A 10-YEAR FOLLOW-UP OF ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS IN A REGIONAL PEDIATRIC HEMATOLOGY CENTER FROM ROMANIA

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Abstract

Introduction: Childhood ALL is not a single disease, but a group of diseases with a variety of genetic and molecular abnormalities in the leukemic cells leading to a wide range of clinical presentations and outcomes. Histochemistry, immunophenotyping, cytogenetics and molecular biology of the blast cells are able to identify different types of ALL. The aim of the paper is to present the improvements made in the diagnosis and risk stratification of the patients as well as the analysis of survival correlated to a number of risk factors.

Material and methods: We studied 59 ALL patients diagnosed between 2001 January – 2010 December, treated in the Haematology Department of the Pediatric Clinic nr. 1 from Targu-Mures. We studied age, gender, white blood cell (WBC) count at diagnosis, absolute lymphoblast count (AlyC) on the 8th day of treatment, blast immunophenotype, BCR-ABL gene expression with qRT-PCR analysis, early treatment response, CNS involvement, relapses and survival. Descriptive analysis, chi-square test, Kaplan- Meier survival curves were performed.

Results: Out of the 59 ALL patients, four were diagnosed before 2 years of age (7%), 34 were between 2-6 years (58%) and 21 were older than 6 years (35%). 36 patients were boys and 23 girls (ratio 1,6:1). WBC count at diagnosis varied between 1.300-770.000/mm^3. Fifteen patients (25%) presented CNS involvement at diagnosis. Pre-B lymphoblasts were noticed in 35 patients, T cell in 9 and mature B cell in 2 patients. Early corticosteroid therapy failure was noticed in 11 children and 3 patients had M2 type BM on day 33. BCR-ABL gene expression was searched in 29 BM samples with 3 positive results. Overall survival after a mean of 41 months was 75%. Survival was strongly influenced by blast immunophenotype (mean survival of 84 months in pre-B ALL, 67 months in T cell ALL and 2 months in mature B cell ALL), early induction failure, CNS involvement at diagnosis (47 versus 104 months) and relapses (29% versus 81%).

Conclusions: 1.During 2001-2010 we diagnosed and treated 59 children with ALL, the overall survival at a medium of 41 months was 75%. 2. We performed blast immunophenotyping in 46 patients and BCR-ABL gene expression assessment with qRT-PCR method in 29 patients with 3 positive results. 3. Statistically significant unfavourable prognostic factors were the mature B immunophenotype, early induction failure, CNS involvement at diagnosis and relapses.

Key words: children, leukemia, risk factors, survival

Introduction

Acute lymphoblastic leukemia (ALL) is a clonal expansion and maturation arrest of lymphoid hematopoiesis, which accounts for 25-30% of childhood cancers. The hallmark of diagnosis in ALL is the lymphoblast in the BM. Histochemistry, immunophenotyping, cytogenetics and molecular biology of the blast cells are able to identify different types of ALL. Age, gender, white blood cell (WBC) count, cytogenetics, immunophenotype and molecular characteristics of the blast cells, central nervous system (CNS) disease, early response to corticosteroid therapy, bone marrow (BM) response to chemotherapy on the 15th and 33rd days, are the basic prognostic factors in ALL. The presence of two specific genetic translocations, the t(9;22) (q34;q11) and the t(4;11) (q21;q23) or their corresponding molecular alterations, the BCR-ABL and MLL-AF4 gene rearrangements indicate a very high malignity.

The aim of the paper is to present the improvements made in the diagnosis and risk stratification of the patients as well as the analysis of survival correlated to a number of risk factors.

Material and methods

We studied 59 ALL patients diagnosed between 2001 January – 2010 December, treated in the Haematology Department of the Pediatric Clinic nr. 1 from Targu-Mures with the ALL-BFM 95 protocol. The studied criteria were the date of onset, age, gender, white blood cell (WBC) count at diagnosis, absolute lymphoblast count (AlyC) on the 8th day of treatment, immunophenotype of lymphoblasts, minor and major BCR-ABL gene expression from bone marrow (BM), the improvement of BM on the 15th and 33th days of treatment, CNS involvement at diagnosis, relapses and survival.

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The real-time PCR method for BCR-ABL gene rearrangement detection was introduced in 2010. BM samples were obtained from the anterior or posterior iliac crista, after previous sedation with 0,1 mg/kg intravenous midazolam and local anaesthesia with 1% lidocain. We collected 2 x 2 ml bone marrow in EDTA tubes for molecular testing and immunophenotyping, and 6-8 smears for morphology. Quantitative RT-PCR analysis was performed in the Molecular Biology Laboratory of our university: RNA extraction was performed using QIAmp RNA Blood Mini Kit 50 (QIAGEN cat.no. 52304) and cDNA transcription with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems cat.no 4374966) according to the supplier's instructions. We studied the b3-a2 and b2-a2 BCR/ABL fusion gene using the primers and protocols recommended by the Europe Against Cancer Program[1]. The RQ-PCR reaction was performed on an ABI 7500 Real Time PCR instrument (Applied Biosystem), using 5 µlcDNA and TaqMan ® Universal PCR Master Mix (Applied Biosystem) in 25 µl end volum. All reactions were made in triplicate. The ABL gene was used as endogenous control and also were used known positive and negative control samples. We performed relative quantification. Data were statistically processed with descriptive analysis, chi-square, Log Rank test and Kaplan- Meier survival curves.

Results

Among the 59 ALL patients diagnosed between January 2001- December 2010, four patients were diagnosed before 2 years of age (7%), 34 were between 2-6 years (58%) and 21 were older than 6 years (35%). We assessed a male predominance: 36 patients were boys and 23 girls (ratio 1,6:1). WBC count at diagnosis varied between 1.300-770.000/mm^3, with an average of 61.140/mm^3 and median of 40.480/mm^3. Twenty nine patients had an initial WBC count below 20.000/mm^3 and 30 above this number. Fifteen patients (25%) presented CNS involvement at diagnosis. After 7 days of corticosteroid therapy, the absolute peripheral lymphoblast count exceeded 1000/mm^3 in 11 patients. Immunophenotyping of marrow lymphoblasts revealed pre-B type in 35 patients, T cell in 9 and mature B cell in 2 patients. On day 15 of chemotherapy, 27 patients had an M1 type marrow (under 5% blasts), 18 had M2 type BM (5-25% blasts) and 9 patients had M3 type BM with more than 25% lymphoblasts. On day 33 of treatment 51 patients had M1 type (86%) and 3 patients M2 type of BM (5%), 5 cases (9%) were missing because of early death. Major and minor BCR-ABL gene expressions were assessed with quantitative real-time PCR method from BM and peripheral blood in 29 patients, out of which 3 results were positive. Based on these criteria, 63% of patients were included in medium malignity and 37% in high risk group. Overall survival after a follow-up of 0-122 months (mean 41,08 months) was 75,9%. (table 1. Figure 1.)

The median survival time was 102 months. The outcome of the 3 patients with BCR-ABL gene expression differed grossly according to treatment options: one patient who was treated with intensive chemotherapy and continuous tyrosine kinase inhibitor is being in first complete remission now for 6 years, the second patient underwent allogeneic stem cell tranplantation in first remission but died after 7 months from the transplantation in systemic herpes infection and the third patient died in early infectious complication.

Table 1. Outcome of childhood ALL (2001-2010).

	Males	Females	Total
Alive	25	19	44
Deceased	10 (28,6%)	4 (17,4%)	14
Total	35	23	58
Missing		1	59

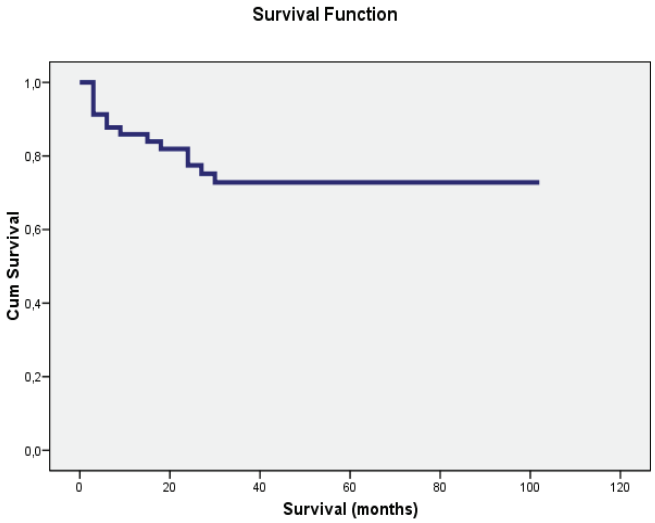

Figure 1. Overall survival in ALL patients during 2001-2010

We lost 14 patients (24,1% mortality) after a mean of 10,4 months and median of 6,5 months of treatment, the longest survival in this goup was 28 months. The causes of deaths were relapses in 6 cases, abandon of treatment in 2 patients, infections (3 patients), tumor lysis syndrome (2 patients) and cerebral inclavation in one patient. Ten boys died out of 35 and 4 girls out of 17. The mean survival in males was 86,7 months and in females 87,4 months, the difference is not significant (p=0,369). Survival correlated to risk groups showed significant difference (Log rank test: p< 0,001): in MR ALL survival was 108 months, while in HR ALL survival was significantly shorter, 55,6 months, as shown in Figure 3. Immunophenotype of the lymphoblast was a strong predictor for survival, in common B ALL the mean survival was 84 months, in T cell ALL 67 months and in mature B cell ALL 2 months (Figure 4). If only pre-B and T cell ALL are compared in respect for survival, the difference is not significant (Figure 5).CNS involvement at diagnosis halved the estimated survival (47 versus 104 months). (Figure 6). BM status at day 15 of treatment was a strong predictor of survival, however, the result was not significant because of the small number of cases in the M3 group. (Figure 7).

Patients with M1 type BM on day 33 had an average survival of 100 months, compared to patients with M2 type BM whose survival was 4-fold lower (25 months). (Figure 8). In the M2 group there were 3 patients, two of them died after 2 years.

Patients who relapsed had a significantly lower survival (Log Rank p=0,011). (Figure 9).

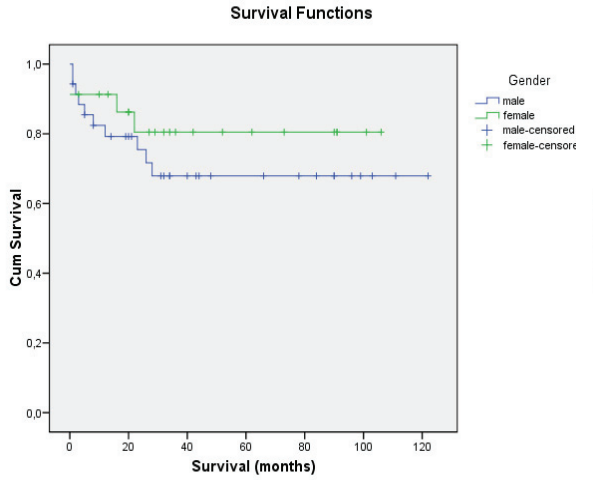

Figure 2. Mean survival according to gender (p=0,369)

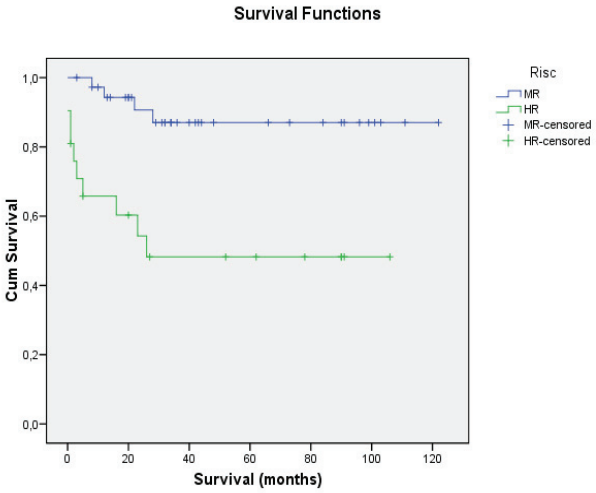

Figure 3. Survival correlated with risk groups (p< 0,001)

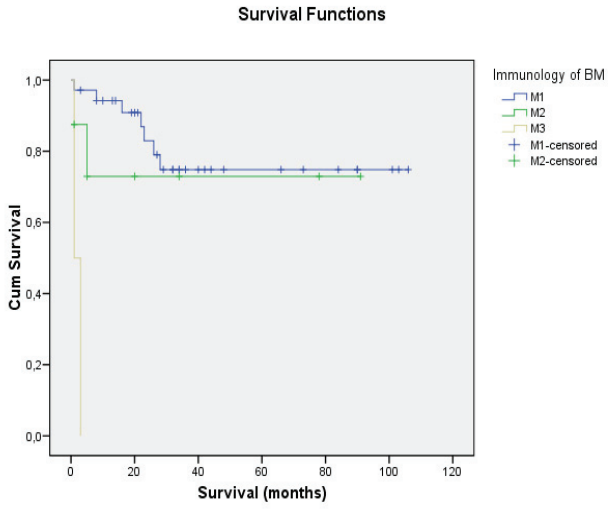

Figure 4. Survival according to blast immunophenotype (M1=pre-B, M2=Tcell, M3=mature B cell)

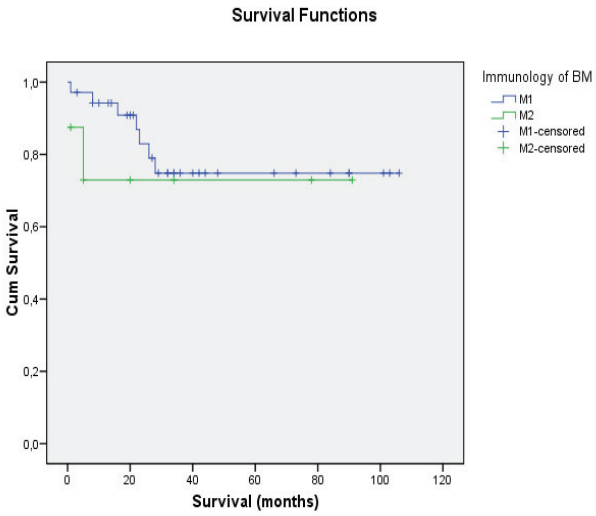

Figure 5. Survival in pre-B and T cell ALL (M1=pre-B, M2=T cell ALL)

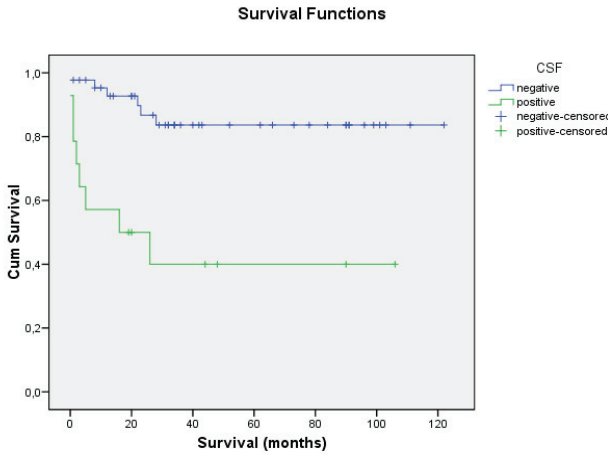

Figure 6. Survival according to CNS involvement.

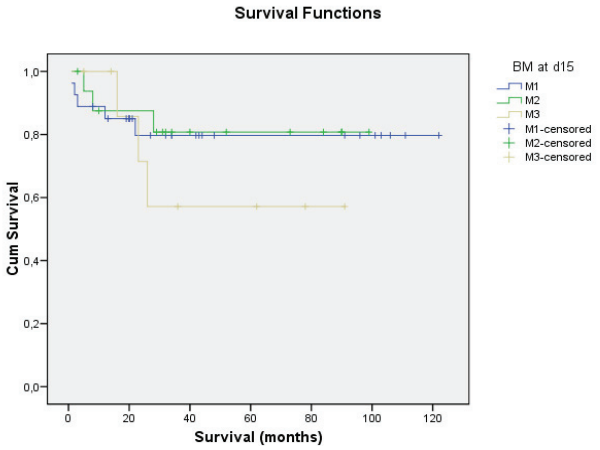

Figure 7. Survival according to bone marrow status on day 15.

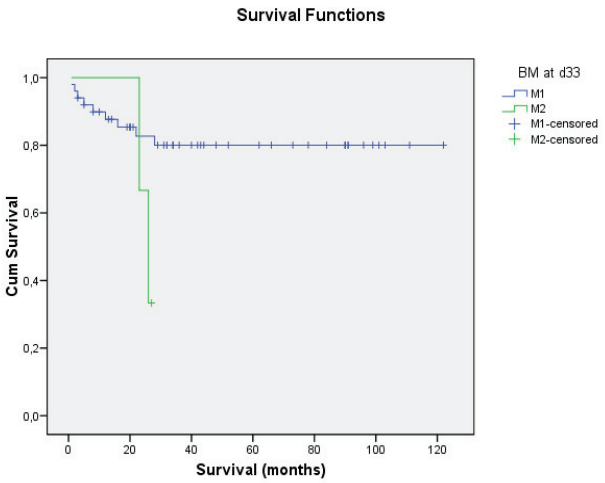

Figure 8. Survival according to bone marrow status on day 33.

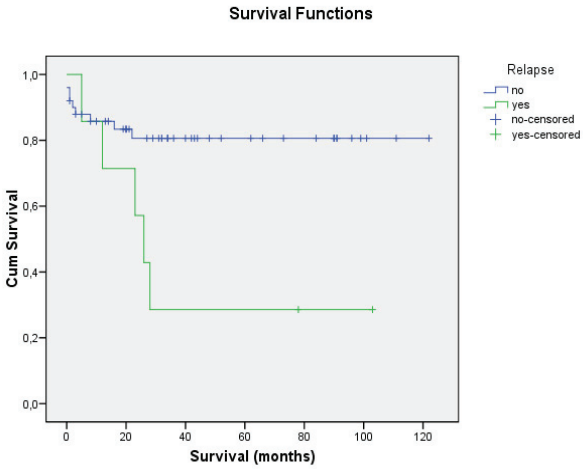

Figure 9. Survival according to relapse (p=0,011).

Discussion

Recently long term survival in childhood ALL is achieved in 80-85% of the patients but the remaining 15-20% can not be cured with current therapies. That is the reason why research is conducted towards identification of more precise risk factors and individualized, targeted therapies [2]. In a large study conducted between 1991-1995 in Japan, the estimated 7-year overall survival rate (OS) and event free survival (EFS) in ALL was 76% and 61,4% respectively [3]. In our study, the OS at 41 months was 75%.

Age younger than 1 and older than 10 years and male gender are unfavourable prognostic factors in childhood leukemia [4].

High WBC count at presentation, usually over 50.000/mm^3 and especially when associated with pre-B immunophenotype, predicts a poor outcome [4].

Blast immunology is an important prognostic feature. Approximately 80-85% of ALL in children are of B lineage and 10-15% of T cell lineage, in 15-30% of cases they coexpress myeloid markers. B cell lineage diagnosed by CD19, CD22, CD79a positivity, is stratified into 4 subgroups: pro-B with no expansion of other B-lineage antigen, common ALL with CD10 positivity, pre-B which has intracytoplasmatic IgM and mature-B which contains cytoplasmatic or surface kappa or lambda. A poor prognostic impact is seen in T-cell ALL, which is less sensitive to methotrexate treatment, caused by its modified metabolism. The mature B cell lymphoblast has a very high rate of proliferation and dismal prognosis. Mixed lineage

leukemias are difficult to diagnose, scoring systems have been developed for better assessment [5,6].

Genetic abnormalities like translocation t(12;21) corresponding to TEL-AML1 fusion gene or hyperdiploidy with 51-65 chromosomes and DNA index above 1,16 are signs for good prognosis. These features are ferquently associated with age between 1-10 years, low presenting WBC count and good response to treatment. Other abnormalities like hypodiploidy (less than 44 chromosomes), MLL gene rearrangements on chromosome 11q23 [(especially t(4;11)], the Philadelphia chromosome [translocation t(9;22)], are associated with poor prognosis, when intensive chemotherapy, allogeneic bone marrow transplantation in the first remission, tyrosine kinase inhibitors may ameliorate the outcome. [4]. In our study we had the possibility to check BCR-ABL gene expression by qRT-PCR in 29 patients with 3 positive results, but could not determine the MLL-AF4 gene expressions because of financial reasons, however the marrow DNA samples are preserved for future examinations.

Early good response to treatment, defined as absolute lymphoblast count in the peripheral blood below 1000/mm^3 after 7 days of corticotherapy, reducing the lymphoblast count below 5% in the bone marrow on day 15 and 33 of treatment, indicates good prognosis. In the french FRALLE-93 study (1993-1999), 3,8% of all ALL patients failed to achieve remission after induction therapy, which greatly affected the 5-year OS of this group, hardly reaching 30% compared to 90% in the group with complete remission. [7]. In our study, early good response to treatment, age, leukemic cell burden at diagnosis and immunophenotyping were used in risk stratification. Similar to the results obtained by the french study, however on a much smaller number of patients, we found a statistically significant difference in survival according to risk groups: in MR group median survival was 108 months (90%) and in the HR group 55 months (45,83%). A nationwide finnish study performed two decades earlier (1989-91), noticed an OS of 76% in SR group and 64% in the merged MR and HR group. [5]. In the same finnish study, 5-year EFS was 78% in the group of patients who achieved M1 bone marrow on day 15 of treatment and only 62% in the group with M2 or M3 BM type. BM status on day 15 and 33 were also significant prognostic factors in our patients, M2 type BM on day 33 lowered fourfold the OS.

Survival rate in cases with relapses was significantly lower compared to patients who did not experience relapses (29% versus 81%).

CNS involvement at diagnosis, as well as traumatic lumbar puncture raises the probability of CNS relapse and lowers the chance of survival (3). This relationship was relevant in our patients, we noticed a reduction of survival to 40% in patients with CNS involvement, compared to OS above 80% in cases when CNS was free from leukemic infiltrates.

Submicroscopic levels of the disease or minimal residual disease (MRD) can be detected by PCR analysis, targeting lymphoblast-specific immunoglobulin or T-cell receptor gene rearrangements or chromosomal translocations in the range of 1/100.000 cells. End-induction MRD is an independent, important predictor of outcome in children with ALL [8].

Conclusions

1. During 2001-2010 we diagnosed and treated 59 children with ALL, the overall survival at a medium of 41 months was 75%.
2. Our diagnostic tools have developed so that we can perform blast immunophenotyping for every patient and minor and major BCR-ABL gene expression assessment with quantitative real-time PCR technique from bone marrow from 2010 on. MLL-AF4 determination has not yet been introduced. Molecular analysis is highly important for accurate risk stratification.
3. Statistically significant unfavourable prognostic factors were blast mature B immunophenotype, early induction failure, CNS involvement at diagnosis and relapses.
4. We did not find significant difference in survival between the pre-B and T cell immunophenotype groups.

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