A pharmacological study on pegylated asparaginase used in front-line treatment of children with acute lymphoblastic leukemia

Carmelo Rizzari
Marco Citterio
Massimo Zucchetti
Valentino Conter
Robert Chiesa
Antonella Colombini
Silvia Malguzzi
Daniela Silvestri
Maurizio D'Incalci

Background and Objectives. Pegylated-asparaginase (PEG-ASP) has been traditionally used as a second-line preparation in children with acute lymphoblastic leukemia (ALL) presenting with clinical allergy to native asparaginase (ASP) products. The main goal of the present study was to investigate the pharmacological effects of the administration of PEG-ASP given as a first-line product in children with ALL.

Design and Methods. PEG-ASP serum enzymatic activity and serum and cerebrospinal fluid (CSF) levels of asparagine were investigated in 20 children with newly diagnosed ALL enrolled in the ongoing AIEOP ALL 2000 protocol and treated with PEG-ASP as a first-line ASP preparation. During induction the drug was administered at the dosage of 1,000 U/m^2 i.v. on days 12 and 27. During reinduction the drug was administered only once at the same dosage.

Results. Among the 20 patients treated in induction serum PEG-ASP activity equalled or exceeded 100 U/L in 18/18, 11/11 and 15/18 of the samples available on days 22, 25 and 27, respectively, and in 16/16, 12/15 and 5/8 samples available on days 36, 39 and 45, respectively. In the 15 patients treated during reinduction serum PEG-ASP activity ≥100 U/L was observed in 14/15, 11/14, 6/10, and 0/12 samples available on days 11, 15, 18 and 23, respectively, after the administration of the drug. Serum asparagine levels were below the detection limit (≤0.2 μM) in all patients/samples and at all time points evaluated during induction; during reinduction only one patient had detectable asparagine levels from day 11. CSF asparagine levels were below the detection limit of the method only in a few patients during both induction and reinduction.

Interpretation and Conclusions. PEG-ASP given as a first-line ASP product in the context of an intensive chemotherapy protocol for pediatric ALL allowed adequate plasma enzymatic activity and asparagine depletion during both exposures to the drug. However, CSF asparagine depletion was inadequate.

Key words: asparaginase, childhood. acute lymphoblastic leukemia, pharmacology, treatment.

Haematologica 2006; 91:24-31

Department of Pediatric Hematology-Oncology, University of Milano-Bicocca, S.Gerardo Hospital, Monza, Italy (CR, MC, VC, RC, AC, SM); Mario Negri Institute for Pharmacological Researches, Milano, Italy (MZ, MD'I); Department of Medicine, Prevention and Biotechnology, University of Milano-Bicocca, Italy (DS).

Correspondence: Carmelo Rizzari, Department of Pediatrics, University of Milano-Bicocca, Hospital of Monza, Via Donizetti 106, 20052, Monza, Italy. E-mail carmelo.rizzari@pediatriamonza.it

Highly purified preparations of asparaginase (ASP) from *Escherichia coli* (*E. coli*) and *Erwinia chrysanthemi* have proven to be active against childhood acute lymphoblastic leukemia (ALL) and lymphomas and have become standard components of the treatment regimens for this disease. An adequate ASP enzymatic activity is required to lower the asparagine concentration in serum below the minimum threshold required for leukemic cell growth.[1-5] Silent inactivation, a phenomenon rather frequently observed after multiple exposures to native ASP products and consisting of a rapid decline of serum enzymatic activity due to the presence of anti–ASP antibodies, may compromise the therapeutic efficacy of the drug. By conjugating the native *E. coli* ASP molecule to polyethylene glycol (PEG) a definite reduction of immunogenic properties, increased non-reactivity to antibodies, a significantly longer half-life and a reduction of the number of injections for the patient have been obtained.[6-8] Due to these features, PEG-ASP has been used, at doses comprised between 1,000 and 2,500 U/m^2 (or about one tenth the dose of the native *E. coli* enzyme), once every one or two weeks, mainly as a second-line product in patients with ALL hypersensitive to the native products.[8-9] More recently PEG-ASP has also been used as a first-line product in ALL front-line protocols for both adults and children.[10-12]

The main goal of the present study was to investigate the pharmacological effects of the administration of PEG-ASP given as a first-line product (i.e. from the induction phase) in children with ALL treated with the intensive AIEOP ALL 2000 study protocol.

Design and Methods

Patients' eligibility and treatment schedule

The recruitment plan of this study was to investigate 20 children, aged < 18 years, with a new diagnosis of ALL. Patients with diabetes, coagulopathy, pancreatitis, or severe neurological and liver diseases pre-existing the start of PEG-ASP treatment were not eligible for the study. The treatment schedule was that used in the AIEOP ALL 2000 study. This treatment schedule is

very similar to that used in the previous AIEOP ALL 95 study[13] consisting of a BFM back-bone including induction (protocol IA+B), consolidation (protocol M), reinduction (protocol II or protocol III) and continuation phase. Patients evaluated in this pharmacological study were not considered eligible for the first randomized study of the AIEOP ALL 2000 protocol (prednisone/control vs dexamethasone/experimental in induction) and were all treated in the prednisone/control arm. Patients could, however, be randomized for the subsequent studies (standard risk: one protocol III vs one protocol II, intermediate risk: two protocols III vs one protocol II, high risk: three intensive blocks plus two protocols II vs three intensive blocks plus three protocols III). Details of the single treatment elements have already been described elsewhere.[13] Patients were stratified based on biological and treatment response parameters, including minimal residual disease evaluation in weeks 5 and 12.[14] Figure 1 shows the outlines of the treatment phases (protocols IA, II and III) and also include the PEG-ASP treatment details and the schedule for cerebrospinal fluid (CSF) and plasma sampling adopted for the present pharmacological study.

The study drug was pegylated *E. coli* asparaginase (Oncaspar®, Medac GmbH, Hamburg, Germany), which was administered intravenously as a 2-hour infusion at the dosage of 1,000 U/m^2 both during the induction (two doses, days 12 and 27) and reinduction phases (one dose only, conventionally considered as given on day 1). No test dose was used before administration. The target level of ASP activity was ≥100 U/L since this level of activity has been found to be adequate to obtain complete depletion of asparagine (≤0.2 μM) in serum and CSF. Patients evaluated in this pharmacological study were also monitored with routine blood analyses performed on the same day as pharmacological monitoring was planned. Informed consent was obtained from parents or legal guardians in all cases.

In order to compare the clinical impact of PEG-ASP in this therapeutic setting, a comparable group of 37 ALL children, previously treated in our institution with the same AIEOP ALL 2000 treatment protocol, was studied. The ratio of the patients in the historical control group to the patients in the study group was two to one. These patients were randomized to receive prednisone during the induction phase IA and were given the native *E. coli* Medac Asparaginase® product (Medac GmbH, Hamburg, Germany) instead of the PEG-ASP product used in our study patients. Data were collected on relevant clinical complications, WHO graded (grade III-IV only) episodes of toxicity associated with the use of native ASP or PEG-ASP, duration of the induction phases IA and IB and the entity of related treatment burden (i.e. days of hospital admission, duration of i.v. antibiotic or antifungal treatment, central venous line placement, administration of packed red blood cells or platelet transfusions).

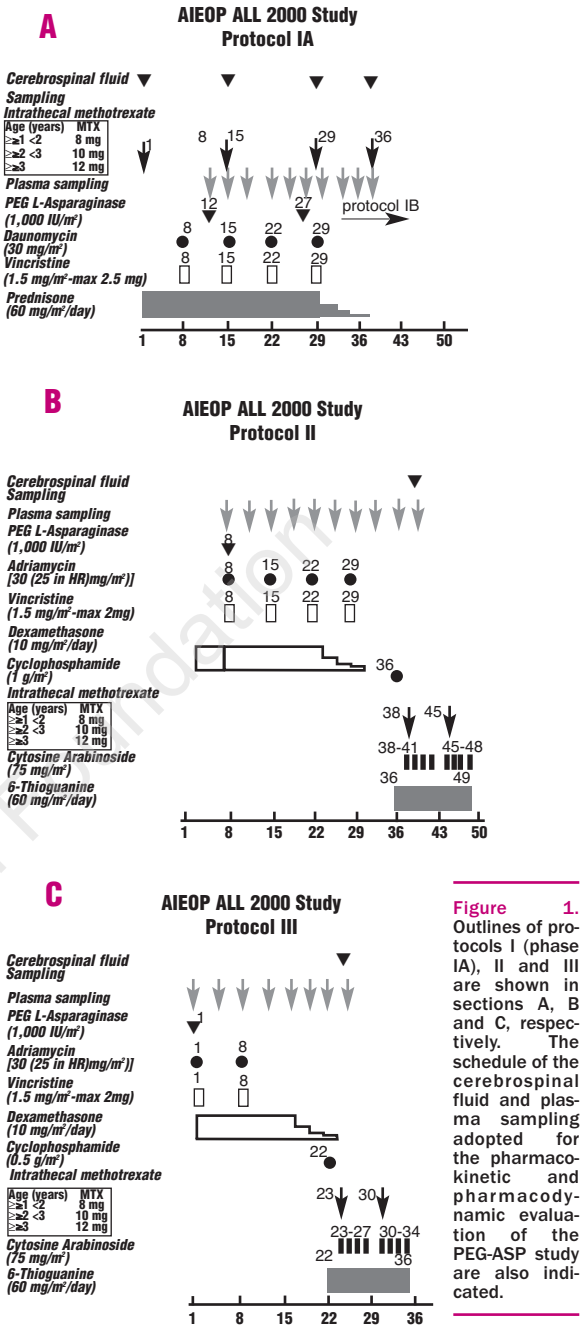

Figure 1. Outlines of protocols I (phase IA), II and III are shown in sections A, B and C, respectively. The schedule of the cerebrospinal fluid and plasma sampling adopted for the pharmacokinetic and pharmacodynamic evaluation of the PEG-ASP study are also indicated.

Sampling

Serum: asparagine levels and ASP activity were evaluated both in induction on day 12 at baseline, on day 12 at peak (i.e. 2 hours after the first PEG-ASP), on days 15, 18, 21, and 24, on day 27 at baseline, on day 27 at peak (i.e. 2 hours after the second PEG-ASP), and on days 30, 33, 36, 38 and 45 for a total of 13 samples, and in reinduction on day 1 at baseline, on day 1 at peak (i.e. 2 hours after PEG-ASP), and on days 4, 8, 11, 15, 18 and 23 for a total of 8 samples. Each blood sample was placed in tubes without anticoagulant and immediately centrifuged; serum was divided in two aliquots and immediately frozen at -80°C and stored until analysis.

Cerebrospinal fluid: asparagine levels were measured when diagnostic lumbar punctures or intrathecal chemotherapy treatment was scheduled (i.e. in induc-

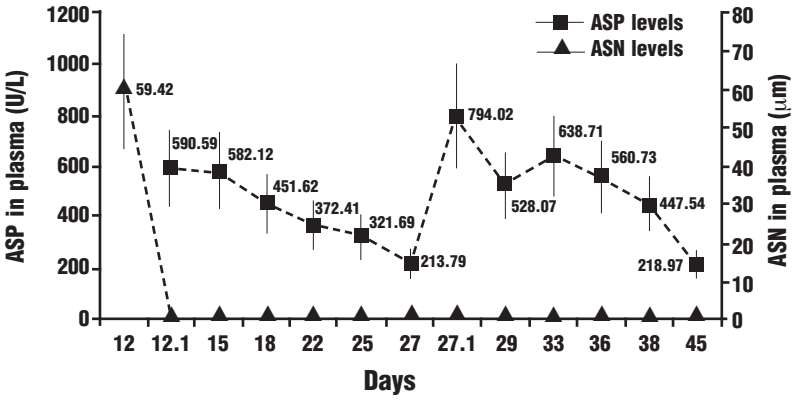

Figure 2. Plasma: asparaginase (ASP) activity levels* (■) vs asparagine (ASN) levels* (▲) during the first exposure (induction, phase IA) to PEG-ASP (given on days 12 and 27). *mean ± SD

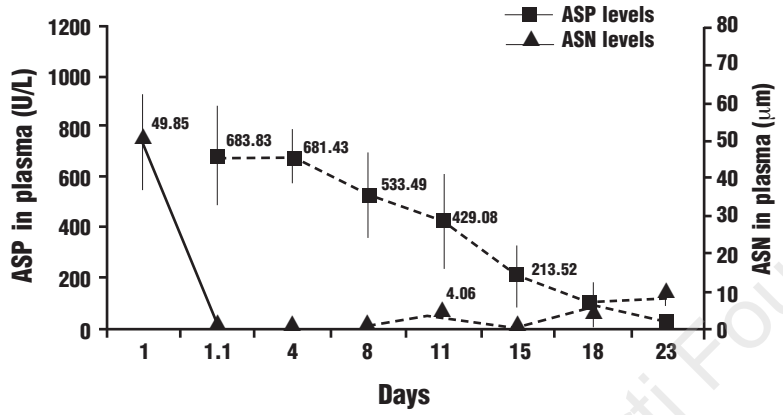

Figure 3. Plasma: asparaginase (ASP) activity levels*(■) vs asparagine (ASN) levels* (▲) during the second exposure (reinduction, protocols II or III) to PEG-ASP (considered to given conventionally given on day 1). *mean ± SD.

tion on days 1, 15, 29, 38 and 52 for a total of 5 samples, and in reinduction on day 38 for patients receiving protocol II and on day 23 for patients receiving protocol III, for a total of one sample for each phase). CSF samples were immediately frozen at -80°C and stored until analysis.

Determination of serum ASP activity

Levels of serum PEG-ASP activity were assessed with the MAAT (Medac Asparaginase-Activity-Test) kit, a quantitative enzyme assay. The way the MAAT test determines PEG-ASP activity is based on the enzymatic hydrolysis of a substrate analogous to asparagine (provided with the MAAT kit) by asparaginase, followed by production of two cleavage products (here called A and B). The B product forms, in a complex reaction with a chromogen, a green dye that can be read by measurement of absorption at 700 nm. The optical density is measured at 700 nm and a specific computerized program plots the absorption values against the activity values, building a *cubic spline* curve. The activity values corresponding to the optical density values of the samples can be read from the calibration curve. The lower limit of quantitation of the MAAT is 30 U/L.

Determination of serum and CSF asparagine levels

Asparagine levels in serum and CSF were measured using a high performance liquid chromatography technique after pre-column derivatization with *o*-phthaldialdeide as described by Turnell and Cooper.[15] The lower limit of detection was ≤0.2 μM for both serum and CSF. Serum and CSF samples were all analyzed in a single laboratory (Mario Negri Institute for Pharmacological Researches) by two of the investigators (M.C. and M.Z.).

Results

Twenty children, 11 males and 9 females aged 2-16 years (mean 9.7), consecutively diagnosed (April 2002 – December 2002) with ALL in our center and eligible for the AIEOP ALL 2000 protocol were investigated in the present study (Table 1). Based on the planned stratification criteria, the 20 patients were stratified into standard risk (n=7), intermediate risk (n=12) and high risk (n=1). All patients received at least the two PEG-ASP doses planned to be administered during induction. Two out of the initial 20 patients developed cerebral sinus thrombosis during the induction phase and were consequently considered off study (i.e. they did not receive any further PEG-ASP dose or other ASP products). One of these patients died three months later because of fatal sepsis which occurred in a phase of severe neutropenia and was thus not related to the use of the study drug. The high risk patient underwent bone marrow transplantation in first complete remission and did not receive any blocks or protocol II or III.

Table 1. Presenting features, treatment response, risk group assignment and current status of the 20 ALL children treated with PEG-ASP.

Initials	*Sex*	*Age (years)*	*WBC/ mm³*	*Hb g/dL*	*Platelets mm³*	*Immuno-phenotype*	*Prednisone response*	*Risk group*	*Status*
V.D.	M	3	6,700	7.7	52,000	Common	Good	Intermediate	Alive in 2nd CR
Y.S.	F	5	2,600	6.3	163,000	pre-B	Good	Intermediate	Alive in 1st CR
G.F.	M	6	7,200	9.4	32,000	Common	Good	Intermediate	Alive in 1st CR
C.F.	M	5	14,020	10.5	210,000	Common	Good	Intermediate	Dead in 1st CR
L.L.	M	3	16,540	7.3	22,000	Common	Good	Intermediate	Alive in 2nd CR
M.G.	M	7	8,690	8.9	61,000	pre-B	Good	Intermediate	Alive in 1st CR
A.A.	M	3	22,000	5.1	18,000	Common	Good	Standard	Alive in 1st CR
A.C.	F	3	10,300	4.7	12,000	Common	Good	Intermediate	Alive in 1st CR
R.P.A	F	2	19,420	4.3	30,000	Common	Good	Intermediate	Dead in 1st CR
Z.A.	M	12	180,000	6.6	25,000	T	Good	Standard	Alive in 1st CR
F.C.	M	16	193,000	9.9	34,000	Common	Good	Intermediate	Alive in 1st CR
G.L.	M	6	4,700	11.6	47,000	pre-B	Good	Standard	Alive in 1st CR
V.F.	F	13	2,700	9.8	43,000	Common	Good	Intermediate	Alive in 1st CR
P.V.	F	10	11,870	5.6	22,000	pre-B	Good	Intermediate	Alive in 1st CR
P.A.	F	15	4,750	6.0	33,000	Common	Good	Intermediate	Alive in 1st CR
B.E.	F	4	13,900	10.1	161,000	pre-B	Good	Standard	Alive in 1st CR
F.M.	M	16	13,690	6.1	25,000	pre-B	Poor	High	Dead after BMT
A.A.	M	4	7,890	5.0	8,000	Common	Good	Standard	Alive in 1st CR
M.C.	F	6	8,900	13.0	186,000	pre-B	Good	Standard	Alive in 1st CR
B.M.	F	2	45,590	11.1	43,000	Common	Good	Standard	Alive in 1st CR
Mean Value		7.05	29,723	7.95	61,350				

WBC: white blood cell count; Hb: hemoglobin; BMT: bone marrow transplantation.

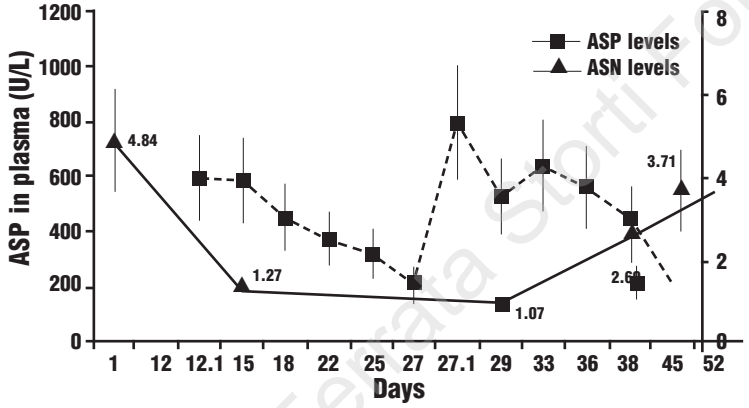

Figure 4. First exposure. Plasma asparaginase (ASP) activity levels*(■) vs cerebrospinal fluid (CSF) asparagine (ASN) levels* (▲) during the first exposure (induction, phase IA) to PEG-ASP (given on days 12 and 27). *mean ± SD.

Two additional patients were not evaluated for further exposure to the drug because they mistakenly received the native *E. coli* ASP product instead of the PEG-ASP. At the time of the present report 17 patients are alive, 15 of them are in continuous first complete remission and two are in second complete remission. The pharmacological data reported in the following sections represent a meaningful subset of the whole amount of available data regarding the sampling points planned in the pharmacokinetic and pharmacodynamic monitoring; all the remaining values obtained from all sampling points are reported as mean ± SD values in Figures 2-4.

Asparaginase activity in serum

First exposure. Two hundred and thirty-two serum samples were obtained and analyzed from the 20 patients (mean number of serum samples per patient = 11). The peak ASP activity in serum samples measured 2 hours after the end of the first PEG-ASP infusion on day 12 was (mean±SD) 590±304 U/L. The levels were 451±217 U/L on day 18 and 213±154 U/L on day 27, just before the second dose of the drug. The peak serum activity measured 2 hours after the end of the second PEG-ASP infusion on day 27 was 794±327 U/L, slightly declining to 638±264 U/L on day 33, to 560±317 U/L on day 39 and to 218±117 U/L on day 45, i.e. 18 days after the second dose of PEG-ASP (Figure 2). Among the cohort of the 20 investigated patients, 18/18, 11/11 and 15/18 for whom samples were available on days 22, 25, and 27, respectively, had serum ASP activity ≥100 U/L. The three patients who did not achieve the level of ≥100 U/L on day 27 had ASP activity values of 90.6, 93.6 and 85.7 U/L. After the second dose (day 27) 16/16, 12/15 and 5/8 samples available on days 36, 39 and 45, respectively, had serum ASP activity ≥100 U/L. The three patients who did not achieve the level of 100 U/L on day 39 had values of 89.6, 76.3 and 31.9 U/L, while the three who did not have ASP activity ≥ 100U/L on day 45 had values of 73.7, 86.1 and 87.3 U/L.

Second exposure. During this phase, considering the 15 investigated patients, 141 serum samples were

obtained and analyzed (mean number of serum samples per patient=5). Asparaginase activity in serum, 2 hours after the single PEG-ASP infusion, was (mean±SD) 683±195 U/L, declined to 429±183 U/L, 213±119 U/L, 194±82 U/L and to 27±20 U/L 11, 15, 18 and 23 days later, respectively (Figure 3). Among this cohort of 15 investigated patients, serum ASP levels were ≥100 U/L in 14/15, 11/14, 6/10 and 0/12 samples available on days 11, 15, 18 and 23, respectively. Samples available on days 11, 15, 18 and 23 of one patient only displayed undetectable serum ASP activity suggesting the occurrence of silent inactivation. The two additional patients who displayed ASP activity levels < 100 U/L on day 15 had ASP activity levels of 29.1 and 32.6 U/L. On day 18 the three additional patients with ASP activity levels < 100 U/L had levels of 86.8 U/L (one patient) and < 30 U/L (two patients). On day 23 the additional 11 patients had ASP activity levels ≥30 and <70 U/L (eight patients) and < 30 U/L (three patients).

Third exposure. Six patients underwent a third exposure and 32 samples were available for pharmacological analysis. On day 11, after the PEG-ASP administration, all these 5 patients had one sample available for ASP activity testing and all of them had serum ASP activity levels ≥100 U/L. On day 15, 4/5 patients had one sample available and in all ASP activity level was ≥100 U/L. No additional samples were available for any of these patients on days 18 and 23.

Serum asparagine levels

Before the first PEG-ASP administration (induction phase IA), baseline asparagine serum levels were (means±SD) 87.3 59.42±23 μM, which were considered within normal values.[15,16]

First exposure. Immediately (i.e. 2 hours) after the first PEG-ASP administration, serum asparagine levels declined below the limit of detection (≤0.2 μM) in all samples from all patients and were still undetectable on day 27, thus before the infusion of the second PEG-ASP dose (i.e. 15 days after the first dose), even in the 3/18 patients with ASP activity levels < 100 and > 85 U/L. Asparagine levels remained undetectable in all samples of all investigated patients after the second dose until day 45 (i.e. 18 days after the second dose) also in the 3/15 and 3/8 patients who had ASP levels < 100 and > 30 U/L on day 39 and < 100 and > 73 U/L on day 45, respectively (Figure 2).

Second exposure. Samples and patients: a similar pattern was observed during the second exposure: the baseline serum asparagine level before PEG-ASP administration was (mean±SD) 49±29 μM, while two hours after the PEG-ASP infusion serum asparagine was undetectable and remained under the limit of detection until day 11 for 14 out of the 15 evaluated patients (Figure 3). In one patient only, out of the 15 evaluated in this phase, did the serum asparagine concentration, after dropping below the limit of detection during the first week after PEG-ASP administration, promptly return to normal values on day +11 and remained normal also on the subsequent days 15, 18 and 23. In this patient, as pointed out in the previous section regarding serum ASP activity levels, an early and rapid decline of serum ASP activity was observed concomitantly with the rise in asparagine levels. Asparagine levels were below the detection limit not only in all patients with ASP activity levels ≥100 U/L at any time point but also in the 2, 3 and 12 patients with ASP activity levels < 100 U/L on days 15, 18 and 23, respectively. Complete asparagine depletion was also observed in the available samples on days 11 and 15 from the five patients who underwent a third exposure (i.e. a second reinduction phase) to the study drug.

Cerebrospinal fluid asparagine levels

During the induction phase, 62 CSF samples were obtained from the 20 investigated patients (mean number of samples per patient=3). Before the first PEG-ASP dose, i.e. at disease onset (day 1), the CSF asparagine level was 4.84 ± 2.05 μM (range 3.01-8.88 μM), which can be considered within the normal range of values.[17,18] The CSF concentrations of asparagine measured on days 15 (3 days after the first PEG-ASP dose),[29,38] and 52 (i.e. 2, 11 and 25 days after the second PEG-ASP dose) were 1.27±0.92 μM, 1.08±0.93 μM, 2.6±2.14 and 3.71±2.53, respectively. In particular, on days 15, 29 and 38 of this phase, CSF samples were available for 17, 10 and 10 patients, respectively, with CSF asparagine levels below the detection limit (≤0.2 μM) in 4/17, 1/10 and 1/10 samples/patients (Figure 4). During the reinduction phase (second exposure), as reported above, 15 out of the 20 patients were investigated and ten CSF samples were obtained. CSF samples were obtained on day 38 from five patients treated with protocol II and on day +16 from five patients treated with protocol III. Asparagine levels in the CSF were below the detection limit in only 2/10 samples/patients, both of which were studied on day 16 (protocol III). Among the five patients (all treated with protocol III) who underwent a third exposure to the drug (i.e. a second reinduction phase) only three CSF samples were available, with one of them being not depleted.

Treatment burden, toxicity and adverse events

To evaluate the clinical impact of the use of PEG-ASP in our study patients (n=20) we performed a comparison with a cohort of 37 patients diagnosed in our center immediately before the PEG-ASP study was started and who were enrolled in the same AIEOP ALL 2000 study. These 37 patients were thus treated with the same chemotherapy schedule but received the native ASP product instead of the PEG-ASP product. The comparison was based on the following parameters: (i) duration of induction (phases IA and IB): the mean (±SD) duration (in days) of phases IA (expected 33 days) and IB (expected 45 days) in the study group vs the historical control group were, respectively: 34.8 (±3.9) vs 35.7 (±4.9) and 53.8 (±7.4) vs 55.9 (±7.5) days; (ii) treatment burden in induction (phases IA and IB): the mean number of days of hospital admission, i.v. antibiotic and antifungal treatment, central venous line placement and the mean number of packed red blood cells and platelet transfusions, in the study group vs the historical control group, were, respectively: in phase IA

13.5 vs 14.7, 4.4 vs 5.8, 0 vs 0.6, 3.1 vs 3.1, and 5.6 vs 4.0 and in phase IB 4.4 vs 5.8, 2.0 vs 3.7, 0 vs 0.4, 3.9 vs 3.5 and 0.4 vs 1.0; (iii) clinical complications: the clinical complications observed during induction (phase IA) and/or reinduction (i.e. protocol II or III) in the study group (n=20) vs the historical control group (n=37) were, respectively: fever of unknown origin during neutropenia: 8 vs 15; varicella 1 vs 0; bacterial pneumonia 0 vs 1; fungal pneumonia: 0 vs 1; cerebral sinus thrombosis: 2 vs 1. Neither symptomatic pancreatitis nor diabetes was encountered among either the historical group or study patients. The two patients of our study group who developed cerebral sinus thrombosis (both during phase IA) underwent specific laboratory investigations aimed at evaluating the presence of genetically determined thrombophilic factors. One of the two patients carried the prothrombin G20210 variant and the MTHFR TT677 genotype (both these genetic factors have been associated with an increased thrombotic risk). Of note, coagulation parameters, routinely evaluated when the two patients presented with the thrombosis, were within normal values; (iv) WHO grade III and/or IV episodes of toxicity in induction (phase IA only) and reinduction phases associated with PEG-ASP or native ASP products: the number of patients presenting with at least one episode of toxicity (as above graded) in the study group (n=20) vs the historical control group (n=37) was, respectively: liver toxicity (raised aminotransferases): 2 vs 4 ; glycemia 1 vs 2; hypofibrinogenemia: 3 vs 7 ; allergic reactions 0 vs 2.

Discussion

PEG-ASP has been marketed since the early 1990s. Due to its longer half-life (i.e. 5.73±3.24 days), the drug has been administered at longer intervals (1-2 weeks) than those used for the native ASP products.[8,20-24] Given its structure and its lower immunogenicity, PEG-ASP has been traditionally used in patients presenting with clinical allergy to native products.[9,25] Initial data in relapsed non-allergic children with ALL showed that PEG-ASP was able to produce, as single drug or in conjunction with other chemotherapy drugs, therapeutic effects comparable to those expected with the use of native products.[26] In a subsequent study it was reported that a weekly PEG-ASP schedule (given at 2,500 U/m^2 i.m.) produced superior induction remission rates in relapsed ALL with respect to a biweekly schedule.[22] PEG-ASP was used in front-line schedules in two different studies conducted in the USA. In the first study, Silverman *et al*. reported similar 5-year event-free survival rates in children with newly diagnosed ALL randomized to receive either a protracted schedule of PEG-ASP (biweekly 2,500 U/m^2 i.m. for 15 doses) or high dose native *E. coli* ASP (25,000 U/m^2 i.m. weekly for 30 weeks).[11] The results of the second randomized study (CCG study 1962) showed that both the efficacy of PEG-ASP (1 single dose of 2,500 U/m^2 i.m.) and native *E. coli* ASP (3 weekly doses of 6,000 U/m^2 i.m. for 3 weeks) in standard risk ALL children and the toxicity profiles of the two products were very similar; in addition, PEG-ASP was able to induce more persistent ASP activity and a faster rate of complete hematologic remission.[10] Most of the studies performed in Europe with PEG-ASP have been conducted in the frame of BFM ALL frontline or relapsed protocols.[12,27-29] One study was performed within the ALL BFM 95 protocol to determine whether one single dose (1,000 U/m^2 i.v.) of Medac Oncaspar® administered during reinduction (protocol II, second exposure to ASP) could reduce the 30% rate of clinical allergic reactions expected to happen in that phase with the use of the native *E. coli* ASP. The study also sought to maintain the target ASP activity levels of ≥100 U/L (and thus adequate serum asparagine depletion) for 2 weeks and ≥50 U/L for 3 weeks (thus comparable to those obtained with four doses of the native *E. coli* ASP product given at a dose of 10,000 U/m^2). No allergic reactions were observed during the reinduction phase (protocol II) among the 66 investigated children but the target ASP activity level of ≥100 U/L was maintained for 14 days in only about 70% of the patients, with a rapid decline of ASP activity levels after 14 days in about 30% of patients, most probably because of the onset of *silent inactivation*.[12]

Our study was designed to evaluate the pharmacokinetic and pharmacodynamic behavior of PEG-ASP given at the dosage of 1,000 U/m^2 in the induction and reinduction phases of a BFM-based treatment to evaluate whether this schedule could provide comparable treatment intensity to that of the native *E. coli* ASP product and avoid the early and fast fall of plasma enzymatic activity (*silent inactivation*) observed by Müller *et al*.[12] In our study, serum ASP activity levels were monitored during induction (protocol I) for 15 days after the first dose (days 12→27), for 18 days after the second dose (days 27→45) and during reinduction (protocols II or III) for 23 days (days 1→23) to evaluate the activity time-course of the schedule adopted. In order to evaluate whether our patients had received a treatment intensity comparable to that provided by a treatment schedule including the native *E. coli* ASP product, we compared data derived from pharmacokinetic monitoring on the use of the native *E. coli* ASP given in induction (protocol I) of the BFM ALL 90 study at the dosage of 5,000 U/m^2 i.v. every 3 days×8 (this schedule is currently used in the front-line induction treatment of the AIEOP ALL 2000 study). Mean trough enzyme activities (measured before the next ASP dose, given every 3 days) averaged 270±109 U/L (median 265 U/L, range 85-552 U/L) with 95.9% of samples completely (i.e.≤0.1 μM) and 4.1% almost completely (>0.1 and ≤0.5 μM) depleted of plasma asparagine, with this depletion persisting for 9 additional days (i.e. until day 42) after the last dose (day 33).[29] In our study ASP activity levels were ≥100 U/L during induction at almost every sampling point, with the exception of samples from three patients on days 27, 39 and 45; although ASP activity levels in these 9 samples were below 100 U/L, they were > 70 U/L in eight patients and 31.9 U/L in only one case. Interestingly the plasma asparagine levels were below the detection limit (i.e. ≤0.2 μM) in all samples. During reinduction (second exposure) one dose (1,000 U/m^2 i.v.) of PEG-ASP was used in our study at the same dosage as that used in

induction to substitute four doses of the native *E. coli* ASP given at the dosage of 10,000 U/m^2 i.v. every 3-4 days. ASP activity levels were above the targeted 100 U/L in almost all patients for the first 2 weeks with one patient only displaying silent inactivation from day 11. On day 18 most of the patients still had ASP activity levels ≥100 U/L. Of note, serum asparagine levels were below the limit of detection of 0.2 μM in all patients, even in the small number of patients with ASP activity levels below 100 U/L. The only exception was the samples obtained from the patient who displayed the phenomenon of silent inactivation. These findings are very similar to those reported in children with ALL treated in the reinduction phase (i.e. protocol II) of the BFM ALL 90 study with the native *E. coli* ASP product given at the dose of 10,000 IU/m^2 i.v. every 3-4 days x 4.[18] In that report trough ASP activity levels (measured every 3 days immediately before the next dose) were ≥100 IU/L in almost all samples (mean 542±243 U/L, median 328 U/L), with 90.7% of the samples showing complete (i.e. ≤0.1 μM) and 9.3% almost complete (>0.1 and ≤0.5 μM) plasma asparagine depletion.[18]

Our study shows that the PEG-ASP time-schedule used (two doses given intravenously at a dose of 1,000 U/m^2 every 2 weeks in induction and one dose only given at the same dosage during reinduction) was adequate to replace the native *E. coli* ASP schedule currently used in the AIEOP ALL 2000 study. From this study, it can also be concluded that a second exposure to PEG-ASP, after a first exposure with the same product, almost completely prevents the onset of the silent inactivation phenomenon expected to happen in over 30% of patients.[12] This favorable drug profile is most likely due to the reduced immunogenicity of PEG-ASP, which could have reduced, since the first exposure, the amount of foreign bacterial protein offered to the immune system for the production of neutralizing antibodies against the native product.[10] The finding that asparagine levels were below the limit of detection even when ASP activity levels were occasionally <100 U/L confirms the suggestion of our previous reports that the lack of achievement of ASP levels <100 U/L does not necessarily imply that adequate serum asparagine depletion is not obtained.[24]

Several groups have reported that adequate CSF asparagine depletion (< 0.2 μM) can be obtained with serum native ASP activity values ≥100 U/L[18,30-33] In this regard, Riccardi *et al.* suggested that ASP activity in the CSF never exceeds 0.2% of the serum ASP activity.[34] In one pharmacological study performed in The Netherlands in the frame of the DCLSG-ALL-9 trial, a single dose of Oncaspar® Medac (1,000 U/m^2, i.v.) given 5 days before the start of induction chemotherapy (window study) was able to produce serum ASP activity levels >100 U/L and serum asparagine levels < 0.2 μM for at least 10 days in all the investigated patients. However, asparagine depletion (< 0.2 μM) was not achieved in the CSF either 5 or 19 days after drug administration.[35] Furthermore, in a study reported by Avramis *et al.*, in which PEG-ASP was given at a different dosage and by a different route of administration, inadequate asparagine depletion occurred in the CSF despite prolonged adequate levels of serum ASP activity.[10] Our study showed similar results. In fact, even with persistently high levels of ASP activity and adequate asparagine depletion in the serum, asparagine was not depleted from the CSF in the vast majority of patients; this finding challenges the opinion that asparagine depletion in the CSF during ASP treatment is mainly due to an exchange between the two compartments, serum and CSF. One possible explanation for the lack of asparagine depletion in CSF may be that PEG-ASP, due to its high molecular weight, does not cross, even in the very small amounts postulated by Riccardi *et al.*,[34] the blood-brain barrier. It is not yet known whether this lack of depletion of asparagine in CSF could have an impact on protection from CNS relapses; modern chemotherapy treatment strategies in fact provide effective prevention of CNS relapses with prolonged intrathecal chemotherapy, high dose methotrexate or, in selected cases, cranial radiotherapy. The role of a single drug is difficult to assess in such a context.

In order to compare the clinical impact of PEG-ASP in the study patients (n=20), data on the duration of treatment phases, treatment burden, toxicity episodes and relevant clinical complications were collected on the cohort of 37 ALL children diagnosed in our institution immediately before the PEG-ASP study was started; these patients received the same chemotherapy treatment (AIEOP ALL 2000) but the ASP product was the native *E. coli* ASP instead of the PEG-ASP. This comparison suggests that the clinical impact of PEG-ASP in the cohort of study patients was similar to that observed in children receiving the native *E. coli* ASP product. Two cerebral thromboses were observed among the study patients (compared with one in the historical group); one of these two patients carried two genetic anomalies (the prothrombin G20210 variant + the MTHFR TT677 genotype) which have been shown to be strongly associated with thrombosis in children with ALL.[36] The issue of the thrombotic events occurring during chemotherapy treatment (including ASP and steroids) in children with ALL has been carefully evaluated by several investigators.[5,36-41] In some reports, the incidence of thrombotic events was higher than 10% with a significantly increased risk among children with central venous lines and/or with a genetic predisposition.[36,37] The small number of patients evaluated in our study does not allow definite conclusions to be drawn on this aspect. However, the analysis of the data available in the literature on the use of PEG-ASP, either as a first-line or second-line ASP product, in larger numbers of patients shows that the rate of thrombotic episodes associated with PEG-ASP is equivalent to that reported with the use of the native *E. coli* ASP product.[10,22,26,35,42,43]

In our study no patient had any clinically relevant allergic reaction. The rate of allergic reactions in the historical group was 5% (2 out of 37 patients). However, the incidence of allergic reactions is much higher when the drug is given intravenously. The rate of allergic reactions reported by the BFM group, which administered the drug intravenously (chemotherapy schedule similar to the AIEOP ALL 2000 protocol and the same native *E. coli* ASP product given at the same dosage) is comprised

between 25 and 39%.[5,18]

In conclusion, PEG-ASP given at the dose and time-schedule used in this study was able to effectively substitute, during multiple exposures to the drug, the native ASP product in terms of serum activity levels and asparagine depletion, to fully abolish clinically overt allergic reactions and to prevent almost completely the phenomenon of immunologically mediated silent inactivation. In addition, patients benefited from requiring fewer injections than necessary with the native preparations. The lack of adequate asparagine depletion in CSF confirmed preliminary information already reported; its impact on protecting the CNS from local relapses still remains uncertain and warrants further investigations.

MAC, MZ: analysis of samples and interpretation of data; CR, VC, MD'I: interpretation of data, drafting the article or revising it critically; AC, RC, SM: collection of samples. The authors reported no potential conflicts of interest. This work was supported by Comitato Maria Letizia Verga per lo Studio e la Cura della Leucemia del Bambino and by Medac GmbH, Hamburg, Germany which provided the study drug. The authors thank all the parents and the children who agreed to participate in this study and the physicians and the nurses of the departments who were indirectly involved in this study.

Manuscript received April 5, 2005. Accepted October 19, 2005.

References

1. Asparaginase. In: DeVita, Hellman, & Rosenberg, Eds. Cancer: Principles & Practice of Oncology, 4th ed, Volume 1, Philadelphia, PA, USA. 1993. p. 387-9.
2. Kidd JG. Regression of transplant lymphomas induced in vivo by means of normal guinea pig serum. J Exp Med 1953;98:565-82.
3. Broome JD. Evidence that the asparaginase of guinea pig serum is responsible for its antilymphoma effects. J Exp Med 1963;118:99-120.
4. Ertel IJ, Nesbit ME, Hammond D, Weiner J, Sather H. Effective dose of asparaginase for induction of remission in previously treated children with lymphocytic leukaemia: a report from Children Cancer's Study Group. Cancer Res 1979;9:3893-6.
5. Muller HJ, Boos J. Use of asparaginase in childhood ALL. Crit Rev Oncol/Hematol 1998;28:97-113.
6. Cao SG, Zhao QY, Ding ZT, Ma L, Yu T, Wang JH, et al. Chemical modification of enzyme molecules to improve their characteristics. Ann NY Acad Sci 1990;613:460-7.
7. Kawashima K, Takeshima H, Higashi Y, Hamaguchi M, Sugie H, Imamura I, et al. High efficacy of monomethoxy-polyethylene glycol-conjugated asparaginase (PEG2-asparaginase) in two patients with hematological malignancies. Leuk Res 1991;15:525-30.
8. Asselin BL, Whitin JC, Coppola DJ, Rupp IP, Sallan SE, Cohen HJ. Comparative pharmacokinetic studies of three asparaginase preparations. J Clin Oncol 1993;1:1780-6.
9. Asselin BL. The three asparaginase s. Comparative pharmacology and optimal use in childhood leukemia. Adv Exp Med Biol 1999;457:621-9.
10. Avramis VI, Sencer S, Periclou AP, Sather H, Bostrom BC, Cohen LJ, et al. A randomized comparison of native Escherichia coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukaemia: a Children's Cancer Group study. Blood 2002;99:1986-94.
11. Silverman LB, Gelber RD, Dalton VK, Asselin BL, Barr RD, Clavell LA, et al. Improved outcome for children with acute lymphoblastic leukaemia: results of Dana-Farber Consortium Protocol 91-01. Blood 2001;97:1211-8.
12. Müller HJ, Loning L, Horn A, Schwabe D, Gunkel M, Schrappe M, et al. Pegylated asparaginase (Oncaspar®) in children with ALL: drug monitoring in reinduction according to the ALL/NHL-BFM 95 protocols. Br J Haematol 2000; 110:379-84.
13. Masera G, Conter V, Rizzari C, Aricò M, Pession A, Paolucci G. AIEOP Non-B ALL trials. Int J Ped Hematol/Oncol 1999;6:101-11.
14. van Dongen JJ, Seriu T, Panzer-Grumayer ER, Biondi A, Pongers-Willemse MJ, Corral L, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet 1998; 352:1731-8.
15. Turnell DC, Cooper JHD. Rapid assay for amino acids in serum or urine by pre-column derivatization and reversed-phase liquid chromatography. Clin Chem 1982;28:527-31.
16. Fukuda K, Nishi Y, Usui T. Free amino acid concentrations in plasma, erythrocytes, granulocytes, and lymphocytes in umbilical cord blood, children, and adults. J Pediatr Gastroenterol Nutr 1984;3:432-9.
17. Heiblim DI, Evans HE, Class L, Agluyani M. Amino acids concentrations in cerebrospinal fluid. Arch Neurol 1978;35:765-8.
18. Boos J, Werber G, Ahlke E, Schulze-Westhoff P, Nowak-Gottl U, Würthwein G, et al. Monitoring of asparaginase activity and asparagine levels in children on different asparaginase preparations. Eur J Cancer 1996; 32:1544 -50.
19. Vieira Pinheiro JP, Ahlke E, Nowak-Göttl U, Hempel G, Müller HJ, Lumkemann K, et al. Pharmacokinetic dose adjustment of *Erwinia* asparaginase in protocol II of the paediatric ALL/NHL-BFM treatment protocol. Br J Haematol 1999;104:313-20.
20. Kurtzberg J, Asselin B, Pollack B. PEG-asparaginase vs native E. coli asparaginase for reinduction of relapsed acute lymphoblastic leukaemia: POG #8866 phase II trial. Proceedings of ASCO 1993;12:325.
21. Cheung NK, Chau IY, Coccia PF. Antibody response to Escherichia coli asparaginase . Prognostic significance and clinical utility of antibody measurement. Am J Pediatr Hematol Oncol 1986;8:99-104.
22. Abshire T, Pollock B, Billet A, Bradley P, Buchanan GR. Weekly polyethylene glycol-conyugated asparaginase compared with biweekly dosing produces superior induction remission rates in childhood relapsed acute lymphoblastic leukaemia: a Pediatric Oncology Group study. Blood 2000;96:1709-14.
23. Vieira Pinheiro JP, Müller HJ, Schwabe D, Gunkel M, Henze G, von Schutz V, et al. Drug monitoring of low-dose PEG-asparaginase (Oncaspar™) in children with relapsed acute lymphoblastic leucemia. Br J Haematol 2001;113:115-9.
24. Rizzari C. asparaginase treatment. In: Ching-Hon Pui, Ed. Current clinical oncology: treatment of acute leukemias: new directions for clinical research. Humana Press Inc, Totowa, NJ, USA; 2002. p. 381-91.
25. Ho DH, Wang CY, Lin JR, Brown N, Newman RA, Krakoff IH. Polyethylene glycol-asparaginase and asparaginase studies in rabbits. Drug Metab Dispos 1998;16:27-9.
26. Ettinger LJ, Kurtzberg J, Voute PA, Jurgens H, Halpern SL. An opel-label, multicenter study of polyethylene glycol-asparaginase for the treatment of acute lymphoblastic leukemia. Cancer 1995;75:1176-81.
27. Müller HJ, Beier R, da Palma JC, Lanvers C, Ahlke E, von Schutz VM, et al. PEG-asparaginase (Oncaspar®) 2500 U/m² BSA in reinduction of relapse treatment in the ALL(NHL-BFM protocols. Cancer Chemother Pharmacol 2002;49:149-54.
28. Vieira Pinheiro JP, Lanvers C, Wurthwein G, Boos J. Drug monitoring of PEG-asparaginase treatment in childhood acute lymphoblastic leukaemia and non-Hodgkin's lymphoma. Leuk Lymphoma 2002;43: 1911-20.
29. Ahlke E, Nowak-Göttl U, Schulze-Westhoff P, Werber G, Borste H, Wurthwein G, et al. Dose reduction of asparaginase under pharmacokinetic and pharmacodynamic control during induction therapy in childhood acute lymphoblastic leukemia. Br J Haematol 1997; 96:675-81.
30. Rizzari C, Zucchetti M, Conter V, Diomede L, Bruno A, Gavazzi L, et al. Asparagine depletion and asparaginase activity in children with acute lymphoblastic leukemia receiving i.m. or i.v. Erwinia c. or E. coli asparaginase as first exposure. Ann Oncol 2000; 11:189-93.
31. Dibenedetto SP, Di Cataldo A, Ragusa R, Meli C, Lo Nigro L. Levels of asparagine in CSF after intramuscular administration of asparaginase from Erwinia in children with ALL. J Clin Oncol 1995;13:339-44.
32. Gentili D, Conter V, Rizzari C, Tschuemperlin B, Zucchetti M. Asparagine depletion in plasma and cerebro spinal fluid of children with acute lymphoblastic leukaemia during subsequent exposures to Erwinia asparaginase . Ann Oncol 1996; 7:725-30.
33. Woo MH, Hak LJ, Storm MC, Gajjar AJ, Sandlund JT, Harrison PL, et al. Cerebrospinal fluid asparagine concentrations after Escherichia coli asparaginase in children with acute lymphoblastic leukaemia. J Clin Oncol 1999;17:1568-73.
34. Riccardi R, Holcenberg JS, Glaubiger DL, Wood KH, Poplack DG. asparaginase pharmacokinetics and asparagine levels in CSF of Rhesus monkeys and humans. Cancer Res 1981;41:4554-8.
35. Appel IM, Pinheiro JP, den Boer ML, Lanvers C, Reniers NC, Boos JI. Lack of asparagine depletion in the cerebrospinal fluid after intravenous dose of PEG-asparaginase: a window study at initial diagnosis of childhood ALL. Leukemia 2003;17:2254-6.
36. Nowak-Göttl U, Wermes C, Junker R, Koch HG, Schobess R, Fleischhack G, et al. Prospective evaluation of the thrombotic risk in children with acute lymphoblastic leukemia carrying the MTHFR TT 677 genotype, the prothrombin G20210 variant, and further prothrombotic risk factors. Blood 1999;93:1595-9.
37. Nowak-Göttl U, Heinecke A, von Kries R, Nurnberger W, Munchow N, Junker R. Thrombotic events revisited in children with acute lymphoblastic leukemia: impact of concomitant Escherichia coli asparaginase/prednisone administration. Thromb Res 2001; 103: 165-72.
38. Nowak-Göttl U, Ahlke E, Schulze-Westhoff P, Boos J. Changes in coagulation and fibrinolysis in childhood ALL: a two-step dose reduction of one E. coli asparaginase preparation. Br J Haematol 1996;95:123-6.
39. Nowak-Göttl U, Werber G, Ziemann D, Ahlke E, Boos J. Influence of two different E. coli asparaginase preparations on fibrinolytic proteins in childhood ALL. Haematologica 1996;81:127-31.
40. Sutor AH, Niemeyer C, Sauter S, Witt I, Kaufmehl K, Rombach A, et al. Gerinnungsveränderungen bei Behandlung mit den Protokollen ALL BFM 90 und NHL-BFM-90. Klin Pädiatr 1992;204:264-73.
41. Nowak-Göttl U, Kuhn N, Wolff JE, Boos J, Kehrel B, Rath B, et al. Inhibition of hypercoagulation by antithrombin substitution in E. coli asparaginase-treated children. Eur J Haematol 1996;56:35-8.
42. Keating MJ, Holmes R, Lerner S, Ho DH. asparaginase and PEG asparaginase-Past, present and future. Leuk Lymphoma 1993; 10:153-7.
43. Holle LM. Pegaspargase: an alternative? Ann Pharmacother 1997;31:19-27.