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Original Article

Comparison of efficacy and safety of different asparaginases in adult acute lymphoblastic leukemia based on nano-magnetic beads immunoassay

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Abstract: Objective: To compare the efficacy and safety of different asparaginase formulations in the treatment of acute lymphoblastic leukemia (ALL) based on nano-magnetic bead immunoassay. Methods: Retrospective analysis of adult ALL patients' clinical data who admitted to The Affiliated Hospital of Changsha Health Vocational College from August 2020 to August 2023. Finally, 65 adult ALL patients were included in this study, including the polyethylene glycol conjugated asparaginase (PEG-ASNase) group (n = 32) and the L-asparaginase (L-ASNase) group (n = 33). Enzyme-linked immunosorbent assay (ELISA) based on magnetic nanoparticles was used to determine the activity of ASNase in both groups. The levels of asparagine or glutamine in two groups were detected by automatic biochemical analyzer during induction therapy, and the adverse events of the two groups were observed during the treatment. Results: PEG-ASNase demonstrated a slower decrease in enzyme activity, longer action duration, and higher safety profile compared to L-ASNase. PEG-ASNase group and L-ASNase group demonstrated a similar complete remission rate (71.88% vs. 60.61%). Event-free survival was higher in patients receiving PEG-ASNase than those receiving L-ASNase (42.4% and 18.7%). The observed adverse reactions included allergic reactions, pancreatic lesions, gastrointestinal reactions and liver function damage. The incidence of gastrointestinal reactions and liver function damage was higher in the L-ASNase group than that in PEG-ASNase group (45.45% and 33.33%). Conclusion: This study provides valuable insights into the asparaginase treatments in clinical, highlighting the importance of PEG-ASNase for improving treatment protocols in adult ALL patients.

Keywords: Acute lymphoblastic leukemia, asparaginase, nano-magnetic beads immunoassay, efficacy, safety

Introduction

Acute lymphoblastic leukemia (ALL) is a hematological malignancy arising from lymphoid progenitor cells of the B and T cell subtypes, primarily affecting children, with adults accounting for approximately 20% of all leukemia cases. Adult ALL is associated with a poorer prognosis, fewer treatment options and a cure rate of less than 40% [1, 2]. Asparagine, a non-essential amino acid vital for leukemia cells but not normal cells, is crucial for the survival of these malignant cells as they rely on exogenous sources. Asparaginase, an enzyme therapy, depletes asparagine levels, thereby inhibiting protein synthesis and cell proliferation in tumor cells, effectively exerting anti-tumor activity [3].

Clinical experience has demonstrated that incorporating asparaginase into multidrug chemotherapy regimens improves the clinical outcome of ALL patients [4]. L-asparaginase (L-ASNase), derived from natural Escherichia coli and used to treat ALL since 1970s, has a well-documented efficacy [5, 6]. However, its application in adult patients is largely hampered by its toxic properties, including severe immune and non-immune-related toxicities, such as pancreatitis, hepatotoxicity, coagulation and neurotoxicity. These toxic effects may

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delay induction therapy after remission and necessitate dose reduction of other antileukemic drugs with similar toxicity [6]. Polyethylene glycol asparaginase (PEG-ASNase) offers an alternative with its extended plasma half-life and reduced antibody formation, prolonging asparagine depletion and potentially decreasing toxicity [7]. However, definitive evidence comparing its anti-tumor effectiveness to L-ASNase and its impact on reducing complications remains limited [8]. Moreover, ASNase can induce the production of anti-ASNase antibodies and neutralize enzyme activity, leading to clinical side effects. Monitoring ASNase activity is the sole evidence-based method to identify patients with silent enzyme inactivation, with activity levels directly correlating with clinical efficacy. A target activity level of ≥ 0.1 IU/mL, which ensures complete asparagine degradation, is crucial for clinical decision-making, emphasizing the importance of precise ASNase activity monitoring in therapeutic management [9].

Immunoassay-based bioanalysis holds promising potential across various fields including biological research, pharmaceutical science, biomarker discovery, clinical treatment and diagnostic targets [10]. Enzyme-linked immunosorbent assay (ELISA) is a commonly used technique for pharmacokinetic assessment of proteins. However, ELISA's reliance on diffusion-dependent heterogeneous reactions typically requires several hours to form detectable immune complexes, and the presence of high concentrations of organic solvents can destabilize the antibodies used in these assays [11]. Immunomagnetic beads separation offers a highly specific and sensitive alternative. This technique involves immunomagnetic beads (IMBs) that selectively capture target analytes from multi-component samples to form antigen-antibody-magnetic beads immune complexes. By simply operating an external magnetic field, the bacteria-bound IMBs can be isolated from the matrix or redispersed in wash and elution buffer for purification and subsequent analysis. Studies have shown that the large specific surface area of nanoparticles can improve the efficiency of removing toxic compounds from the environment. Advances in nanomaterials and nanotechnology have led to the development of nano-sized magnetic beads, which can drastically reduce incubation times from hours to minutes. These nanomagnetic beads are increasingly being used directly in the separation and purification of both small molecular targets and macromolecules, proving to be excellent carriers [12, 13].

In light of the critical need to monitor ASNase activity, we have developed a rapid nano-magnetic beads immunoassay to evaluate serum ASNase activity. This method enables us to compare the efficacy and safety of different ASNase formulations in treating adult acute lymphoblastic leukemia, aiming to provide essential guidance for clinical practice.

Materials and methods

Clinical data

This study was approved by the Medical Ethics Committee of Changsha Health Vocational College. We conducted a retrospective analysis of adult ALL patients' clinical data who admitted to The Affiliated Hospital of Changsha Health Vocational College from August 2020 to August 2023. The diagnostic stratification was guided by the Chinese Adult Acute Lymphoblastic Leukemia diagnosis and treatment guidelines (2021 Edition): (1) Persistent or recurrent fever; (2) Progressive anemia, bleeding, and joint pain; (3) Enlargement of liver, spleen and lymph nodes; (4) White blood cell count (WBC) > 10×10^9/L; (5) Active bone marrow hyperplasia; (6) A bone marrow smear showing a lymphocyte ratio of primitive/immature cells at 20% or higher [14]. Inclusion criteria: patients aged between 18 and 60, who were diagnosed with ALL for the first time, had not received related treatment in the past, and signed the informed consent form for the study. Exclusion criteria: patients who had contraindications to the drugs used in treatment; patients with abnormal organ function indexes such as heart, liver, kidney and lung; patients with malignant tumors of other systems; patients who had received cytotoxic chemotherapy in the past; patients with craniocerebral organic lesions and mental disorders; patients who failed to complete the chemotherapy regimen; patients with incomplete clinical data and lost to follow-up.

Mode of treatment

During the induction remission and regular intensive therapy phases, patients were administered the VDLP regimen, which included vin-

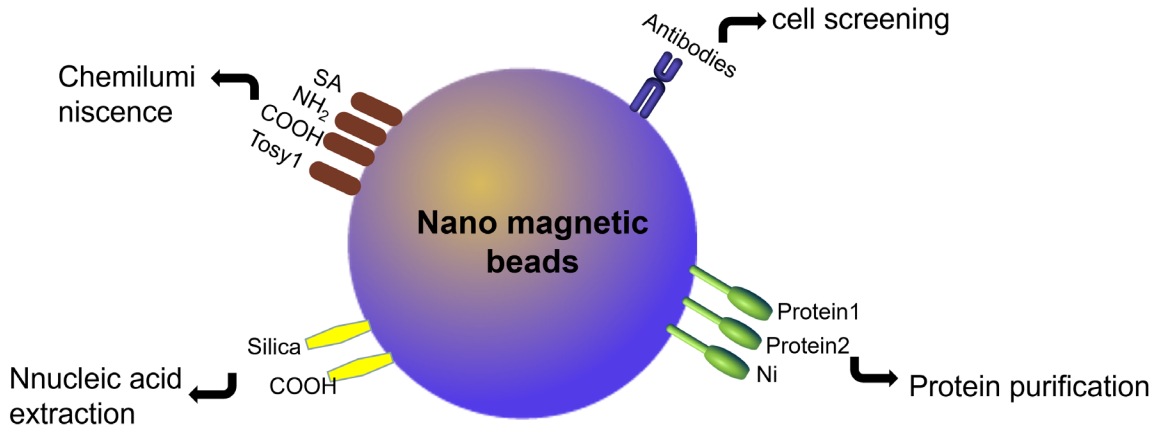

Figure 1. Schematic diagram of immunoassay with nano-magnetic beads.

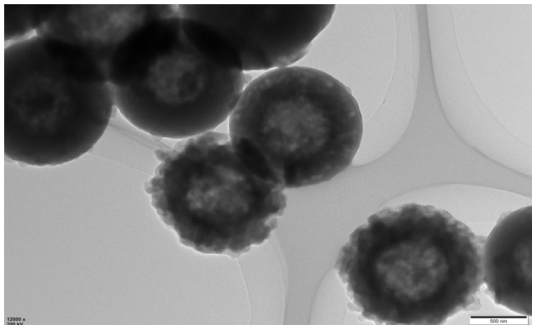

Figure 2. Transmission electric competition diagram of nano-magnetic beads. The diameter of the iron nitride particles in the magnetic core of the bead is about 10-40 nm. The shell of the graphene sheet is a multi-layer structure, and the outer graphene shell can protect the internal iron nitride.

cristine (Haizheng Pfizer Pharmaceutical Co., Ltd., H20043326), daunorubicin (Shenzhen Wangle Pharmaceutical Co., Ltd., H44024361), asparaginase [PEG-ASNase (Jiangsu Hengrui pharmaceutical Co., Ltd., H20153215) or L-ASNase (Jiangsu Hengrui pharmaceutical Co., Ltd., H20090015)], and prednisone (Yichang Renfu Pharmaceutical Co., Ltd., H42022111). All asparaginases were administered via intravenous drip. HyperA/B regimen was used alternately in the consolidation stage.

Preparation of nanometer magnetic beads

Preparation of nano-magnetic beads: 0.5% aqueous solution of iron salt (II) and (III) was prepared at a molar ratio of at 2:1 (III:II). 30% ammonia hydrate solution was added dropwise to a concentration of 8%. The mixture was incubated for 15 minutes at room temperature. Then, the particles were collected with magnets and resuspended in double distilled water. After removing the supernatant, the beads were washed five times with excess distilled water [11].

Nano-magnetic beads (500 μL) were mixed with 3 mg/mL and anti-ASNase antibody solution to obtain a final antibody concentration of 8-70 μg/mL. The mixture was incubated with vigorous stirring for 30 minutes. Subsequently, the magnetic beads were separated by a magnetic frame, the supernatant was discarded and the beads were washed 3 times with PBS. After completion, the 70 μg/ml antibody solution was added to the activated magnetic beads to oscillate for 2 h at room temperature, and the magnetic beads were washed thoroughly post-reaction. Finally, 100 μL of 50 mmol/L Tris solution (pH 7.4) was added and oscillated for 20 minutes at room temperature to quench the unreacted carboxyl group. After beads collection, the product was re-suspended in 100 μL PBS buffer (pH 7.4) and stored at 4°C. The principle of immunoassay of nano-magnetic beads is shown in **Figure 1**. The transmission electric competition diagram of nano-magnetic beads is shown in **Figure 2**, showing spherical, equal size, and uniformly distributed beads.

ASNase activity assay

The serum samples of the two groups were collected at 0, 3, 7 and 14 days after induction chemotherapy and 3, 7 and 14 days following the cessation of treatment. These samples were stored at -80°C. The ASNase activity of

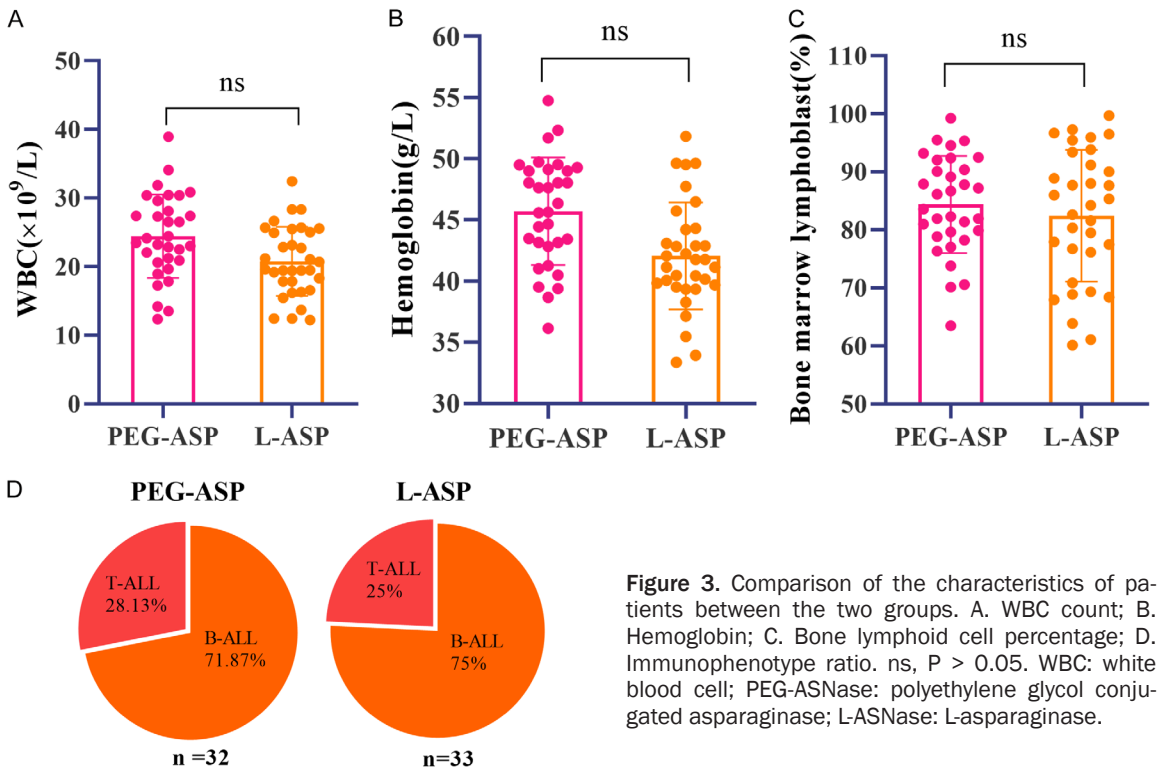

Figure 3. Comparison of the characteristics of patients between the two groups. A. WBC count; B. Hemoglobin; C. Bone lymphoid cell percentage; D. Immunophenotype ratio. ns, P > 0.05. WBC: white blood cell; PEG-ASNase: polyethylene glycol conjugated asparaginase; L-ASNase: L-asparaginase.

the two groups was measured by ELISA based on nano-magnetic beads, and the OD value of the enzyme activity solution was detected in ELISA plate.

Observation index

The levels of asparagine (Asp) or glutamine (Glu) after one course of induction therapy were monitored by automatic biochemical analyzer, and the adverse events were observed. The therapeutic effects were graded according to the Chinese Adult Acute Lymphoblastic Leukemia Diagnosis and Treatment Guidelines (2021 Edition) [14]: complete remission (CR), partial remission (PR) and non-remission (NR).

Follow-up statistics

The patients were followed up through outpatient clinic or other forms of communication for 24 months, and the relapse-free survival rate was recorded, that is, the time from the first CR to recurrence, mortality or final follow-up.

Statistical analysis

Statistical analysis was performed using SPSS22.0 software. The measurement data were described as mean ± standard deviation ($\bar{x}$±Sd). T-test was performed for inter-group comparison, and multiple time data were analyzed by repeated measures analysis of variance with post hoc Bonferroni test. The count data were expressed as n (%) and χ^2 test was used. The survival curve was analyzed by Kaplan-Meier method and Log rank test. $P < 0.05$ was considered with statistical difference.

Results

Analysis of patient characteristics

This study included 65 adult ALL patients, including polyethylene glycol conjugated asparaginase (PEG-ASNase) group (n = 32) and L-asparaginase (L-ASNase) group (n = 33). The clinical characteristics of patients in PEG-ASNase group and L-ASNase group were analyzed. There was no significant difference in initial WBC count, bone marrow hyperplasia and immunophenotype between the two groups (all P > 0.05) (**Figure 3A-D**). Among the 32 patients in PEG-ASNase group, there were 23 cases of B-ALL and 9 cases of T-ALL; and among the 33 patients in L-ASNase group, there were 25 cases of B-ALL and 8 cases of T-ALL (**Figure 3D**).

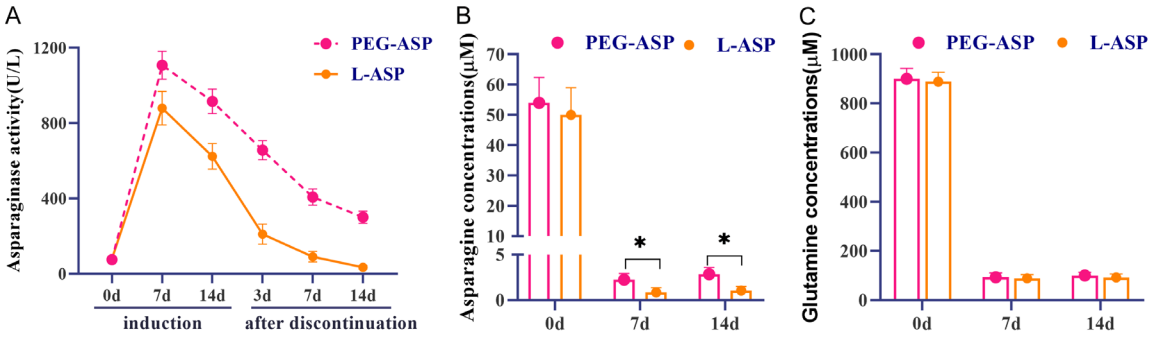

Figure 4. Serum ASP activity and ASP/Glu amino acid level. A. ASP activity; B. ASP amino acid level; C. Glu amino acid level. *P < 0.05. ASP: asparaginase; PEG-ASNase: polyethylene glycol conjugated asparaginase; L-ASNase: L-asparaginase.

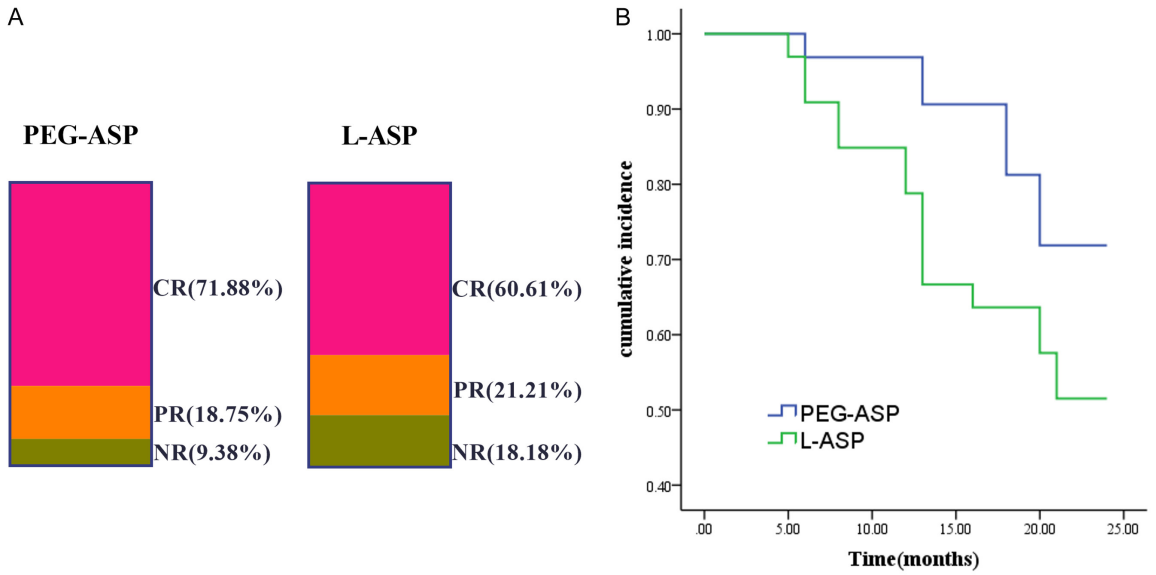

Figure 5. Comparison of curative effects between the two groups. A. Short-term efficacy; B. Event-free survival curve analysis. PEG-ASNase: polyethylene glycol conjugated asparaginase; L-ASNase: L-asparaginase; CR: complete remission; PR: partial remission; NR: non-remission.

Comparison of ASNase activity, ASP and Glu amino acid level between the two groups

The results of repeated measures analysis of variance indicated significant effects of both group (F_{group} = 2996.686, P < 0.001) and time (F_{time} = 2967.679, P < 0.001) on ASNase activity, with a significant interaction between the two ($F_{group \times time}$ = 85.244, P < 0.001). Both groups (F_{group} = 11.079, P = 0.002) and time (F_{time} = 2429.341, P < 0.001) significantly influenced serum ASP level (**Figure 4A**); however, there was no interaction between the two ($F_{group \times time}$ = 2.443, P = 0.095). Group (F_{group} = 0.016, P = 0.900) had no effect on serum Glu level, while time (F_{time} = 2831.901, P < 0.001) had an effect on serum Glu, and there was no interaction between the two ($F_{group \times time}$ = 0.254, P = 0.776) (**Figure 4B**, **4C**).

Comparison of curative effects between the two groups

The short-term efficacy of the two groups showed that most of the patients achieved the first remission, and there was no significant difference between the two groups. The CR ratio of the PEG-ASNase group was 71.88%, and the CR ratio of the L-ASNase group was 60.61% (**Figure 5A**). All patients were followed up for 24 months. The event-free survival rate was significantly higher in the PEG-ASNase group at

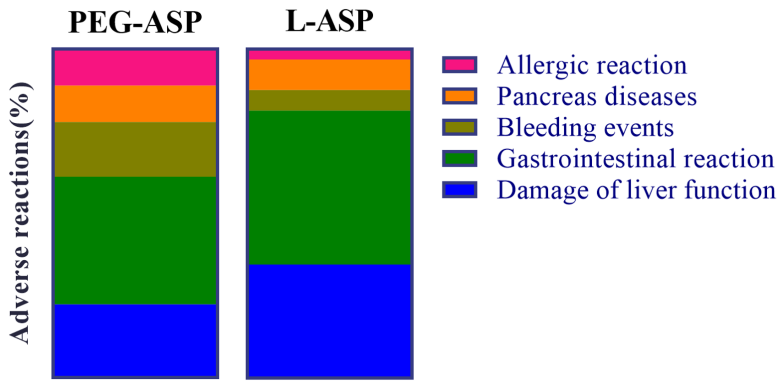

Figure 6. Comparison of the incidence of chemotherapy-related adverse reactions between the two groups. PEG-ASNase: polyethylene glycol conjugated asparaginase; L-ASNase: L-asparaginase.

42.4% compared to 18.7% in the L-ASNase group, as indicated by a statistically significant chi-square test. However, the event-free survival curves of the two groups analyzed by Kaplan-Meier and Log rank test indicated no significant difference between the two groups (**Figure 5B**).

Comparison of adverse reactions between the two groups

The adverse reactions in PEG-ASNase group included allergic reaction (6.25%), pancreatic lesion (6.25%), bleeding reaction (9.38%), gastrointestinal reaction (21.88%) and liver function damage (12.5%). The adverse events in L-ASNase group included allergic reaction (3.03%), pancreatic lesion (9.10%), bleeding reaction (6.10%), gastrointestinal reaction (45.45%) and liver function damage (33.33%). There was significant difference in the incidence of gastrointestinal reaction and liver function damage between the two groups (all $P < 0.05$) (**Figure 6**).

Discussion

Acute lymphoblastic leukemia (ALL) is a common hematological malignancy characterized by malignant proliferation, impaired differentiation and blocked apoptosis of proto-and juvenile lymphocytes, with high heterogeneity [15]. Compared with childhood ALL, adult ALL has a poorer prognosis and short survival time [16]. Adult ALL can occur at different stages of cellular metabolism, exhibiting variations in clinical features, immunophenotype, biological characteristics and response to therapy [17]. The natural progression of ALL is typically rapid, spanning approximately 2 to 6 months. The main causes of death in ALL patients include infection, bleeding and systemic failure. In recent decades, the application of combined chemotherapy and active prevention and treatment of CNSL has significantly increased the survival time of ALL patients [18-20].

ALL tumor cells, lacking asparagine synthase, depend on extracellular ASP for survival and proliferation. ASNase exerts its anti-leukemia effect by depleting circulating ASP and inhibiting cancer cells' amino acids. The clinical benefit of incorporating ASNase therapy into ALL therapy has been well-documented [21]. Reduced ASNase activity may lead to poor therapeutic effects and poor outcomes. A growing number of studies have shown that adult ALL treatment regimens supplemented with ASNase significantly increase remission rates [22]. Due to the broad pharmacokinetic variability of ASNase and the narrow therapeutic window between effective drug concentrations and side effects, regular monitoring of ASNase activity is crucial to ensure optimal timing and dosing, thereby maximizing therapeutic benefits while minimizing toxicity [23].

L-ASNase is one of the key drugs in combined ALL chemotherapy. Recent studies have suggested that PEG-ASNase is superior to traditional L-ASNase preparations due to the extended half-life, allowing for sustained asparagine depletion when asparaginase level falls below 0.1 IU/mL (100 U/L). In this study, the activity of ASNase was measured regularly in patients treated with PEG-ASNase and L-ASNase. The data showed that the activity of ASNase in both groups remained above 100 Umax L during treatment, but after drug withdrawal, the activity of PEG-ASNase decreased gradually and the activity of L-ASNase decreased rapidly, reflecting PEG-ASNase's longer half-life. Previous studies have reported that ASNase activity levels are inversely proportional to ASP levels and are usually used to determine asparagine consumption in patients [24]. Recent studies high-

lighted the importance of glutaminase activity for effective ALL treatment. Compared with the level of ASP, the level of Glu in serum is higher, necessitating a toxic dose of ASNase to achieve the required consumption [25]. In our study, we observed that the levels of ASP and Glu of the two groups decreased significantly during treatment, and higher levels of ASNase activity consumed more ASP and Glu. This underscores the role of monitoring ASNase activity levels in controlling drug administration timing and dosage, thus maintaining efficacy and preventing toxicity.

Asparaginase (ASNase), a foreign protein when administered to patients, can trigger an immune response that is associated with decreased asparagine (ASP) activity. PEG-ASNase is a synthetic compound produced by chemical coupling of polyethylene glycol and Asp, which not only maintains the enzyme activity, but also reduces the immunogenicity of protein. Its half-life is as long as 6 days, which avoids the pain of repeated injection and reduces the risk of allergy [26]. Compared with L-ASNase, PEG-ASNase can reduce renal excretion, prolong half-life, increase bioavailability, reduce immunogenicity and reduce drug administration frequency [27]. Clinical trial data show that PEG-ASNase and L-ASNase can be used in the treatment of ALL in adults. A large number of studies have proved that ASNase therapy has satisfactory remission (CR) rate and better event-free survival time, and PEG-ASNase is better than L-ASNase [28]. PEG-ASNase exhibits a slower decrease in activity, longer action time, and higher safety profile. In this study, the efficacy and safety of different ASNases in the treatment of adult ALL were tested based on nano-magnetic beads ELISA, demonstrating a consistent curative effect. ASNase treatment had a higher CR rate and event-free survival time, but the CR rate and event-free survival rate of PEG-ASNase group were higher than those of L-ASNase group. In our study, common adverse reactions observed in both groups during ASNase treatment included gastrointestinal reactions, pancreatitis, abnormal blood coagulation, allergic reactions and liver function damage. The incidence of gastrointestinal reactions and liver function damage was higher in the L-ASNase group (45.45% and 33.33%), possibly due to side effects caused by increased mitochondrial stress and changes in lipoprotein metabolism induced by Asp and Glu consumption.

To sum up, PEG-ASNase demonstrated superior short-term efficacy in the treatment of adult ALL, with lower incidence of liver function damage and gastrointestinal reaction. However, this study has some limitations, including a small sample size and a short follow-up period, which hinder our ability to accurately assess long-term efficacy and safety. To address these shortcomings, future research should aim to expand the sample size and extend the duration of follow-up.

Conclusion

Incorporating ASNase to the treatment of adult ALL patients can effectively achieve the anti-tumor activity. PEG-Asparaginase (PEG-ASNase) shows a slow decrease in enzyme activity, and both groups exhibit similar CR rate and event-free survival time. However, in terms of adverse reactions, PEG-ASNase is associated with lower adverse reactions compared to that of L-ASNase, indicating its higher clinical treatment value. This suggests that PEG-ASNase holds significant potential for further development in clinical diagnostics and treatment strategies.

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Disclosure of conflict of interest

None.

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