Improvement of Risk Stratification in Acute Lymphoblastic Leukemia Patients by the Determination of the BCR-ABL Gene Expression

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Introduction: The BCR-ABL fusion gene [t(9;22) (q34;q11)] occurs in 3–5% of pediatric acute lymphoblastic leukemia (ALL) and predicts a very poor prognosis.
Material and methods: 2 ml samples of bone marrow (BM) and peripheral blood (PB) in EDTA tubes from 24 ALL patients were examined in the molecular biology laboratory of our university with quantitative real-time PCR (qRT-PCR) method for BCR-ABL gene expression. Prognostic factors, like age, leucocyte count, lymphoblast morphology and immunology, absolute lymphoblast count on day 8, remission status on day 33 as well as treatment results were recorded from every patient.
Results: All 24 qRT-PCR analysis for major and minor BCR-ABL gene expression from BM and PB were negative. Immunophenotyping performed in 25 patients revealed common B ALL in 12 patients, T-cell immunology in 3 and pre-B immunophenotype with aberrant myeloid marker expression and/or CD10 negativity in 11 patients. L1 morphology appeared in 85.7% of the pre-B ALL cases, while other immunophenotypes were more likely associated with L2 cytology (62.5%) (p=0.033). Early cortisone response was favourable in 22 patients, all 26 patients achieved complete remission on day 33. Common B immunophenotype was associated with lower WBC (mean 37,770, median 8,200) than other immunophenotypes (WBC mean 63,783, median 50,680).
Conclusions: A new method, the qRT-PCR test was introduced in the investigation of pediatric ALL in our university from 2010. We found a statistically significant correlation between L1 blast morphology and common B immunophenotype. Poor cortisone response was found more frequently in T-cell ALL and pre-B ALL with aberrant myeloid markers or CD10 negativity. All our patients achieved complete remission on day 33. Lower WBC count at presentation was associated with L1 morphology and pre-B immunophenotype.

Keywords: lymphoblastic leukemia, BCR-ABL gene expression

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. Patients are stratified into standard, medium and high risk groups. The 4-year event-free survival (EFS) varies from 80% in most favourable cases to 65% in high risk patients.

Two specific gene rearrangements or the corresponding cytogenetic translocations, namely the BCR-ABL gene rearrangement t(9;22) (q34;q11) and the MLL-AF4 gene rearrangement t(4;11) (q21;q23) when present, place patients into the high risk group. A realistic risk stratification is crucial to obtain optimal results because treatment-intensity varies among risk groups.

The aim of this study is to perform a screening for BCR-ABL gene expression level in the bone marrow and peripheral blood of the ALL patients treated in our center in order to identify these very high risk patients.

Material and methods

We studied 26 pediatric ALL patients treated at the Pediatric Clinic I from Tîrgu Mureş between October 2010 – February 2011. We collected prognostic data such as age at diagnosis, gender, year of diagnosis, WBC count at presentation, absolute lymphoblast count in the peripheral blood on the 8th day of corticotherapy, BM remission status on the 33rd day of chemotherapy, CNS involvement, lymphoblast morphology and immunology. Informations about treatment, cranial radiotherapy and outcome were also recorded. Seventeen bone marrow samples and 24 peripheral blood samples were tested for major and minor BCR-ABL gene expression with the qRT-PCR technique. 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 × 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

Table I. Relationship between blast immunophenotype and early cortisone response (Chi-square test, p=0.273)

Absolute blast count in peripheral blood on day 8	Blast immunophyenotype		Total
	Common B	Other (T-immunology, pre-B plus CD10 neg, CD13, CD33)	
<1000/mm^3	11 (55.0%)	9 (45.0%)	20 (100.0%)
>1000/mm^3	1 (25.0%)	3 (75.0%)	4 (100.0%)
Total	12 (50.0%)	12 (50.0%)	24 (100.0%)

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 volume. All reactions were made in triplicate. The ABL gene was used as endogenous control and known positive and negative control samples were also used. We performed relative quantification. Data was statistically processed with Chi-square test and descriptive analysis.

Results

Between January 2007 – February 2011, 26 patients were diagnosed with ALL, two of them with early CNS involvement. The mean age at diagnosis was 5.52 years (4 months – 17 years). In the favourable age group of 1–6 years there were 15 patients, while one patient was a 4 months old infant and the other 10 patients had ages above 6 years. There was a male predominance with a ratio of boys versus girls of 1.6:1. The initial WBC count ranked between 1,500 and 239,460/mm^3 with a median of 14,800/mm^3 and mean of 50,378/mm^3. Twenty-three bone marrows were evaluable for blast morphology, out of which L1 type lymphoblast was found in 7 cases and L2 type in 16 cases. Immunophenotyping was performed on 25 bone marrow samples, 3 of them were of T-cell, 12 were of pre-B cell morphology and 11 samples were of pre-B morphology but with CD10 negativity or they expressed additional aberrant myeloid markers (CD13, CD33). Seventeen BM samples were screened for BCR-ABL gene expression (p201) and all were negative. Major (p210) and minor (p190) BCR-ABL gene expression was tested in the peripheral blood of 24 patients and all test results were negative. Four patients had an absolute lymphoblast count above 1000/mm^3 in the peripheral blood on the 8th day of corticotherapy. All bone marrows showed complete remission on the 33rd day of chemotherapy (< 5% lymphoblasts). Four patients were stratified into high risk leukemia and 22 into the medium risk group. Seven patients were given cranial radiotherapy. None of the patients experienced bone marrow or extramedullary relapses. One two year old patient died of severe pneumonia.

A strong, however statistically not significant relationship (Chi-square test, p=0.273) was found between early cortisone response and the immunophenotype of lymphoblasts. Poor cortisone response appeared in only 25% of common B ALL, but in 75% of patients with T-cell ALL and pre-B ALLs which expressed aberrant myeloid markers or were CD10 negative.

Table II. Significant statistical relationship between other than common B immunology of the blasts and L2 cell morphology (Chi-square test, p=0.033)

Blast morphology	Blast immunophyenotype		Total
	Common B	Other (T-immunology, pre-B plus CD10 neg, CD13, CD33)	
L1 Count	6	1	7
% within BM morphology	85.7%	14.3%	100.0%
% within blast immunophenotype	50.0%	9.1%	30.4%
L2 Count	6	10	16
% within BM morphology	37.5%	62.5%	100.0%
% within blast immunophenotype	50.0%	90.9%	69.6%
Total Count	12	11	23
% within BM morphology	52.2%	47.8%	100.0%
% within blast immunophenotype	100.0%)	100.0%	100.0%

L1 morphology appeared in 85.7% of the pre-B ALL cases, while other immunophenotypes were more likely associated with L2 cytology (62.5%). This association was significant with Chi-square test (p=0.033) (Table II).

L1 morphology was associated with lower WBC count at diagnosis (mean 28,131.25/mm^3; median 10,700/mm^3) compared to the L2 morphology of the blasts (mean WBC 60,136.67/mm^3; median 38,900/mm^3).

Common B immunophenotype appeared more likely with lower WBC (mean 37,770, median 8,200) than other immunophenotypes (WBC mean 63,783, median 50,680).

Discussion

ALL patients are stratified into risk groups, based on prognostic factors, which predict the outcome and define the intensity of the treatment [2].

Seventy five percent of childhood ALL cases have chromosomal abnormalities and consecutive molecular rearrangements. Novel therapies targeting aberrant cytokine receptor signalling may have therapeutic benefits in high-risk ALL [3].

The TEL-AML1 fusion gene [t(12;21)] which predicts excellent prognosis, can be detected in 1/1000 cases by cytogenetics and up to 25% of pre-B ALL by molecular techniques [4].

The BCR-ABL fusion gene [t(9;22) (q34;q11)] occurs in 3–5% of pediatric ALL cases where the ABL translocates to the minor breakpoint cluster region on chromosome 22, resulting a 190 kd protein (p190) in contrast to chronic myeloid leukemia (CML) where a different breakpoint produces a 210 kd protein (p210). Philadelphia chromosome positive ALL appears at older age, with higher WBC count, CNS involvement at diagnosis and a very poor prognosis with a 5-year event-free survival (EFS) of only 14% [4]. A large study enrolling 326 patients showed that despite good cortisone response in 72% and complete postinduction remission in 82% of the patients, the 5-year

EFS did not exceed 25% [5]. They may benefit from treatment with tyrosine kinase inhibitors, intensive chemotherapy and bone marrow transplantation in their first remissio n[6,2]. Our 24 patients tested negative for BCR-ABL gene expression, which is a realistic result as soon, as only 3–5% of pediatric ALL patients are Philadelphia chromosome positive, in contrast with adults.

MLL rearrangement appears in 80% of infant ALL and 3–8% of ALL in older children. It corresponds to the t(4;11), t(11;19) and (9;11), and hyperleucocytosis, CD10 negativ immunophenotype and dismal outcome with chemotherapy are its main features. Better results (EFS 43.6%) were achieved with early bone marrow transplantation [7,8]. We had one single patient with infant leukemia, diagnosed at the age of 4 months with hyperleucocytosis, yet she achieved good cortisone response and complete remission with the Interfant 99 protocol. She is being currently under maintenance therapy.

Four of our patients had poor cortisone response but their bone marrow was free of blasts on the 33rd day of chemotherapy. In a large trial 2–23% of the patients did not achieved complete remission after one month of treatment, and they were 1.5–6.1 × more likely to have adverse events than patients with more rapid treatment response. The slow 33-day response is an independent poor prognostic factor in ALL [9].

Conclusions

1. The diagnosis of childhood ALL has become more precise by introducing the quantitative real-time PCR examination for the detection of BCR-ABL gene rearrangements from bone marrow and peripheral blood. We performed this test on 17 BM and on 24 PB samples, all tests were negative.
2. We performed immunophenotyping on 25 BM out of 26, common B ALL was diagnosed in 12 patients, T-cell ALL in 3 and pre-B with aberrant myeloid markers or CD10 negativity in 11 patients.
3. We found a statistically significant correlation between L1 blast morphology and common B immunophenotype.
4. Poor cortisone response (n=4) was found more frequently in T-cell ALL and pre-B ALL with aberrant myeloid markers or CD10 negativity.
5. All our patients achieved complete remission on day 33.
6. Lower WBC count at presentation was associated with L1 morphology and pre-B immunophenotype.

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References

1. Gabert J et al – Standardization and quality control studies of real time quantitative reverse transcriptase polimerase chain reaction of fusion gene transcripts for residual disease detection on leukemia – a Europe Against Cancer Program, Leukemia 2003, 17: 1318–1357.
2. Silverman Lewis B – Childhood Acute Lymphoblastic Leukemia: Currently Applied Prognostic Factors, in: Agarwal Bharat R, Calaminus Gabriele, Diller Lisa, Egeler Maarten: SIOP Education Book 2010, 11–18. 42nd Congress of the International Society of Paediatric Oncology, Boston, USA, October 21–24, 2010.
3. Mullighan Charles – Genetic Alterations in High-Risk B-Progenitor Acute Lymphoblastic Leukemia, in: Agarwal Bharat R, Calaminus Gabriele, Diller Lisa, Egeler Maarten: SIOP Education Book 2010, 77–88. 42nd Congress of the International Society of Paediatric Oncology, Boston, USA, October 21–24, 2010.
4. Lanzkowski Philips – Manual of Pediatric Hematology and Oncology, Fourth Edition, Elsevier, 2004, 415–452.
5. Aricò M, Valsecchi MG, Camitta B, et al.– Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med, 2000, 342 (14): 998–1006.
6. Hunger Stephen – Philadelphia Chromosome Positive ALL: Use of Tyrosine Kinase inhibitors, in Agarwal Bharat R., Calaminus Gabriele, Diller Lisa, Egeler Maarten: SIOP Education Book 2010: 67–72. 42nd Congress of the International Society of Paediatric Oncology, Boston, USA, October 21–24, 2010.
7. Kosaka Y, Koh K, Kinukawa N, et al. – Infant acute lymphoblastic leukemia with MLL gene rearrangements: outcome following intensive chemotherapy and hematopoietic stem cell transplantation. Blood 2004, 104(12): 3527–3534.
8. Biondi A, Cimino G, Pieters R, Pui CH – Biological and therapeutic aspects of infant leukemia. Blood 2000, 96: 24–33.
9. Gaynon PS, Desai AA, Bostrom BC, et al. – Early response to therapy and outcome in childhood acute lymphoblastic leukemia: a review. Cancer 1997, 80(9): 1717–1726.