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Analysis of the influence of decrease of L-asparaginase activity and hypersensitivity reaction on the treatment outcome in children with acute lymphoblastic leukemia

Ocena wpływu obniżenia aktywności L-asparaginazy i reakcji alergicznej na wyniki leczenia ostrej białaczki limfoblastycznej u dzieci

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Key words

L-asparaginase, acute lymphoblastic leukemia, children, treatment outcome, hypersensitivity

Słowa kluczowe

L-asparaginaza, ostra białaczka limfoblastyczna, dzieci, wyniki leczenia, nadwrażliwość

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Summary

Introduction. L-asparaginase (L-ASPA) is one of the basic drug in the treatment of the acute lymphoblastic leukemia (ALL) in children.

Aim. The aim of the study was to analyze the influence of decrease of L-ASPA activity and allergic reaction on the treatment outcome in children with ALL.

Material and methods. Eighty seven patients treated with ALL IC-BFM 2002 Protocol were enrolled to the study. L-ASPA activity was measured during induction. In course of all chemotherapy cycles comprising L-ASPA symptoms of allergic reactions to the drug were registered. Treatment outcome was assessed after observation lasting 62-102 months.

Results. Activity below therapeutic values (< 100 IU/l) was noticed in 19 (21%) patients, including 7 (8%) patients with undetectable activity (< 30 IU/l). Disease free survival (DFS) did not differ significantly between the groups with therapeutic and low L-ASPA activity (5-years DFS 88.9 and 84.5% respectively; p = 0.69). Allergic reaction occurred in 42 (49%) patients. Children with low and undetectable L-ASPA activity were at especially high risk of allergic reaction (hazard ratio respectively: 1.86 and 2.23). Occurrence of hypersensitivity to L-ASPA was not associated with outcome deterioration (5-years DFS in patients with allergic reaction 85%; in patients without hypersensitivity: 85.8%; p = 0.94).

Conclusions. Decrease in L-ASPA activity in children treated for ALL was not associated with outcome deterioration but was significant risk factor of hypersensitivity to this drug.

Streszczenie

Wstęp. L-asparaginaza (L-ASPA) to jeden z podstawowych leków stosowanych w terapii ostrej białaczki limfoblastycznej (ALL) u dzieci.

Cel pracy. Celem pracy była ocena wpływu obniżenia aktywności L-ASPA i reakcji alergicznej w odpowiedzi na ten lek na wyniki leczenia ALL u dzieci.

Materiał i metody. Do badania włączono 87 dzieci leczonych według protokołu ALL IC-BFM 2002. Podczas indukcji oznaczano aktywność L-ASPA. W trakcie wszystkich cykli chemioterapii zawierających L-ASPA obserwowano pacjentów w kierunku wystąpienia reakcji alergicznej na ten lek. Po okresie obserwacji trwającym od 62 do 102 miesięcy oceniono wyniki leczenia.

Wyniki. Aktywność L-ASPA poniżej wartości terapeutycznych (100 IU/l) stwierdzono u 19 (21%) pacjentów, w tym u 7 (8%) dzieci stwierdzano aktywność nieoznaczalną (< 30 U/l). Wskaźnik ponad 5-letniego przeżycia wolnego od choroby (DFS) nie różnił się istotnie pomiędzy grupą pacjentów z aktywnością L-ASPA poniżej 100 IU/l a pacjentami z aktywnością terapeutyczną (DFS odpowiednio 88,9 i 84,5%; p = 0,69). Reakcja alergiczna wystąpiła u 42 (49%) pacjentów. Szczególne ryzyko wystąpienia reakcji nadwrażliwości dotyczyło pacjentów z niską i nieoznaczalną aktywnością leku (współczynnik ryzyka HR odpowiednio: 1,86 i 2,23). Wystąpienie reakcji nadwrażliwości na L-ASPA nie wiązało się

z istotnym pogorszeniem wyników leczenia u obserwowanych pacjentów (ponad 5-letnie DFS: 85 i 85,8%; p = 0,94).

Wnioski. Obniżenie się aktywności L-ASPA u dzieci leczonych z powodu ALL nie wiązało się z pogorszeniem wyników leczenia, natomiast istotnie zwiększało ryzyko wystąpienia reakcji nadwrażliwości na ten lek.

INTRODUCTION

L-asparaginase (L-ASPA) is one of the basic agents in the treatment of acute lymphoblastic leukemia (ALL). Efficacy of L-ASPA therapy is related to the grade and duration of asparagine decrease in serum and cerebrospinal fluid (CSF), which depends on the enzyme activity. Activity above 100 IU/l, assuring complete asparagine depletion, is concerned as therapeutic (1). Although, total removal of asparagine was observed in some patients with L-ASPA activity below 100 IU/l (2-4).

L-ASPA as a protein of bacterial origin can cause development of antibodies, which leads to allergy with local or generalized symptoms (5-7), or to inactivation of the enzyme and shortening of its half-life without symptoms of hypersensitivity ("silent inactivation") (5-9). The reported frequency of anti-asparaginase antibodies is variable and ranges up to 70% (7-10). The frequency of hypersensitivity reactions ranges from 0 to 45% (7-10). Incidence of the hypersensitivity reaction depends on preparation of L-ASPA, doses, way of administration, number of L-ASPA administrations during one treatment phase (4-7, 9, 11-13). It is recommended to change L-ASPA preparation when hypersensitivity or silent inactivation occurs. Conflicting data exist regarding influence of immunologic reaction to L-ASPA on the treatment outcome (7, 14-17). Effects of the hypersensitivity can be minimized by fast switching to another preparation after allergy occurrence (7).

AIM

The aim of the study was analysis of the influence of decrease of L-ASPA activity and allergic reaction to this drug on the treatment outcome in children treated for ALL.

MATERIAL AND METHODS

Ninety seven children with ALL began the treatment according to the international protocol ALL IC-BFM-2002 in the Department of Pediatric Oncology and Hematology in the Children's University Hospital in Cracow from 1th June 2005 to 31th October 2008. Eighty seven patients were eligible to the study (10 were excluded because not enough blood samples were available for L-ASPA activity measurement). General characteristic of the analyzed children is shown in table 1.

Observation was finished on 31th December 2013. Median follow-up was 87 months (range: 62-102 months). On the day of the finish of observation 72 patients remained in first complete remission (I CR), lasting 60-100 (median 82) months.

L-ASPA was administrated during induction (Protocol I), reinduction (Protocols II and III) and HR cycles (tab. 2). Blood for L-ASPA activity test was collected before each administration of the drug during Protocol I. Plasma was centrifuged and frozen in -80°C till examination. L-ASPA activity was assessed using MAAT test (Medac Asparaginase-Aktivitäts-Test) test in the Department of Clinical Biochemistry of the Polish-American Institute of Pediatrics (Jagiellonian University

Table 1. Clinical characteristics of the 87 patients with acute lymphoblastic leukemia analyzed in the study.

Parameters		cALL	proB-ALL	Transitional ALL	T-ALL	All
Number of patients (percentage)		71 (81.6)	3 (3.4)	2 (2.3)	11 (12.7)	87 (100)
Age: median (range) [years]		5.5 (1.7-17)	14 (3.5-16)	1,5 (1.2-1.9)	7.5 (2-13.5)	6 (1.2-17)
		Number of patients (percentage)				
Risk groups	**SRG**	25 (35)	1 (33)	2 (100)	0	28 (32)
	IRG	31 (44)	2 (67)	0	7 (64)	40 (46)
	HRG	15 (21)	0	0	4 (36)	19 (22)
Down syndrome		4 (5.6)	0	0	0	4 (4.6)
HSCT in the treatment of first line		2 (2.8)	0	0	3 (27)	5 (5.7)
Relapses		10 (14)	1 (33)	0	0	11 (12.6)
Progression before remission		1 (1.4)	0	0	0	1 (1.1)
Lasting first remission		59 (83)	2 (67)	1 (50)	10 (91)	72 (82.7)
Lasting second remission		4 (5.6)	0	0	0	4 (4.6)
Deaths **– of ALL** **– of toxicities**		7 (9.8) 3 (4.2) 4 (5.6)	1 (33) 1 (33) 0	1 (50) 0 1 (50)	1 (9) 0 1 (9)	10 (11.5) 4 (4.6) 6 (6.9)

Table 2. Treatment protocols comprising L-ASPA.

Protocol I	5000 IU/m^2/24 h – day 12., 15., 18., 21., 24., 27., 30., 33.
Protocol II	10 000 IU/m^2/24 h – day 8., 11., 15., 18.
Protocol III	10 000 IU/m^2/24 h – day 1., 4., 8., 11.
HR1-3 cycles	25 000 IU/m^2/24 h – day 6. and 11.

Medical College, Kraków). For each patient average value of L-ASPA activity in induction was counted, and this value was used in specified analysis.

Patients were strictly observed for early recognition of the symptom of hypersensitivity to L-ASPA during all treatment protocols with L-ASPA administrations.

Early response to the chemotherapy was assessed basing on the percentage of blasts in bone marrow on the day 33 of induction therapy. Treatment outcome was estimated on the basis of survival rates: overall survival (OS) counted from the date of the beginning of the treatment to the date of the finish of observation or to the death, event free survival (EFS) – from the beginning of the treatment to the finish of the observation or to the unfavorable event (progression, relapse, death of any reason), disease free survival (DFS) – from beginning of the treatment to the finish of observation or to the treatment failure (early progression, relapse), relapse free survival (RFS) – from remission to the finish of observation or to relapse. Comparative analysis concerning influence of low (< 100 IU/l), undetectable (< 30 IU/l) L-ASPA activity and hypersensitivity reaction on DFS was performed.

STATISTICA 8 software was used for statistical analysis. U Mann-Whitney test, chi-square test, Yates' corrected chi square test, V-square test, Fisher's exact test, Kaplan-Meier survival curves analysis and log-rank test were performed.

RESULTS

From 87 enrolled patients 84 received all L-ASPA doses scheduled in the ALL IC-BFM-2002 program. Two children died before finishing the treatment with L-ASPA, during HR cycles. In one patient L-ASPA was contraindicated after acute pancreatitis which occurred after 7th dose of L-ASPA during induction therapy.

Three hundred seventy four samples collected 3 days after L-ASPA administration were tested for the drug activity. L-ASPA activity ranged from < 30 to 2063 (median 248) IU/l. Activity < 100 IU/l was found in 59 (16%) samples, in 19 (21%) patients. The group of patients with low L-ASPA activity in at least one examined sample (on average in 68% of samples) did not differ from children with therapeutic L-ASPA activity in all samples concerning sex, age, phenotype of leukemia and risk groups (tab. 3). In 15 (4%) samples in 7 (8%) patients activity was undetectable (< 30 IU/l). In 2 of 7 patients activity was undetectable only in one sample (collected after the last dose of L-ASPA in induction), in 3 patients it was undetectable in 2 samples (after the 2 last doses of L-ASPA in induction), in 1 patients it was in 4 of 6 samples and in one child in all 5 collected samples. No significant difference in sex, age, phenotype of leukemia and risk groups was found between group of patients with undetectable L-ASPA activity in at least one examination and children with activity above 30 IU/l in all tested samples. Clinical characteristics of the defined groups of patients is show in the table 3.

Hypersensitivity reaction to L-ASPA was observed in 42 (49%) from 85 patients (2 patients without allergy, who did not received all L-ASPA doses, were excluded). Hypersensitivity was more

Table 3. Clinical characteristics of the defined groups of patients.

Parameters		L-ASPA < 100 IU/l (n = 19)	L-ASPA ≥ 100 IU/l (n = 68)	L-ASPA < 30 IU/l (n = 7)	L-ASPA ≥ 30 IU/l (n = 80)	Allergy to L-ASPA (n = 42)	No allergy (n = 43)
Age: median (range) [years]		6.3 (1-13)	7 (2-17)	5 (2.5-8)	7 (1-17)	6.5 (2-16)	6.7 (1-16)
Number of patients							
cALL		14	57	7	64	35	35
proB-ALL		0	3	0	3	2	1
Transitional ALL		1	1	0	2	1	1
T-ALL		4	7	0	11	4	6
Sex	**boys**	9	37	2	44	17*	27*
	girls	10	31	5	36	25*	16*
Risk groups	**SRG**	6	22	4	24	12	16
	IRG	9	31	2	38	20	19
	HRG	4	15	1	18	10	8
Relapses		2	9	0	11	6	5
Progression before remission		0	1	0	1	0	1
Deaths		2	8	0	10	3	6

*p = 0.0042

common among girls than boys (chi-square test: p = 0.0042). Patients with hypersensitivity did not differ from other patients concerning age, phenotype of leukemia and risk groups (tab. 3).

Among 42 patients with allergy to L-ASPA, in 32 (76%) hypersensitivity reaction occurred during first L-ASPA administration in the Protocol II, III or HR, after more than one month of interval from preceding dose of the drug. In the rest of patients allergic reaction was preceded by extension of break between L-ASPA administrations caused by infectious complication of the therapy. In 14 patients (33% of children with hypersensitivity) development of the symptoms of allergy during Protocols II, III or HR cycles was preceded by decrease of L-ASPA activity < 100 IU/l (in 7 patients < 30 IU/l) during induction.

In the group of patients with low (< 100 IU/l) L-ASPA activity detected in at least one sample, hypersensitivity reaction was observed more frequently (14/18 patients, 78%) than in children with therapeutic activity detected in all samples (28/67 patients, 42%; V-square test: p = 0.007; fig. 1). Two patients without hypersensitivity, who did not received all scheduled L-ASPA doses, were excluded from this analysis. Hazard ratio (HR) of allergic reaction in patients with decreased L-ASPA activity was 1.86 (95% confidence interval [CI]: 1.22-2.36).

All patients with undetectable (< 30 IU/l) L-ASPA activity in at least one sample developed allergy to L-ASPA. Among 78 remaining patients, who had detectable activity of the enzyme in all samples, in 35 (45%) hypersensitivity was observed (chi-square test: p = 0.016; fig. 2). Two patients without hypersensitivity, who did not received all scheduled L-ASPA doses, were excluded from this analysis. Hazard ratio of allergic reaction in patients with undetectable L-ASPA activity was 2.23 (95% CI: 1.38-2.23).

Among 42 patients with allergic reaction to native *E. coli* L-ASPA, 41 were treated subsequently with equivalent dose of PEG-ASPA, one patients developed allergy during administration of the last dose of L-ASPA. Eleven children receiving PEG-ASPA developed hypersensitivity to this preparation and continued therapy with Erwinaze. No allergy to Erwinaze was observed in described group of patients.

In 82 patients (94%) bone marrow on the day 33. of the induction was classified as M1 (< 5% of blasts), in 5 hildren (6%) as M2 (5-25% of blasts). These groups did not differ concerning patients' average value of L-ASPA activity in induction (median/range: 224/94-423 IU/l and 269/0-644 IU/l respectively; p = 0.61). There was no significant relation between decrease of L-ASPA activity below 100 IU/l (p = 0.60) or below 30 IU/l (p = 0.87) and result of bone marrow examination (M1 vs M2) on the day 33. of induction or subsequent bone marrow examination after reconstitution of hematopoiesis.

Among 87 observed patients, 86 (99%) achieved complete remission (CR) during Protocol I or HR cycles. In one case progression of the disease was found before achieving CR. Three children died in I CR because of toxicities. Relapse was recognized in 11 patients. At the

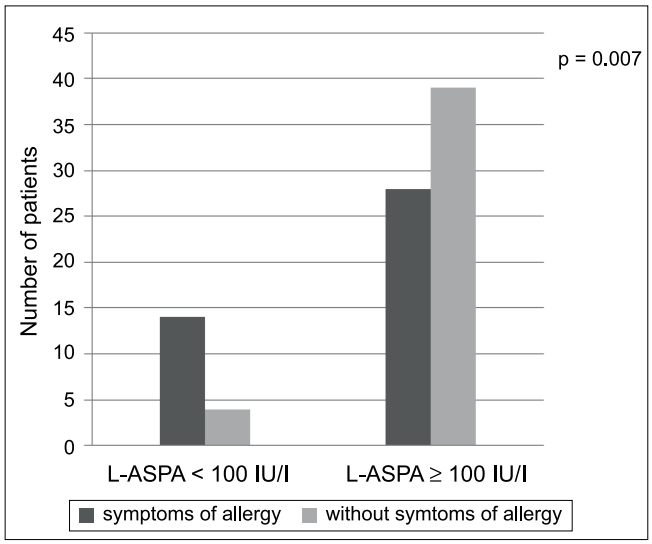

Fig. 1. Relationship between L-ASPA activity (< 100 IU/l vs. ≥ 100 IU/l) in the Protocol I and occurrence of the allergy symptoms after consecutive L-ASPA administrations.
L-ASPA < 100 IU/l – group of patients with L-ASPA activity < 100 IU/l in at least one examination (n = 18)
L-ASPA ≥ 100 IU/l – group of patients with L-ASPA activity ≥ 100 IU/l in all examinations (n = 67)

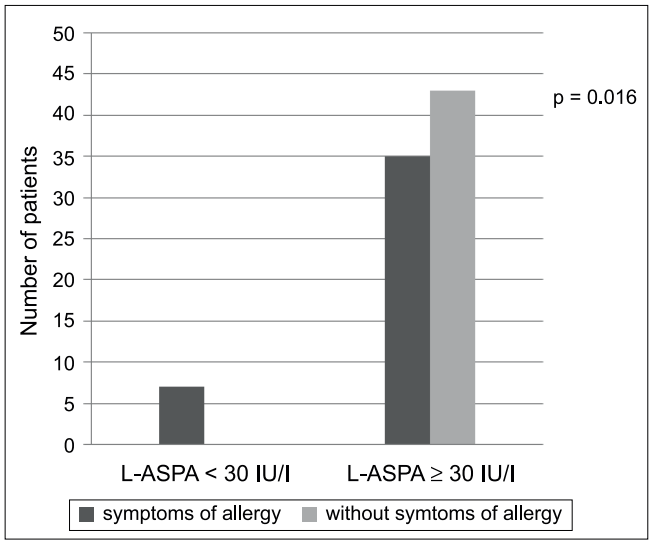

Fig. 2. Relationship between L-ASPA activity (< 30 IU/l vs ≥ 30 IU/l) in the Protocol I and occurrence of the allergy symptoms after consecutive L-ASPA administrations.
L-ASPA < 30 IU/l – group of patients with L-ASPA activity < 30 IU/l in at least one examination (n = 7)
L-ASPA ≥ 30 IU/l – group of patients with L-ASPA activity ≥ 30 IU/l in all examinations (n = 78)

end of observation 72 patients (82.7%) remained in I CR lasting 60-100 months (median 82 months). The probability of 5-years RFS was 86.7 ± 0.04%. The probability of 5-years OS was 90.8 ± 0.03%. Ten children (11.5%) died. Two of them because of sepsis in neutropenia during HR cycles, one patients of hemophagocytic syndrome during remission maintenance therapy. Four patients died in relapse because of progression of leukemia. In 3 children the death was caused by transplantation related toxicities (2 in II, 1 in III CR).

The probability of 5-years EFS was 82.7 ± 0.04%. Unfavorable events occurred in 15 patients (1 early

progression, 3 deaths from toxicities in I CR, 11 relapses). The probability of 5-years DSF was 85.7 ± 0.04%.

No relationship between decrease of L-ASPA activity below therapeutic values and treatment outcome was observed. The probability of 5-years DFS in patients with therapeutic and low (< 100 IU/l) L-ASPA activity was 84.5 and 88.9% (p = 0.69) respectively and in children with measurable and undetectable (< 30 IU/l) activity it was 84.4% and 100% (p = 0.26) respectively (fig. 3 and 4). Treatment failure occurred in 2 of 19 patients (10.5%) with L-ASPA activity below 100 IU/l and in 10 from 68 (14.8%) children with therapeutic activity, the difference was no statistically significant (p = 0.92). No treatment failure was observed in the group of patients with undetectable L-ASPA activity. Median of average values of L-ASPA activity in induction in patients with and without treatment failure was 324 IU/l and 260 IU/l respectively (p = 0.35).

No evidence of influence of allergy to L-ASPA on the treatment outcome was found. The probability of 5-years DFS in the group of patients with and without hypersensitivity reaction was 85 and 85.8% respectively (p = 0.94; fig. 5). There was no relationship between allergic reaction and the treatment failure which was observed in 6 of 42 children with hypersensitivity (14.3%) and in 6 among 43 patients without allergy (13.9%).

DISCUSSION

Therapeutic activity of the enzyme was maintained in the majority (79%) of the observed patients treated with L-ASPA according to ALL IC-BFM-2002 Program. We found activity below 100 IU/l in at least one of samples collected 3 days after administration of the drug in 19 (21%) children, comprising 7 (8%) patients with undetectable activity in at least one of samples.

L-ASPA activity above 100 IU/l is regarded as therapeutic, leading to complete asparagine depletion in the blood (1). We proved that decrease of L-ASPA activity below 100 IU/l was not associated with deterioration of the treatment outcome. It can be explained by the possibility of the enzyme action even with activity lower than 100 IU/l. In prior reports complete asparagine depletion necessary for antileukemic effect was described in some patients with L-ASPA activity below 100 IU/l (2-4). Tsurusawa et al. (18) reported that minimal L-ASPA activity for complete removal of asparagines was 6-180 IU/l (median 16 IU/l). In own study in the group of patient with low L-ASPA activity (< 100 IU/l), in most children activity of the enzyme was detectable (> 30 IU/l), therefore it could have been sufficient for complete asparagine depletion. Furthermore, low activity of the drug was detected only in the part of samples collected from individual patient. Moreover, 78% of patients with low L-ASPA activity were switched to the PEG-ASPA because of the symptoms of hypersensitivity during consecutive phase of the therapy, what probably assured maintenance of the therapeutic activity of the enzyme (ac-

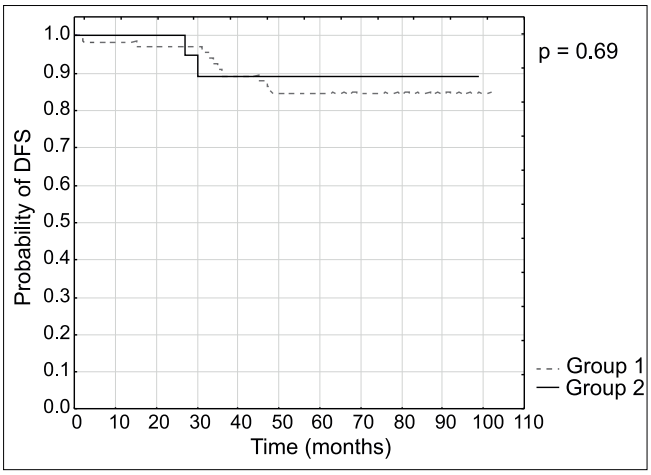

Fig. 3. Disease free survival (DFS) in the group of patients with therapeutic (≥ 100 IU/l) and low (< 100 IU/l) L-ASPA activity.
Group 1 – patients with therapeutic (≥ 100 IU/l) activity of L-ASPA in all samples (n = 68)
Group 2 – patients with low (< 100 IU/l) activity of L-ASPA in at least 1 sample (n = 19)

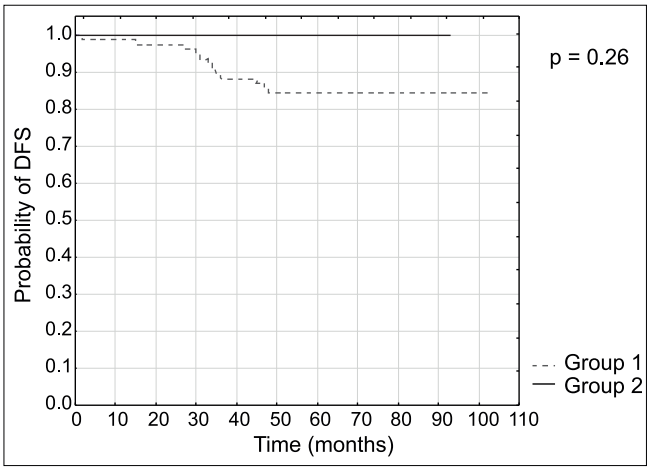

Fig. 4. Disease free survival (DFS) in patients with undetectable (< 30 IU/l) L-ASPA activity and activity > 30 IU/l.
Group 1 – patients with L-ASPA activity ≥ 30 IU/l in all samples (n = 80)
Group 2 – patients with L-ASPA activity < 30 IU/l in at least one sample (n = 7)

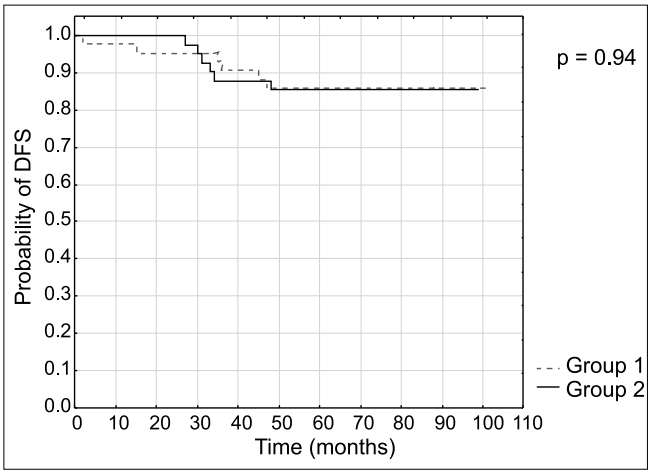

Fig. 5. Disease free survival (DFS) in patients with and without allergic reaction to L-ASPA.
Group 1 – patients without allergic reaction to L-ASPA (n = 43)
Group 2 – patients with allergic reaction to L-ASPA (n = 42)

tivity of PEG-ASPA was not measured). Panosyan et al. reported that patients with silent inactivation (recognized on the basis of antiasparaginase antibodies presence) continuing the treatment with same preparation had higher leukemic events rate comparing with children without antibodies and patients with allergy, but switched to another preparation (19). Similarly, Woo and al. described no difference in clinical outcome of patients with hypersensitivity continuing the treatment with another preparation of L-ASPA compared with patients without hypersensitivity (7).

Interestingly, we observed very good treatment outcome in the group of 7 patients with undetectable L-ASPA activity in at least one sample. It could have been expected that lack of activity of L-ASPA (one of the basic antileukemic agent) should influence the clinical outcome. However, no treatment failure nor death from toxicities was observed in this group. It can be explained by the fact, that majority of children with undetectable activity of L-ASPA were SRG patients, however the difference in proportion of the risk groups was not statistically significant. Moreover, undetectable activity was found only in part of the examined samples. Finally, because of the symptoms of allergy all 7 patients were switched to PEG-ASPA during the consecutive treatment stage, what could have assure therapeutic activity of the enzyme and influenced favorably on the treatment outcome.

We observed hypersensitivity reaction in 49% of patients, in slightly higher proportion than described elsewhere (0-46%) (5, 7, 12, 16, 20). The majority of reactions occurred during readministration of L-ASPA in Protocols II, III or HR cycles, after a period of no asparaginase therapy for more than one month. The remaining patients developed hypersensitivity during induction in case of delay in realization of the treatment program and prolonged interval between L-ASPA doses caused by toxicities. This is consistent with observations of other authors (5-7, 9, 12).

Lower frequency of allergy during induction therapy compared to reinduction can be explained by the difference in L-ASPA doses in particular stages of the therapy. L-ASPA dose in induction (5000 IU/m^2) is lower than in consecutive phases of the treatment (Protocol II/III – 10 000 IU/ m^2, HR cycles 25 000 IU/m^2). It is hypothesized that lower doses of L-ASPA cause rather silent inactivation, while higher doses can be responsible for symptomatic hypersensitivity (5, 11, 21).

It was previously reported that patients with antiasparaginase antibodies were more likely to develop allergic reaction than those without antibodies (7). We confirmed that decrease of L-ASPA activity, probably as the effect of antibodies forming (we did not measured antibodies levels), can precede symptoms of allergy. In our study majority of patients with low and all with undetectable L-ASPA activity developed clinical hypersensitivity.

We observed no relationship between hypersensitivity reaction and the treatment outcome. It is consistent with results obtained by other authors (7, 16, 17). Adverse effect of allergy could have been minimized by quick switching to pegylated preparation of L-ASPA assuring maintenance of therapeutic activity of the enzyme.

CONCLUSIONS

L-ASPA is one of the basic drug in the therapy of acute lymphoblastic leukemia. With regard to variability of the enzyme activity in particular patients and the risk of hypersensitivity reaction, monitoring of the treatment by regular control of activity of L-ASPA seems to be essential.

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