



A Pilot Study Conducted at a Tertiary Cancer Care Center, Evaluating the Serum Asparaginase Activity in Children Suffering from Acute Lymphoblastic Leukemia after the Administration of Biosimilar Pegaspargase

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Abstract

Introduction L-asparaginase is considered to be the most important component in the treatment of acute lymphoblastic leukemia (ALL). Intensifying the use of L-asparaginase during treatment for ALL has resulted in a significant rise in the percentage of children and adolescents who are cured of the disease. Asparaginase trough activity more than or equal to 100 IU/L on day 7 has been found to be the desired activity level in all childhood leukemia patients.

Objectives Due to the paucity of data on biosimilar pegaspargase in the upfront setting, we planned this prospective pilot study to evaluate the levels of serum asparaginase activity (SAA) after biosimilar pegaspargase infusion.

Materials and Methods It is a prospective, single-center, pilot study of 10 pediatric ALL patients for the duration of 6 months. All children less than 18 years with ALL on treatment with curative intent and receiving pegaspargase and who provided informed consent were included in this study. The enzymatic spectrophotometric method was used to determine SAA, and it was measured on the 7th and 14th days after the first dosage of pegaspargase-asparaginase, as well as on the 14th day after the second dose of pegaspargase-asparaginase, while toxicity was charted according to **Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.**

Results From 10 patients with a median age of 5.5 years, a grand total of 29 samples were taken for analysis. Children who received pegaspargase had either B-ALL or T-ALL. After the first dose, mean \pm SD (standard deviation), SAA levels at day 7 was 131.3 ± 38 IU/L and at Day 14 was 94.8 ± 8 IU/L. After the second dose, mean \pm SD SAA level at day 14 was 86.1 ± 15 IU/L. No patient had clinical hypersensitivity reaction and no patient reported any asparaginase-related toxicity. One patient died due to sepsis, infection with multidrug-resistant gram-negative bacteria.

Keywords

- ▶ pegaspargase
- ▶ acute lymphoblastic leukemia
- ▶ serum asparaginase activity
- ▶ childhood leukemia

Conclusions Biosimilar pegaspargase maintained good SAA levels 7 and 14 days after infusion.

Drug Trial Registration: Clinical Trial Registry of India vide reference CTRI/2021/08/036033 and available at <https://ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=59285&EncHid=&userName=>

Introduction

Acute lymphoblastic leukemia, also known as ALL, is an extremely rare form of hematologic malignancy that is characterized by an increase in the production of abnormal lymphoid progenitor lymphoblast cells. These cells can be either B cells or T cells. ALL is more prevalent in children, making up nearly 30% of all cases of pediatric cancer, while only accounting for 1% of all cases of adult cancer. When taking into account the effects of age, 5-year overall survival rate for children is greater than 90%, while it is less than 20% for older adults.¹⁻³

L-asparaginase is the pivotal drug used in the treatment of ALL. The patients who have been diagnosed with ALL are given *Escherichia coli* in either its native form or in conjugation with poly(ethylene glycol) (PEG) as their treatment. Reference biologic pegaspargase is the approved first-line asparaginase treatment for pediatric ALL. *E. coli* is PEGylated by reacting with either succinimidyl carbonate PEG (calaspargase pegol) or succinimidyl succinate PEG (biologic pegaspargase). L-asparaginase uses the substrate l-asparagine (Asn) to catalyze the production of free l-aspartic acid (Asp). The main goal of l-asparaginase therapy is to lower or eliminate endogenous circulating l-asparagine from the blood, depriving circulating blast cells of this crucial nutrient. PEG incorporation, on the other hand, increases steric hindrance, which restricts access of circulating peptidases and proteases and significantly lengthens half-life. This also lessens its immunogenicity; however, compared with the more than 30% incidence rate as observed with native enzyme, pegaspargase is only used in 3% of first-line and 10% of relapsed ALL patients without any prior reactions. Patients' immune systems can still produce antibodies against the linker.¹

Intensification of L-asparaginase during therapy has led to dramatic increase in cure rates. Asparaginase trough activity more than or equal to 100 IU/L on day 7 has been found to be the desired activity level. Biologic pegaspargase (Oncospar) is not easily available in India, while other biosimilar pegaspargase is expensive compared with native formulations. Biosimilar native L-asparaginase have shown poor activity in pilot study.⁴

Serum asparaginase activity (SAA) has developed into a valid pharmacodynamic tool because there is a direct correlation between the level of L-asparaginase activity and decrease in asparagine concentration. Following a 1981 study that showed plasma and cerebrospinal fluid (CSF) asparagine were undetectable at this level, which was later validated in numerous studies, SAA 0.1IU/mL was

proposed as the minimum desired threshold for meaningful efficacy.^{1,3} Therefore, SAA that reflects the enzyme's ability to deplete asparaginase should be compulsorily monitored in every pediatric ALL patients. Comparisons should be included in prospective work and identification of new microbiological sources of L-asparaginase to maximize the clinical efficacy of the drug while minimizing the side effects.^{1,5}

Forty-six B-ALL patients who had relapsed were randomly assigned to receive pegaspargase (2500 IU/m²) either weekly (four doses) or biweekly (two doses) during reinduction in the POG 9310 study. The overall complete response (CR) rate was 90%, and the cohort receiving weekly (97%) rather than biweekly (82%) dosing experienced higher CR, $p = 0.003$. Asparaginase activity was more active when the CR rate was higher ($p = 0.012$).^{1,6}

In this pilot study, we have inducted biosimilar pegaspargase as the intensification therapy followed by comparing assays at different intervals after first and second dose to keenly observe the trough activity; desired level is more than or equal to 100 IU/L on day 7 so that any needful changes can be met with the existing regimens.

Materials and Methods

It is a prospective, single-center pilot study done at tertiary cancer care center by the Division of Pediatric Haematology and Oncology Manipal Comprehensive Cancer Care Centre and Department of Biochemistry. The duration of this study was 6 months.

Inclusion and Exclusion Criteria

All children less than 18 years of age with ALL on treatment with curative intent and receiving pegaspargase who provided informed consent were included in this study of 6 months duration. However, any child with ALL with Down syndrome was excluded from this study as per the exclusion criteria.

All the patients were administered two doses of biosimilar pegaspargase (manufactured by Indian Pharmaceutical company) intravenously (IV) with a gap of 14 days in between. Assent form along with legally authorized representative form was obtained by subjects' parents or guardians.

Primary and Secondary Outcome

The primary outcome of the study was to measure the trough level activity of peg L-asparaginase at the decay stage at

7 days and 14 days after first dose and 14 days after second dose. While the secondary outcomes in terms of clinical hypersensitivity and asparaginase-related toxicity were measured at day 35 and for more clinical significance to the descriptions of grades, the toxicity charting was done as per the guidelines of Common Terminology Criteria for Adverse Events (CTCAE) V 4.0 for which the grading scale also includes a quantitative component.

Statistical Analysis

Descriptive statistics were used to evaluate the data, including percentages and frequencies for demographic parameters, and median for laboratory parameters and lastly mean and standard deviations for SAA values. Statistical analysis was done using Microsoft Excel. SAA being the key observation estimated using enzymatic spectrophotometric method. Other hematological parameters, which were accounted for, included hemoglobin, platelet count, total bilirubin and direct bilirubin with values taken before first and second dose as part of routine blood tests taken prior to chemotherapy.

Spectrophotometric Estimation of L-Asparaginase Activity

The activity of SAA was estimated by quantifying the levels of Indoxine generated after the substrate L-asparagine in the presence of 8-hydroxyquinolone (Indoxine method).

Standards for the assay were prepared in pooled plasma obtained from the blood bank. A total of seven standard concentrations were decided based on the dosage administered to the patients (2, 1, 0.5, 0.25, 0.125, 0.06, 0.03 IU/mL). The standard curve was plotted using the software mycurvefit.com (►Fig. 1). The patient samples were treated the same way as the standards and the optical density (OD) values were plotted against the standard using the same software.

Results

Ten children with B-ALL (7 patients) and T-ALL (3 patients) were included in the study, and 29 samples were collected (►Table 1).

After the first dose, mean \pm SD (standard deviation) levels of asparaginase on day 7 and 14 were 131.3 ± 38 IU/L and 94.8 ± 8 IU/L followed by mean value of 86.1 ± 15 IU/L on day 14 after the second dose (►Fig. 2). Furthermore, in the linear graph, the L-asparaginase activity can be seen well above the expected value and in the 14th day evaluation, the trough levels seem to fall below 100IU/L in certain patients after first dose and in most patients after the second dose. No case of clinical hypersensitivity reported. Asparaginase-related toxicity that includes any thromboembolic event, seizures, vomiting, pancreatitis, encephalitis, and hyperglycemia was not observed in any patient. However, sepsis/febrile neutropenia reported in nine out of ten patients and one patient died due to infection- multidrug-resistant (MDR) gram-negative sepsis. Another study concluded that *Pseudomonas aeruginosa* and *Klebsiella* species were the most frequently isolated organisms, of which most were gram-negative organisms while few were fungal. However, the antibiotic response was good with only some episodes requiring a third-line antibiotic.⁷

Discussion

In 1994, Food and Drug Administration first approved pegaspargase for use in the treatment of ALL patients who were hypersensitive to native forms of L-asparaginase.⁸ Asparaginase's tumor-inhibitory properties were discovered nearly 50 years ago when researchers noticed that lymphoma-bearing mice treated with guinea pig serum quickly underwent complete regression. This observation later led to the isolation of asparaginase of bacterial origin.⁹

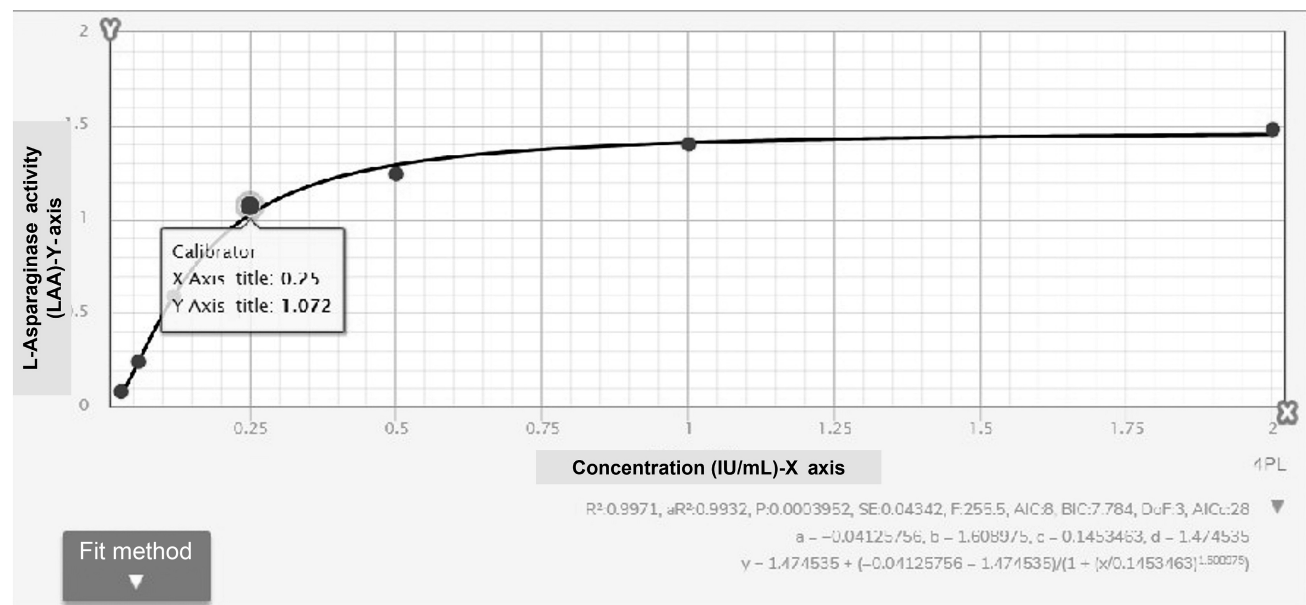


Fig. 1 Standard curve plotted for spectrophotometric estimation of L-asparaginase activity.

Table 1 Demography and laboratory parameters before pegaspargase-L-asparaginase dose

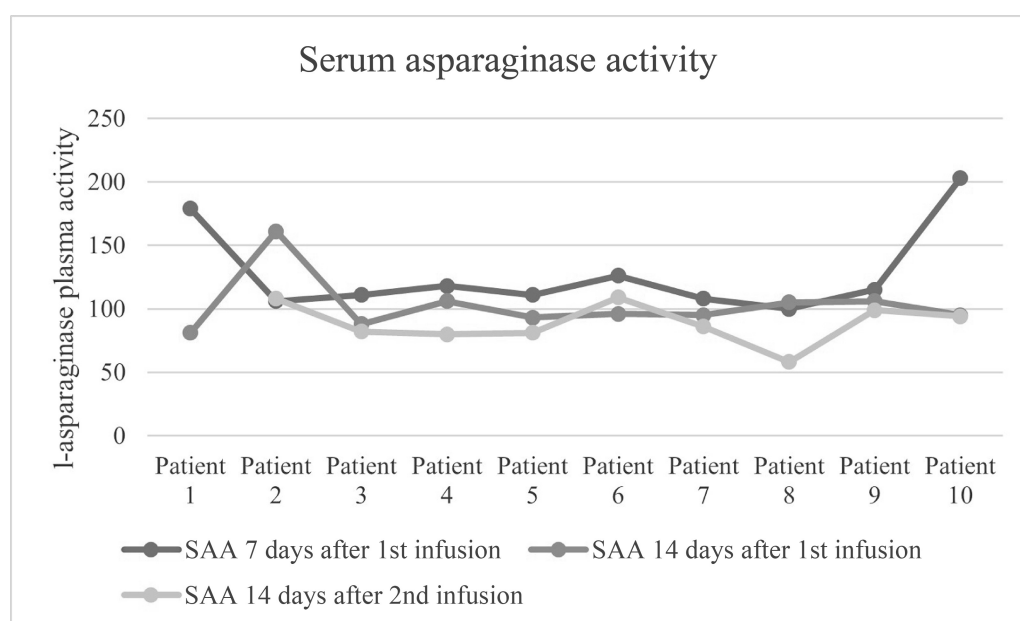
Demography		
Age in years (Median)		5.5
Gender n (%)	Male	5 (50%)
	Female	5 (50%)
Diagnosis	B-cell acute leukemia	7 (70%)
	T-cell acute leukemia	3 (30%)
Laboratory parameters		
	Prior to 1st dose (median)	Prior to second dose (median)
Hemoglobin (g/dL)	12.1	9.75
Platelet count ($10^9/L$)	110	128
Total bilirubin (mg/dL)	0.585	1.01
Direct bilirubin (mg/dL)	0.145	0.34
Albumin (g/dL)	4	3.2

In this pilot study of 10 ALL patients, all were given biosimilar pegaspargase through IV route.¹⁰ In a comparative study, comparison of IV versus intramuscular [IM] route of administration of pegaspargase, Children's oncology group [COG] leukemia 51 trials (2003–2015), the rate of grade 3 hypersensitivity reaction was 3.2% for IV administration whereas it was 5.4% with IM route. Increased IV infusion time of 10% pegaspargase over the first hour and the remaining 90% over the second hour can further reduce the infusion reaction caused by pegaspargase.⁸ In the induction stage, the dosage used was 2500 IU/m². The administration of IM and IV doses of pegylated *E. coli* asparaginase and *Erwinia* asparaginase is authorized.¹⁰

Up to 44 to 60% of patients may develop anti-asparaginase neutralizing antibodies in response to bacterial-derived asparaginases, which can inhibit a specific enzyme's activity and prevent the target amino acid from being deaminated

within the serum. There is evidence of immunological cross-reaction between the antibodies in various formulations of native *E. coli*-asparaginase and PEG-asparaginases, as suggested by laboratory preclinical findings, but not in *Erwinia* asparaginase. Due to the liver's involvement in de novo Asn biosynthesis, the pharmacodynamic analyses strongly suggest that more than or equal to 90% of the glutamine must be deaminated before optimal asparaginase deamination can occur.¹⁰

Enzymes as therapeutic drugs, however, have drawbacks related to the purity of bacterial proteins and the limited pharmacokinetic (PK) distribution in the central compartment of the plasma volume, as well as the potential to induce immunogenicity in the host. Extensive purification is always required to minimize the immune reactions as these proteins also have limited biodistribution in circulation followed by

**Fig. 2** Serum asparaginase activity (SAA) after first and second infusion of pegaspargase-L-asparaginase.

rapid elimination.⁹ The approximate level of Asn in the serum is 50 μM that is de novo synthesis derived from the liver by catalysis of Asp and glutamine.⁹ The development of clinical hypersensitivity with the native form is nearly 3 to 78%. Clinical allergy is most commonly seen in asparaginase therapy when initiated without steroids and would be limited if preinduction with the steroid immunosuppression is done pre-emptively resulting in greater Asn depletion and improved outcomes.⁹ For prevention of hypersensitivity reactions, in a study, the administration of IV pegaspargase and premedication containing acetaminophen, hydrocortisone, and diphenhydramine was advised.⁸ To counter the immunogenicity and the rapid decline, PEG is conjugated to native L-asparaginase thereby increasing the elimination half-life.⁹

Pegaspargase has an approximately 6-day elimination half-life, which is five times longer than native *E. coli* and nine times longer than Erwinia ASNase.¹¹ Another study found that PEG Asparaginase could lower the frequency of administration because it has a longer half-life (7 ± 2 days) than *E. coli* L-Asparaginase (20 hour).¹² Asparaginase activity as low as 0.05 IU/mL has been reported by several researchers to cause Asn depletion or positive outcomes in patients, challenging the strict 0.1 IU/mL requirement. The target level of plasma asparaginase activity to be attained has been set between 0.05 and 0.4 IU/mL, despite the fact that this range is necessary to achieve sufficient Asn depletion. However, data indicate that a level of 0.02 IU/mL should be maintained for effective plasma asparaginase activity. Pegaspargase clearance is observed to be multiphasic, with a rapid decline during the first day, a slower decline during days 1 to 7, a still slower decline during the second week, and then an increasingly more rapid decline at 22 to 29 days. The activity might fall below 0.02 IU/mL, showing a considerable acceleration of clearance after day 21.¹³

Pegaspargase 2,500 IU/m² was administered once or twice weekly in a study for the treatment of ALL. It was found that the trough serum enzymatic activity levels averaged 750 to 800 IU/L at trough times, or on day 7, and increased to higher concentrations of 1,200 to 900 IU/mL on days 21 and 28 post-induction, or after the third and fourth doses, respectively. Additionally, during induction, serum Asn was markedly decreased ($p < 0.002$ for all comparisons) from day 0 to days 7, 14, 21, and 28.⁹

In our study, the trough level was maintained above 100 IU/L post a week of medication that dropped down to a mean level of 94.8 IU/L and further to 86.1 IU/L at the end of 2 weeks post second dose. Moreover, the percentage of samples with adequate ASNase activity on day 21 of delayed intensification above 0.03 IU/mL were 95 and 31% in pegylated and native, respectively; furthermore above 0.1 IU/mL, it was 95 and 19%, respectively.¹⁴ In the secondary outcome, nine out of ten patients reported sepsis/febrile neutropenia and there was one fatality due to infection—MDR gram-negative sepsis—while no other major events were reported for this duration.

Studying the correlation between SAA and Asn concentration is crucial because the main goal of asparaginase

therapy is to deplete serum Asn. However, it has been debatable to measure Asn in the presence of asparaginase due to rapid ex vivo hydrolysis. Although the ideal asparaginase depletion level and duration for leukemic cell death are unknown, several studies legitimately identify a target asparaginase activity level of 100 IU/L.¹⁰ There are some studies that contradict the strict 0.1 IU/mL criteria, such as the Avramis and Panosyan study, which contend that asparaginase activity levels of more than 0.4 to 0.7 IU/mL are necessary for the best asparaginase depletion.¹¹ According to a recent study, however, an activity level of 20 IU/L can efficiently deplete plasma asparagine, and the 95% confidence interval for plasma asparagine depletion following a pegaspargase dose is close to 22 to 29 days. In line with this information, this pilot study's patients maintained SAA levels above 100 IU/L at 7 days and above 20 IU/L at 14 days, resulting in all patients remaining in remission.¹³

Since it is known that patients with anti-asparaginase antibodies have higher asparaginase clearance, there were some restrictions on the number of patients in our pilot study. Additionally, antibody titer was not assessed.⁹ PEG Asparaginase was used in our study as the first-line therapy for the diagnosis and management of children with ALL, as recommended by the Chinese guidelines. By the analysis of the collected data, lower hypersensitivity rate and hepatic injury have been shown in the patients of the PEG Asparaginase groups. Additionally, the use of PEG Asparaginase in pharmacotherapy may lessen the financial burden associated with using medical resources because of decreased administration frequency and a shorter length of hospital stay.¹²

Levocarnitine and vitamin B complex are being tested in numerous ongoing studies for their ability to treat hyperbilirubinemia linked to PEG. To accurately measure plasma, asparagine is not mostly feasible outside the clinical trial context. The optimal therapeutic level where plasma Asparagine is fully depleted, but the measurement of its plasma values faces uncertainty due to technical issues that call the validity into question. There is still room for improvement even though asparaginase is a well-established cornerstone of ALL/LBL therapy. Some approaches, like encapsulating L-asparaginase in donor-derived erythrocytes, appear promising for overcoming potential hypersensitivity. When the cleaved asparagine enters the erythrocyte, it does so while the drug is hidden from the patient's immune system. However, on observing the current available data, the information regarding the asparaginase levels and their PK value is needed in a larger group so that asparaginase dose regimens can be optimized. A more direct evaluation of the SAA levels required for the best outcomes might be made in future studies by correlating SAA values with outcomes in sizable uniformly treated cohorts.³

Conclusions

Pegaspargase is more tolerable, less immunogenic, and equally effective when compared with native L-asparaginase. If at all possible, all patients with childhood leukemia should have their levels of SAA, an indicator of the enzyme's capacity

to deplete asparaginase, if at all possible, all patients with childhood leukemia should have their levels of SAA, an indicator of the enzyme's capacity to deplete asparaginase monitored. Biosimilar pegaspargase maintained good SAA levels at 7 and 14 days after infusion. However, further evaluation should be considered regarding therapeutic SAA levels and its correlation with complete plasma depletion of asparagine (including CSF values). Since it has been found that pegaspargase has a prolonged effect, is convenient to administer, and has a good safety profile, it can be recommended to replace native asparaginase. However, larger studies are warranted for exploring further scope.

Note

The abstract of this text was published in the Supplementary issue: Abstracts of the 25th Annual Conference of the Pediatric Hematology Oncology chapter of the Indian Academy of Pediatrics (PHOCON 2022), N Delhi in November 2022 with doi: [10.1016/j.phoj.2022.10.176](https://doi.org/10.1016/j.phoj.2022.10.176) The manuscript has been read and approved by all the authors, that the requirements for authorship (Pg. 18) have been met, and that each author believes that the manuscript represents honest work, if that information is not provided in another form.

Patient Consent

None declared.

Ethics Approval

This study was approved by Institutional Ethics Committee at Kasturba Hospital, Manipal and the procedures used in this study adhere to the tenets of the Declaration of Helsinki. All procedures performed in studies involving human participants were in accordance with the ethical standards and by approval from the Kasturba Medical College and Kasturba Hospital Institutional Ethics Committee (DHR Registration No. EC/NEW/INST/2019/374, Dated 14th April 2021, IEC: 356/2021) and was registered at CTRI- REF/2021/08/046121. It was performed in line with the latest Helsinki Declaration's guiding principles from 2013.

Authors' Contributions

Vasudeva Bhat K conceptualized and designed the study. Archana M.V., Arjun Asok, and Krishnananda Prabhu helped in data acquisition and manuscript preparation.

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None declared.

Conflicts of interest/Competing Interest

None declared.

Acknowledgment

None declared.

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