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PROGNOSTIC SIGNIFICANCE OF *BCR-ABL* REARRANGEMENT IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

ZNACZENIE PROGNOSTYCZNE REARANŻACJI *BCR-ABL* W OSTREJ BIAŁACZCE LIMFOBLASTYCZNEJ U DZIECI

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Summary

Background. Acute lymphoblastic leukemia (ALL) is the most frequent pediatric malignancy. Presence of adverse risk factors determines risk group stratification in this disease.

Objective. The aim of study was the analysis of results of therapy and role of prognostic risk factors in treatment of childhood ALL in kujawsko-pomorskie region in 1995-2010.

Patients and methods. During this period, ALL was diagnosed in 223 patients. With respect to time period and therapy protocol, the patients were divided into two groups: group 1 A/B (1995-2002) and group 2 (2002-2010). Probability of overall survival (OS), event-free survival (EFS) and relapse-free survival (RFS) were analyzed. Uni- and multivariate analyzes for risk factors were performed.

Results. Over the analyzed 17-year period, OS has increased from 77.9% in group 1A and 73.7% in group 1B to 86.2% in group 2. Results of RFS and EFS have also increased during this time. The death rate has decreased from 26% in group 1A and 26.3% in group 1B to 10.2% in group 2. The most important adverse prognostic risk factors during the first period included involvement of liver, spleen, lymph nodes as well as poor response to initial therapy, while during the second period the most important independent risk factor was *BCR-ABL* rearrangement in lymphoblasts.

Conclusions. The most important independent prognostic risk factors in pediatric ALL include advanced disease, *BCR-ABL* rearrangement, and initial response to therapy. These factors are used for stratification to treatment groups, intensification of therapy and hematopoietic stem cell transplantation.

Streszczenie

Wstęp. Ostra białaczka limfoblastyczna (ALL) jest najczęstszym nowotworem wieku dziecięcego. W tej chorobie obecność czynników ryzyka decyduje o stratyfikacji pacjentów do grup ryzyka.

Cel pracy. Analiza wyników terapii i roli czynników prognostycznych w ALL u dzieci w regionie kujawsko-pomorskim w latach 1995-2010.

Pacjenci i metodyka. Analizą objęto 223 dzieci, które podzielono na 2 grupy w zależności od okresu terapii i stosowanych protokołów leczenia: grupa 1A/B

(1995-2002) i grupa 2 (2002-2010). Wyznaczono prawdopodobieństwo przeżycia wolnego od zdarzeń (pEFS), prawdopodobieństwo przeżycia (pOS) i prawdopodobieństwa przeżycia wolnego od wznowy (pRFS). Przeprowadzono analizę jedno- i wielowariantową czynników ryzyka niepowodzenia terapii.

W y n i k i . W analizowanym okresie 17 lat, OS wzrosło od 77,9% w grupie 1A i 73,7% w grupie 1B do 86,2% w grupie 2. Wyniki RFS i EFS również uległy poprawie. W tym czasie odsetek zgonów obniżył się z 26% w grupie 1A i 26,3% w grupie 1B do 10,2% w grupie 2. W analizie czynników ryzyka wykazano, że w pierwszym analizowanym okresie zajęcie wątroby, śledziony i węzłów chłonnych oraz niekorzystna początkowa odpowiedź na terapię były związane z gorszymi ostatecznymi wynikami terapii, natomiast w drugim okresie czasu najsilniejszym niezależnym niekorzystnym czynnikiem rokowniczym była obecność rearanżacji *BCR-ABL* w limfoblastach.

W n i o s k i . Najważniejszymi czynnikami prognostycznymi w ALL u dzieci są: zaawansowanie choroby, odpowiedź na leczenie oraz obecność rearanżacji *BCR-ABL*, które są podstawą stratyfikacji do grup ryzyka, intensyfikacji leczenia w grupach wysokiego ryzyka oraz kwalifikacji do przeszczepiania komórek krwiotwórczych.

Key words: acute lymphoblastic leukemia, children, prognostic factors
Słowa kluczowe: ostra białaczka limfoblastyczna, dzieci, czynniki ryzyka

BACKGROUND

Leukemia is the most frequent children cancer, which estimates 30% of all cases of cancers at the age of 1-18 years. Acute lymphoblastic leukemia (ALL) is the most common and occurs in 80-85% of all cases of leukemia. In the developed countries, there are annually 30-40 new cases of ALL per one million children. ALL is a heterogeneous group of disorders that can be subtyped by morphology, immune-phenotyping, cytogenetic study, and molecular techniques [1]. The Philadelphia chromosome (Ph) resulting from a translocation t(9;22) is the hallmark of chronic myeloid leukemia and also—occurs in approximately 5% of children and 20% of adults with ALL [2]. This reciprocal translocation juxtaposes the *BCR* gene on chromosome 22 with the *ABL* gene on chromosome 9, producing a fusion gene that encodes the *BCR-ABL* fusion protein. Ph-positivity in ALL is associated with aggressive disease and has been shown to be a poor prognostic factor, especially in children. Thus, even in spite of therapy with tyrosine kinase inhibitors, Ph-positive patients are candidates for more aggressive treatment regimens such as hematopoietic stem cell transplantation. The relapse rate for Ph+ALL is still high, ranging from 30 to 70% [3, 4].

The objective of the study was the analysis of results of therapy and the role of prognostic risk factors, including BCR-ABL rearrangement, in treatment of childhood ALL in kujawsko-pomorski region in 1995-2010.

PATIENTS

A total number of 223 children under 18 years of age diagnosed and treated for ALL in Department of Pediatric Hematology and Oncology in Bydgoszcz between 1995-2010 were included into the study.
The follow-up was completed by 2011. The Department is the only center in the kujawsko-pomorski region for children with malignant diseases.

With respect to the time period and therapeutic protocols ("therapeutic era") used, the patients were divided into 2 groups (Table 1). Infants (age below 1 year at diagnosis) and patients with mature B-cell ALL were excluded from this analysis, as they are treated according to different protocols.

Group IA (1995-2002). 96 patients were treated according to ALL-BFM-90 protocol (group IA) [5]. Patients treated with ALL-BFM-90 protocol were stratified into 3 risk groups: standard (SR), intermediate (IR) and high (HR), depending on risk factor (RF), response to one week prednisone therapy, bone marrow (BM) response by day 15, complete remission (CR) by day 33 and presence of *BCR-ABL*, hypodiploidy or *MLL-AF4* rearrangement, central nervous system (CNS) involvement and/or pre-T/T-ALL immunophenotype. Follow-up ranged from 10 days to 15.2 years, median 9.9 years.

Grupa IB. In 1999-2002, patients with initial white blood cell (WBC) count >50 G/L were treated according to New York I (1999, n=7), and New York II (1999-2002; n=12) protocols (group IB) [6]. Follow-up ranged from 1.2 to 11.8 years, median 9.1 years. This group included also three patients with initial WBC below 50 G/L.

Group II (2002-2010) 108 children were treated according to ALL-IC-2002 protocol [8]. The patients were stratified into 3 risk groups (depending on age, WBC count, response to one week prednisone therapy, BM response by day 15, CR by day 33 and presence of *BCR-ABL* or *MLL-AF4*). Follow-up ranged from 24 days to 8.2 years, median 2.8 years.

Table 1. *Groups of patients*
Tabela 1. *Grupy pacjentów*

YEARS	PROGRAM	NUMBER OF PATIENTS	GROUP
1995-2002	ALL-BFM-90 [5]	96	1A
	New York I i II [6,7]	19	1B
2002-2010	ALL-IC-2002 [8]	108	2

Table 2. *Patients characteristics*
Tabela 2. *Charakterystyka pacjentów*

CHARACTERISTICS	GROUP 1A (n=96)	GROUP 1B (n=19)	GROUP 2 (n=108)
Age (range, median) [years]	0.1-18.8/5.1	1,3-8.6/5.4	1,1-17.6/5.0
Sex male/female	49 / 47	11 / 8	58 / 50
Initial WBC [G/L] (range/median) <20 G/L ≥20 G/L	0.5-387.0/9.2 57 (59.4%) 39 (40.6%)	11,2-325.0 / 75.5 3 (15.8) 16 (84.2%)	0.59-305.0/9.25 67 (62.0%) 41 (38.0%)
Initial HB concentration (g/dl) < 8.0 ≥ 8.0	2.3-15.9/7.6 54 (56.3%) 42 (43.7%)	3.5-14.1/8.7 6 (31,6%) 13 (68.4.7%)	2.1-14.3/8.0 53 (49.1%) 55 (50.9%)
FAB classification* L1 L2	 67 (69.8%) 39 (30.2%)	 12 (63.2%) 7 (36.8%)	 65 (60.2%) 43 (39.8%)
Immunophenotype common/pre-B pro-B T undifferentiated	 76 (79.2%) 5 (5.2%) 15 (15.6%)	 11 (57.9.1%) 1 (5.3%) 6 (31,5%) 1 (5.3%)	 93 (86.1%) 3 (2.8%) 11 (10.2%) 1 (0.9%)
Response to steroids good poor	 93(96.9%) 3 (3.1%)	 19 (100%) 0 (0.0%)	 97 (89.8%) 11 (10.2%)
Bone marrow at day 14/15* M1 M2 M3 no data	 72 (75.0%) 12 (12.5%) 4 (4.2%) 8 (8.3%)	 10 (52.6%) 2 (10.6%) 7 (36.8%)	 90 (83.3%) 14 (13.0%) 3 (2.8%) 1 (0.9%)
Bone marrow at day 28/33* M1 M2 no data	 88 (91,7%) 1 (1,0%) 7 (7.3%)	 11 (57.9%) 8 (42.1%)	 107 (99.1%) 1 (0.9%)
Hypodiploidy present absent	 14 (14.6%) 82 (85.4%)	 1 (5.3%) 18 (84.7%)	 12 11,1%) 96 (88.9%)
BCR-ABL rearrangement present absent	 0 96 (100%)	 1 (5.3%) 18 (94.7%)	 7 (6.5%) 101 (93.5%)
Initial CNS status no involvement involvement	 91 (94.8%) 5 (4.2%)	 19 (100%) 0 (0.0%)	 86 (79.6%) 22 (20.4%)
Mediastinum no involvement involvement	 91 (94.8%) 5 (4.2%)	 16 (84.2%) 3 (15.6%)	 98 (90.7%) 10 (9.3%)
Hepatomegaly no involvement < 4 cm ≥ 4 cm	 22 (22.9%) 34 (35.4%) 40 (41,7%)	 3 (15.6%) 7 (37.0%) 9 (47.4%)	 36 (33.3%) 41 (38.0%) 31 (28.7%)
Splenomegaly no involvement < 4 cm ≥ 4 cm	 40 (41,6%) 28 (29.2%) 28 (29.2%)	 6 (31,5%) 5 (26.4%) 8 (42.1%)	 50 (46.3%) 28 (25.9%) 30 (27.8%)
Lymph nodes no involvement < 3 cm ≥ 3 cm	 53 (55.2%) 36 (37.5%) 7 (7.3%)	 9 (47.4%) 9 (47.4%) 1 (5.2%)	 50 (46.3%) 48 (44.4%) 10 (9.3%)

*not performed in all patients

METHODS

Diagnosis of ALL. ALL was diagnosed based on the result of blasts morphology, immunophenotype, cytochemistry, and cytogenetic analysis. The immunophenotype of ALL has been diagnosed by APAAP method until 1996, and then by flow cytometry using an acute leukemia panel of monoclonal antibodies.

Clinical analysis. Following risk factors were analyzed: age and sex, involvement of CNS, mediastinum, lymph nodes, hepatomegaly or splenomegaly >4 cm, risk group based on BFM risk index, initial WBC, initial hemoglobin concentration, blast immunophenotype, *BCR-ABL*, *TEL-AML1*, *MLL-AF4* rearrangements, hypodiploidy or hyperdiploidy, response to one-week steroid therapy, and bone marrow response at day 14/15 and 28/33.

Cytogenetic Analysis. Cytogenetics and molecular methods (FISH and RT-PCR) were used to identify genetic aberrations such as *BCR-ABL*, *TEL-AML*, and *MLL-AF4*. Heparinized bone marrow aspiration samples were cultured in RPMI-1640 with sodium bicarbonate for 24 and 48 h. Metaphase chromosomes were banded by the conventional GTG-banding technique, and karyotypes were analyzed to assess chromosome abnormalities.

RT-PCR Analysis. RT-PCR was used to detect the *BCR-ABL* fusion gene (b3a2, b2a2, and e1a2). Total RNA was extracted from white blood cells using Trizol reagent. Random primers were used for cDNA synthesis from 2µg of total RNA. The amplifications of *BCR-ABL* and *ABL* as internal control were performed according to BIOMED1 [9]. The PCR products were analyzed on 2% agarose gel and visualized by ethidium bromide staining under UV illuminator.

Statistical analysis. The probability of 5 years event-free survival (EFS), relapse-free survival (RFS) and overall survival (OS) were determined with Kaplan-Meier method. Event free survival (EFS) was defined as time from diagnosis to the date of failure (relapse, secondary neoplasm or death from any cause). Overall survival (OS) was defined as time from diagnosis to death or the date of the last contact. Relapse free survival (RFS) was defined as time from

complete remission to relapse of disease. The Kaplan-Meier method was used to estimate survival curves for pEFS, pOS and pRFS. Differences between groups were determined by log-rank test. All potential factors (mentioned in Table 2) that could influence the treatment results were analyzed in univariate analysis of risk factors in Cox regression model. Odds ratios (OR) were calculated with 95% confidence interval (CI). All significant factors (p-value<0.05) were put together and analyzed in multivariate analysis. SPSS 21 (SPSS Inc, Chicago, Illinois) statistical package was used.

RESULTS

Survival

Over the analyzed 17-year period, OS has increased from 77.9% in group 1A and 73.7% in group 1B to 86.2% in group 2 (Tab. 3). Results of RFS and EFS has also increased during this time. The death rate has decreased from 26% in group 1A and 26.3% in group 1B to 10.2% in group 2 (Tab. 4).

Table 3. *Results of 5-year therapy*
Tabela 3. *Wyniki leczenia 5-letnie*

Group	pEFS	pRFS	OS
1A	0.688±0.047	0.806±0.044	0.779±0.043
1B	0.684±0.107	0.719±0.107	0.737±0.101
2	0.766±0.045	0.814±0.045	0.862±0.042

pEFS, probability of event-free survival; pOS, probability of overall survival; pRFS, probability of relapse free survival

Table 4. *Deaths of patients*
Tabela 4. *Zgony pacjentów*

	GROUP 1A	GROUP 1B	GROUP 2
Number of patients	96	19	108
Alive	71 (74.0%)	14/19 (73.7%)	97 (89.8%)
Deaths	25 (26.0%)	5/19 (26.3%)	11 (10.2%)
No remission	3 (3.2%)	2 (10.5%)	2 (1,9%)
Remission	10 (10.4%)	0	4 (3.7%)
At relapse	12(12.4%)	3 (15.8%)	5 (4.6%)

Group 1A. 94/97 (97.9%) children treated according to ALL-BFM-90 protocol achieved CR. Survival in this group was from 10 days to 15.2 years, median 10.0 years. Mean EFS was 10.8 years (95% CI=9.6-12.2). Twenty-five patients died, including 3 before achievement of remission, 10 in CR1, and 12 in relapse. 62 patients stay in long-term CR. Relapse of disease occurred in 16 (16.7%) patients. Median RFS was 2.5 years (range, 85 days – 5.4 years). Mean OS was 11.7 years (95% CI=10.6-12.8).

Group 1B. All 19 children treated with New York protocols achieved CR, for 0.7-11.8 years, median 8 years. Mean EFS was 7.8 years (95% CI= 5.7-9.8). Five patients died (2 in CR and 3 due to relapse). Mean OS was 8.9 years (95% CI=6.9-10.9). Five relapses were observed. Median RFS was 9.1 years (range, 207 days – 11.8 years).

Group 2. CR was achieved by 106/108 patients. Survival in this group was from 24 days to 8.3 years, median 3.1 years. Mean EFS was 6.5 years (95% CI=5.8-7.1). 11 (10.2%) deaths occurred: 2 before CR, 4 in CR and 5 due to relapse. Relapse occurred in 16 children, at a median time 1.4 years (range, 92 days – 6.5 years). Mean OS was 7.4 years (95% CI=6.8-7.8).

Risk factors analyses

Event-free survival (EFS)

Group 1A. In univariate analysis following risk factors had adverse influence on EFS: response to one-week steroid therapy (OR=10, 95%CI=2.9=36; p=0.001), risk group (OR=3.6, 95%CI=1.7-7.7; p=0.001), initial WBC>20 G/L (OR=1.5, 95%CI=1.03-2.1; p=0.033), hepatomegaly ≥4 cm (OR=2.3, 95%CI=1.1-4.7; p=0.022), splenomegaly ≥4 cm (OR=2.8, 95%CI=1.4-5.7; p=0.004), T-ALL immunphenotype (OR=3.7, 95%CI=1.7-7.9; p=0.001), bone marrow M3 at 14 day of therapy (OR=3.9, 95%CI=1.2-13; p=0.027) and bone marrow M2/M3 at 33 day of therapy (OR=14, 95%CI=1.7-100; p=0.014). In multivariate analysis, risk group, and splenomegaly ≥4 cm retained their significance (Tab. 5).

Table 5. *Multivariate analysis of risk factor for event-free survival*
Tabela 5. *Analiza wielowariantowa czynników ryzyka wystąpienia niekorzystnego zdarzenia*

Group	Characteristics	Risk factor	OR (95%CI)	p
1A	Risk group	SR/IR HR	OR=1 OR=3.5 (1,5-7.9)	p=0.003
	Splenomegaly >4 cm	<4cm ≥4cm	OR=1 OR=2.9 (1,3-6.3)	p=0.008
1B	Bone marrow at 15 day	M1 M2	OR=1 OR=23 (1,6-100)	p=0.022
	Lymph nodes	No involvement involvement	OR=1 OR=4.2 (1,3-13)	p=0.011
2	BCR-ABL rearrangement	Absent Present	OR=1 OR=3.7 (1,03-13.2)	p=0.044

OR – odds ratio, SR – standard risk group, IR – intermediate risk group, HR – high risk group

Group 1B. In univariate analysis lymph nodes involvement (OR=2.4, 95%CI=1.2-4.9; p=0.016) and

bone marrow M2/M3 at 15 day of therapy (OR=7.7, 95%CI=1.07-56; p=0.042) had adverse influence on EFS. Both factors retained their significance in multivariate analysis (Tab. 5).

Group 2. In univariate analysis, the presence of BCR-ABL (OR=3.7, 95%CI=1.05-13.0; p=0.041) was the only prognostic risk factor with adverse influence on EFS, while initial WBC>20 G/L showed a trend towards significance (OR=2.3, 95%CI=0.97-5.1; p=0.059). In multivariate analysis, the presence of BCR-ABL was the only prognostic risk factor (OR=3.7, 95%CI=1.05-13.0; p=0.041) (Tab. 5).

Overall survival (OS)

Group 1A. In univariate analysis following risk factors had adverse influence on OS: response to one-week steroid therapy (OR=2.3, 95%CI=1.8=6.5; p=0.001), risk group (OR=4.7, 95%CI=2.1-10; p=0.001), initial WBC>20 G/L (OR=1.6, 95%CI=1.1-2.4; p=0.017), hepatomegaly ≥4 cm (OR=3.0, 95%CI=1.3-6.7; p=0.009), splenomegaly ≥4 cm (OR=2.6, 95%CI=1.2-5.8; p=0.015), T-ALL immunphenotype (OR=4.0, 95%CI=1.7-9.1; p=0.001), bone marrow M3 at 14 day of therapy (OR=4.7, 95%CI=1.4-16; p=0.014) and bone marrow M2/M3 at 33 day of therapy (OR=12, 95%CI=1.5-98; p=0.020). In multivariate analysis, risk group, and hepatomegaly ≥4 cm retained their significance (Tab. 6).

Table 6. *Multivariate analysis of risk factors for death*
Tabela 6. *Analiza wielowariantowa czynników ryzyka zgonu*

Group	Characteristics	Risk factor	OR (95%CI)	p
1A	Risk group	SR/IR HR	OR=1 OR=4.7 (2.21-14)	p<0.001
	Hepatomegaly	<4 cm ≥4 cm	OR=1 OR=3.0 (1,7-12)	p=0.002
	Bone marrow at 33 day	M1 M2	OR=1 OR=10 (1,03-96)	p=0.047
1B	No significant factors			
2	BCR-ABL rearrangement	Absent Present	OR=1 OR=7.2 (1,4-37)	p=0.020

OR – odds ratio, SR – standard risk group, IR – intermediate risk group, HR – high risk group

Group 1B. None risk factor showed prognostic significance for OS in univariate analysis; thus, multivariate analysis was not performed.

Group 2. In univariate analysis, the presence of BCR-ABL (OR=4.9, 95%CI=1.01-23.0; p=0.049) was the only prognostic risk factor with adverse influence on OS, while initial WBC>20 G/L showed a trend towards significance (OR=3.2, 95%CI=0.94-11; p=0.062). In multivariate analysis, the presence of BCR-ABL was the only prognostic risk factor (OR=7.2, 95%CI=1.4-37; p=0.020) (Tab. 6).

Relapse-free survival (RFS)

Group 1A. In univariate analysis following risk factors had adverse influence on RFS: response to one-week steroid therapy (OR=5.2, 95%CI=1.7-16; p=0.004), risk group (OR=3.3, 95%CI=1.1-10; p=0.040), hepatomegaly ≥4 cm (OR=2.9, 95%CI=1.1-8.1; p=0.037), splenomegaly ≥4 cm (OR=4.1, 95%CI=1.5-11; p=0.005), T-ALL immunphenotype (OR=4.5, 95%CI=1.6-13; p=0.005), and bone marrow M3 at 14 day of therapy (OR=6.7, 95%CI=1.5-30; p=0.013). In multivariate analysis, T-lineage immunophenotype and splenomegaly ≥4 cm retained their significance (Tab. 7).

Table 7. *Multivariate analysis of risk factor for disease relapse*
Tabela 7. *Analiza wielowariantowa ryzyka wznowy choroby*

Group	Characteristics	Risk factor	OR (95%CI)	p
1A	Immunophenotype	B-lineage T-lineage	OR=1 OR=4.3 (1,4-13)	p=0.009
	Splenomegaly	<4 cm ≥4 cm	OR=1 OR=5.0 (1,3-6.3)	p=0.002
1B	Bone marrow at 15 day	M1 M2	OR=1 OR=7.7 (1,04-56)	p=0.042
2	BCR-ABL rearrangement	Absent Present	OR=1 OR=6.6 (1,3-34)	p=0.024

OR – odds ratio

Group 1B. In univariate analysis, bone marrow M2/M3 at day 15 of therapy (OR=7.7, 95%CI=1.04-56; p=0.042) was the only prognostic risk factor with adverse influence on RFS (Tab. 6).

Group 2. In univariate analysis, BCR-ABL rearrangement (OR=6.6, 95%CI=1.3-34; p=0.024) was the only prognostic risk factor with adverse influence on RFS (Tab. 7).

DISCUSSION

In this study, it has been shown that over the analyzed period, overall survival in children with acute lymphoblastic leukemia increased from 73.7-77.9% to 86.2%, and the death rate decreased from 26% to 10.2%. This improvement was reached due to progress

in diagnosis, therapy protocols, and improvement in supportive therapy.

During this period, German group BFM reported 10-year overall survival rate of 83% for patients treated with ALL-BFM-90 protocol, and 85% for those treated according to ALL-BFM-95 protocol [10]. International protocol ALL-IC-2002 ended up with overall survival of 82% for 5060 children being included [8]. Results for high-risk group of children treated with the New York protocols between 1999-2002 (group 1B) were even better than those obtained by other Polish centers. Our study showed 5-years event-free survival of 68%, and overall survival of 73% patients, while 5-years EFS for all Polish centers reached 56% in New York I and 73% in New York II protocols [6,7].

Current studies indicate that the most important prognostic factors are cytogenetic/molecular aberrations, response to initial therapy and presence of minimal residual disease (MRD) [11-14]. Our study, which reflects progress in our region over last two decades, was related to available methods of diagnosis and therapy. Detection of MRD has become available in our center only recently, and is still limited by high costs [15,16]. On the other hand, detection of translocation t(9;22) or *BCR-ABL* rearrangement in leukemic blasts, the most important genetic abnormality in ALL, was available almost throughout the whole analyzed period. Subgroups of children with unfavorable genotypes such as Philadelphia positive ALL have an extremely poor prognosis [2, 4]. Arico et al. showed that children aged >10 years with this rearrangement and WBC>20 G/L, are at particularly poor risk of cure [2,11]. In our study 6.5% of children showed BCR-ABL rearrangement, which seems to be higher than the usually reported level of 3-5% [8,13]. Low remission rate is expected if there is a lack of suppression of Ph-positive metaphases with intensive chemotherapy. Our analysis has shown that *BCR-ABL* rearrangement was not only the most important adverse prognostic factor in the last period, but also the only significant factor in multivariate analysis for OS, EFS and RFS. Identification of additional prognostic variables that can be used to tailor therapy more precisely and discovery of drugs that can modify pathways involved in transformation and resistance to therapy are top priorities.

Response to initial therapy determined by three time points (day 8 with estimation of response to one-week steroid monotherapy, day 15 and day 33 with analysis of bone marrow response) is very important for stratification and prognosis. Some of these factors had significance for patients treated between 1995-2002. Nevertheless, response to therapy is easy to perform, thus accessible to all centers worldwide. The significance of these factors is valuable for patients stratification and risk-oriented therapy [11-14].

History of the treatment of childhood acute lymphoblastic leukemia has become a paradigm for cancer cure. Our study showed that relapses still remain the most important failure of therapy. During the analyzed period some prognostic factors in childhood ALL were changing; some of them lost their significance, while new factors appeared. Nevertheless, immunophenotype, initial WBC, *BCR-ABL* rearrangement and early BM response at 15 and 33 day are independent prognostic factors. With the improvement of treatment results in pediatric acute lymphoblastic leukemia in kujawsko-pomorski region in 1995-2010, our results reached those obtained by national and the international study groups in highly-developed countries.

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