletions or hyperdiploid karyotype have had an intermediate prognosis. The prognosis of patients with $t(9;22)$, $t(4;11)$ and $t(1;19)$ is unfavorable [6]. Pullarkat et al. proposed a new classification of chromosome abnormalities in cytogenetic risk groups. They took into account the proposed cytogenetic risk groups from MRC UKALLXII / ECOG 2993

study. Patients with $t(9;22)$ or Philadelphia chromosome (Ph1) have been identified as a distinct risk group because of very severe prognosis. Patients with Ph1 can benefit from treatment with tyrosine kinase inhibitors. This classification included four cytogenetic risk groups for patients with acute lymphoblastic leukemia: very high risk group, the high risk group, intermediate risk group and standard risk group. The very high risk group included translocation $t(4;11)$, complex karyotype, low hypodiploidy (30-39 chromosomes). The high risk group included monosomy 7, $del(7p)$, trisomy 8, $t(1;19)$, $t(17;19)$, $t(5;14)$, 11q abnormalities (with MLL mutation) near triploidy (60-78 chromosomes). Intermediate risk included normal karyotype, low hyperdiploidy (47-50 chromosomes), $del(9p)$ or other karyotypic changes not identified with 11q abnormalities (without MLL mutation), $del(6q)$, $del(9p)$, $del(12p)$, $del(17p)$, $del(13q)$ or monosomy 13, $t(14q32)$, $t(10;14)$, tetraploidy (>80 chromosomes). Standard risk was defined by high hyperdiploidy with 51-65 chromosomes [7].

According to multicenter international trial MRC UKALLXII/ECOG 2993, which included cytogenetic data from 1522 ALL cases, patients with a Ph1 chromosome, complex karyotype, translocation $t(4;11)(q21;q23)$, $t(8;14)(q24.1;q32)$, low hypodiploidy or near triploidy all had lower overall survival when compared with other patients. Contrary, ALL cases with high hyperdiploidy or a $del(9p)$ had a higher overall survival [6].

Materials and methods

Patients

The study included 36 patients with acute lymphoblastic leukemia from Hematology Clinics from Țirgu Mureș. Samples of heparinized bone marrow from ALL patients were sent to the Genetic Laboratory of the University of Medicine and Pharmacy of Țirgu Mureș for cytogenetic evaluation.

Cytogenetic analysis

Heparinized bone marrow was obtained at the time of diagnosis and during drug therapy for monitoring treatment response. Bone marrow was cultured for 1–3 days in RPMI 1640 medium, which was supplemented with 20% fetal calf serum, 1% L-glutamine, 50ng/ml penicillin/streptomycin without mitogens. After incubation, the cells were treated with Colcemid solution (10 µg/ml), followed by treatment with hypotonic solution (0.075M KCl), and were fixed with a mixture of methanol and glacial acetic acid (3:1). We used Giemsa staining (GTG staining) technique. Metaphase cells were captured with the Cytovision System (Applied Imaging). The recommendations of the International System for Human Cytogenetic Nomenclature (ISCN) were used to interpret the karyotype [8]. The cell culture was considered failed in ALL cases with less than ten metaphases available for analysis or with poor quality metaphases.

Statistical methods

Statistical analysis was performed using the software SPSS 17 (Statistical Package for the Social Sciences). Patient overall survival was estimated using the Kaplan-Meier method from the date of ALL diagnosis until death from any cause or until the last patient follow-up. Survival curves were statistically compared using the log-rank test. Differences between two groups were considered statistically significant if *p* values were < 0.05 in a two-tailed test [9].

Results

This work was performed on 36 cases (22 males and 14 females, of which 13 children and 23 adults) with ALL diagnosed and treated in the Hematology Clinics, Tg Mures. We successfully analyzed the leukemic karyotype of 31 (86%) patients, and identified 22 (71%) cases with cytogenetic abnormalities. In our study, the frequency of chromosomal abnormalities was 50% in children and 85% in adults.

Cytogenetic Findings

The most common clonal karyotype aberration in ALL patients was numerical chromosomal abnormalities, detected in 62% of cases. Structural chromosomal aberrations were observed in 38% of our cases and were represented by translocations $t(9;22)(q34;q11)$; $t(7;12)(q22;p13)$; $t(8;14)(q21;q11)$ and deletions $del(11)(q22)$; $del6q$; $del(9)(p21)$; $del(17)(p12)$; $del(14)(q21)$; $del(12p)$ and $del(6)(p21)$, $t(17;22)(p11;q11)$. The most common structural chromosomal abnormalities in our series were deletions.

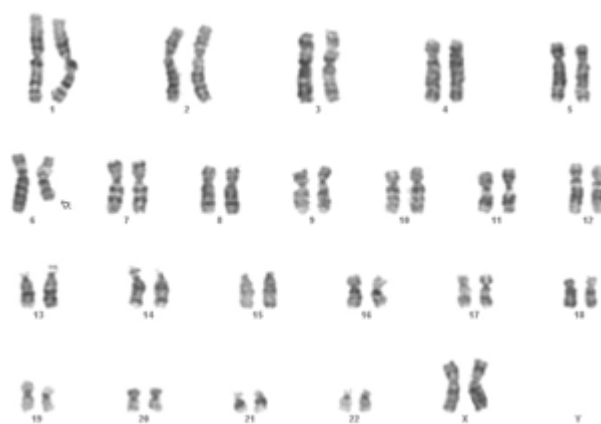


Fig. 1. Karyotype 46,XX,del(6)(p21)

According to MRC UKALLXII/ECOG 2993 study [6] we included our patients in different cytogenetic risk groups (Table I).

We made a comparison of the median overall survival (OS) between the low, intermediate, and high cytogenetic risk groups. Cytostatic treatment used in children was according to ALL BFM protocol and in adults was Hoelzer protocol. Our patients with $t(9;22)$, also known as Philadelphia chromosome (Ph1), were treated according to the national protocol which include Imatinib mesylate (Glivec).

There was no statistically significant difference in the median OS between the three cytogenetic risk groups ($p = 0.863$). The estimate mean for survival time was 37.9 months for ALL patients (CI 26.54–49.25). We noticed that the patient's death occurred more frequently in the first three years after diagnosis, survival at 1, 2 and 3 year was 84%, 72% and 56% respectively.

The estimated means for survival time according to cytogenetic risk group was 31.75 months (CI 0.00–80.45) for patients included in low cytogenetic risk group; 24.38 months (CI 21.14–27.62) for intermediate cytogenetic risk group and 31.08 months (CI 19.7–42.45) for those included in high cytogenetic risk group. The estimated means for survival time in ALL patients with failure chromosomal analysis was 9.31 months (CI 4.55–14.07). As shown above, the survival time for ALL patients with low cytogenetic risk or favorable prognosis was slightly better than in those patients with intermediate or high cytogenetic risk groups, although no statistically significant difference was observed ($p = 0.131$).

Table I. Cytogenetic risk groups in ALL patients

Cytogenetic risk	Cytogenetic abnormality	No. of cases
Low	Hyperdiploidy >50 chromosomes	2
Intermediate	Normal karyotype	9
	Hyperdiploidy < 50 chromosomes	3
	All other structural/numerical abnormalities (hypodiploidy)	11
High	Complex karyotype	1
	$t(9;22)(q34;q11.2)$	3
	Hypodiploidy (<20)	1
	$t(8;14)$	1

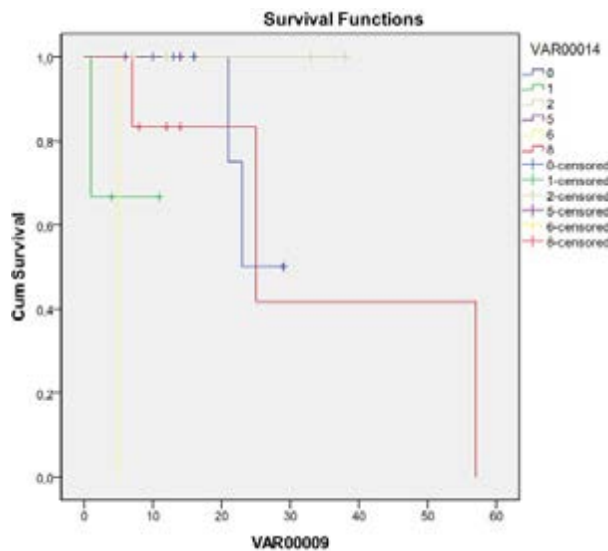


Fig. 2. Kaplan-Meier survival curves in ALL patients according to the chromosomal abnormalities, (0) normal karyotype, (1) deletion, (2) translocation, (5) trisomy, (6) complex karyotype, (8) other chromosomal abnormalities

Differences in sample size of the risk group and treatment protocol between children and adult patients may account for these findings. Comparing survival by gender in patients with ALL, statistical analysis revealed no significant difference ($p = 0.084$).

Overall comparisons between ALL patients with chromosomal abnormalities and those with a normal karyotype revealed no statistical difference ($p = 0.405$). There were significant differences in overall survival (OS) observed between patients who had a normal karyotype compared to patients who had chromosomal abnormalities categories ($p = 0.008$). In our study patients with deletions or complex karyotype aberrations had the lowest survival.

Discussion

The majority of our ALL patients (71%) presented an abnormal karyotype, either in chromosome number (32%) or structural abnormalities (68%) such as deletions and translocations. The references in the literature are variable, chromosomal aberrations are present in 70–98% of patients with ALL, the proportion depending on the techniques used and the type of disease [10,11].

According to MRC UKALLXII/ECOG 2993 study [6] 23 of our patients belong to the intermediate risk group. According to the new classification proposed by Pullarkat et al [7] the most of our patients belong to the intermediate cytogenetic risk group.

In our study, the frequency of chromosomal abnormalities was 50% in children and 85% in adults. According to the literature, the frequency of chromosome abnormalities in adult patients with ALL (64–85%) is higher than in pediatric ALL cases (60–69%) [12].

Cytogenetic analysis of our ALL cases showed that 29% had normal diploid karyotypes; similar to some reports [10, 13]. Pseudodiploidy was found in 38% of ALL pa-

tients in agreement with the findings of Pui et al. [14].

Hypodiploid patients were found in 13% of the cases in the present study. Our results are similar to that reported by other studies who considered hypodiploidy to be a relatively unusual finding in ALL (only 3% - 9% of all patients) [15]. Pseudohaploidy (<30 chromosomes) was found in a young ALL patient. Pseudohaploidy is rarely seen, being associated with a short complete remission and a poor prognosis [10].

In the current study hyperdiploidy represented 22.7% of ALL patients, which is similar to that reported by other authors [16]. Hyperdiploidy was present in 15.8% of adults with ALL, although Faderl et al. [1] reported a higher frequency (25% of cases of ALL in adults), making it one of the most common abnormalities. In children, hyperdiploidy was found in 8.3% of cases consistent with that reported by Settin et al. [10]. In our ALL patients, high hyperdiploidy was associated with a favorable outcome, the average survival being 31.5 months. Additional chromosomes found were chromosomes 4, 8, 21.

French Group of Cytogenetics (Groupe Français de Cytogénétique Hematologique) observed the association of trisomy 4, 6, 8, 10, 14, 17 and 21 with high hyperdiploidy and gain of chromosomes 5, 8, 10 and 21 in hyperdiploidy with 47-50 chromosomes. They established that ALL patients with hyperdiploidy, but without Ph chromosome and those with tetraploidy have a favorable prognosis [17].

Trisomy 8 as single chromosomal abnormality was found in one of our ALL cases, its frequency is similar with that reported in other studies [18]. In "Mitelman Database of chromosome Aberrations in Cancer", are described 50 cases of trisomy 8 as sole cytogenetic abnormality in ALL patients [19]. Although trisomy 8 is rare as sole anomaly in ALL (1%), it is observed in 10% of cases of ALL with additional cytogenetic abnormalities, and is frequently associated with t(9,22) [18]. In the literature, most cases of ALL with trisomy 8 are children and there were described only 3 cases in the elderly. Some reports suggest that trisomy 8 in ALL patients is associated with a relatively good prognosis [20].

In the present work, we have observed deletions reported in literature del(11)(q22); del(6q); del(9)(p21); del(17)(p12); del(14)(q21); del(12p); del(6)(p21) [10, 21].

Deletion del(6q) was found in 3.2% of our ALL cases. Our findings are similar to those found by Faderl et al [1]. According to Pullarkat et al (2008), del(6q) is associated with an intermediate prognosis [7]. The frequency of del(9p) presented in 3.2% of our ALL cases, is inferior to that reported by different studies (7-13%). Our patient was diagnosed with pre-B ALL. Recent data indicate that abnormalities 9p is a severe risk factor for B-cell ALL, but not for T-cell ALL [22]. The frequency of del(12p) in our study group was 3.2%, similar to that observed by UKALLXA study (4%), but less than that observed by the French Group of Cytogenetics (5%). According to published data, del(12p) do not affect prognosis of ALL [6, 17]. Deletion of the long arm of chromosome 11, del(11q), was found in

a young patient diagnosed with T-cell ALL. The frequency of 11q abnormalities were similar to that reported by Faderl et al (<10%). Taking into account the results from the literature and the overall survival of our patient (6 months after diagnosis), we consider that del(11q) is a poor prognostic factor, and we included the patient in the unfavorable cytogenetic risk group.

We found translocation t(9;22)(q34;q11.2) or Ph1 in 11.1% of our ALL cases, which is lower than that detected by Larson et al. They reported that Ph1 appears in 20% - 30% of adults with ALL with higher incidence (> 50%) in ALL cases aged 50 years or older [23]. According to Faderl et al. the Ph1 is the most frequent cytogenetic abnormality in adult patients with ALL [1]. Our results might be explained by the relatively small size of our cohort. The overall survival of our ALL patients with t(9;22)(q34;q11.2) was 7.3 months.

Translocation t(8;14)(q21;q11) was found in one preT-ALL patient. Although the prognostic value of t(8,14) has not been established (in some studies it was associated with an intermediate prognosis, in other with a severe one), we included this structural abnormality in the group with poor prognosis because it was seen in a young ALL patient (23 years old) who had a short survival (6 months) [1].

In our study translocation t(7;12)(q22;p13), as sole cytogenetic abnormality was seen in a young ALL patient. According to the published data t(7;12)(q22;p13), was described in a few cases of adult B cell- ALL, being more frequent in children with ALL. In children with ALL, t(7;12) is often associated with deletion on chromosome 7, del(7q) [24].

Our results demonstrate that cytogenetic is an important prognostic factor in ALL. The importance of cytogenetic analyses, one of the most important prognostic factor in ALL, has been reported previously by the CALGB (Cancer and Leukemia Group B), MRC UKALLXII / ECOG study groups and the Southwest Oncology Group (SWOG)-9400 study [6,7,25].

Cytogenetic abnormalities had important significance related to diagnosis, management and prognosis in ALL [10]. Chromosomal abnormalities confer important informations for prognosis which are useful for risk stratification of acute lymphoblastic leukemia [26]. Risk stratification according to cytogenetics has an important role in planning of treatment strategies.

Conclusion

Chromosomal analysis should be performed in all ALL patients at diagnosis. Our study highlights the importance of cytogenetic analyses as an important prognostic factor in ALL.

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