The Supplementation of Vitamin A for the Prevention of Asparaginase-Associated Pancreatitis in Children With Acute Lymphoblastic Leukemia: A Prospective Single-arm Clinical Trial（The PREVAIL Study）

Rationale

Acute lymphoblastic leukemia (ALL) is the most common cancer in children and adolescents[1]. Asparaginase (ASNase) is one of the preferred drugs for the current treatment of ALL[2]. ASNase catalyzes the hydrolysis of asparagine (Asn) into aspartic acid and ammonia, thereby depleting Asn in the bloodstream, which is an essential amino acid for leukemia cells. Since leukemia cells selectively rely on exogenous Asn, continuous exposure to ASNase leads to amino acid deficiency, cessation of growth, and ultimately induces apoptosis[3, 4]. Since the drug introduced into the treatment of pediatric ALL in the 1960s, the survival rate for pediatric ALL has gradually increased from 50% to nearly 90%[5]. Other reports indicate that intensified ASNase treatment significantly increases the survival rates of adolescents and young adults with ALL who have a poor prognosis [6, 7]. Furthermore, multiple studies have shown that the response to ASNase treatment is an independent prognostic factor for children with ALL[8-10]. Therefore, ASNase plays a critical role in the treatment of ALL, and the degree of response to its treatment is closely related to the prognosis. However, during the course of treatment with ASNase, approximately 2%-10% of patients develop asparaginase-associated pancreatitis (AAP), with one-third of these cases progressing to severe acute pancreatitis, accompanied by symptoms such as pseudocyst formation and pancreatic necrosis. Once AAP occurs, due to the high risk of re-use, the drug is typically discontinued in clinical practice, leading to poor outcomes in children with ALL. To better predict and prevent the occurrence of AAP, several studies have identified risk factors for AAP, including increasing age, severity of ALL, dosage of ASNase, and racial differences[11-13]. However, these risk factors are not accurate predictors of AAP, and the exact mechanism of AAP remains unclear.

Existing studies suggest that the mechanism of AAP may involve ASNase-induced depletion of Asn, leading to an imbalance of amino acids in the serum, which in turn triggers pancreatic injury [14]. Additionally, research in adult acute pancreatitis has confirmed that severe hyperlipidemia can increase the risk of acute pancreatitis [15]. However, it is not yet fully clear whether hyperlipidemia is a risk factor for AAP in children. Due to the unclear pathogenesis, current clinical methods for preventing AAP are limited. Although low-fat, low-oil dietary strategies have been implemented in clinical practice for children undergoing ASNase treatment, they have not proven to be effective in preventing AAP.

Recently, a collaborative team explored the mechanisms of AAP by analyzing large amounts of gene expression data, small molecule data, and electronic health records, elucidating the important role of vitamin A and its analogs in the prevention and treatment of AAP [16]. First, the researchers used a systematic approach to identify risk factors affecting AAP. Connectivity map analysis of transcriptome data suggested that ASNase-induced gene signatures might be reversed by vitamin A and its analogs. Analyses of large electronic health record databases (TriNetX) and the U.S. FDA Adverse Event Reporting System (FAERS) showed that vitamin A intake in patients receiving ASNase treatment could reduce the risk of AAP. Additionally, the research team conducted metabolomic analyses on plasma samples from 24 ALL patients who developed pancreatitis (cases) and 26 ALL patients who did not develop pancreatitis after a single exposure to ASNase. The results showed that ALL patients who developed pancreatitis had lower plasma levels of carotenoids compared to the control group. A 30-day dietary recall survey revealed that these cases had lower vitamin A intake than the control group. The current standard ALL chemotherapy regimens consist of combinations of several drugs. Since ASNase is not used as a single drug in any standard regimen, it is unclear whether ASNase alone reduces the levels of retinoids in patients. Therefore, the researchers used a mouse model exposed to ASNase. In mice, ASNase alone not only reduced circulating retinol in the blood but also decreased retinol levels in the liver, the primary storage site for retinol. In conclusion, the

researchers suggest that ASNase reduces retinoid levels in patients, and intake of retinoids may reduce the risk of pancreatitis in leukemia patients treated with ASNase. These findings provide an important reference for the clinical prevention of AAP, but since the data are based on retrospective studies in European and American children and adult ALL populations, the strength of the evidence needs to be further reinforced. Therefore, this study aims to design a prospective study to evaluate the correlation between exogenous vitamin A levels and AAP incidence, thereby providing data and theoretical support for the clinical prevention of AAP.

Methods

Study Design

This is a single center, prospective, single-arm interventional study performed in Children's Hospital of Soochow University from China. Children participating in this study will receive 3600 unit vitamin A orally per day for 30 days. The dosage will be adjusted based on the blood level of vitamin A but will not exceed a certain limit(8000U/L). Historical cases are used as controls.

Participants

All patients meet the inclusion criteria at the participating sites will be recruited. We will collect patients consecutively admitted to our center within 2-month period. Informed consent is required for each participant and/or the guardian of the participant in this study, either signed by the patient himself and/or the guardian of the patient. All the data stored in the electronic database are de-identified to guarantee patients privacy.

Inclusion criteria

1. One month < age ≤ 18 years.

2.Diagnosis with acute lymphoblastic leukemia, which was confirmed by cell Morphology, Immunology, Cytogenetics and Molecular biology.

Exclusion criteria

1. Previous history of pancreatitis or other conditions that may lead to pancreatitis (such

as hyperlipidemia, hypercholesterolemia, cholelithiasis).

2. Treatment course delays of more than two weeks due to financial issues.

3. Exclusion of cases where acute pancreatitis occurs without the use of PEG-asparaginase (PEG-ASP).

4. The patient switching from PEG-ASP to L-ASP or Erwinia-ASP treatment due to adverse reactions other than AAP.

5. Expected hospital stay of less than 2 days.

6. Use of vitamin A supplements prior to enrollment within 1 month.

Withdrawal of participants

Participants will be withdrawn from the clinical research study in the following circumstances:

1. In cases of withdrawal of informed consent, where capacity exists.

2. In cases of withdrawal of consent by the participant's guardian for children with incapacity.

Participants may withdraw from the trial at any time during the trial. Withdrawal from the trial will not result in fines, discrimination, or retaliation, nor will it affect our future medical treatment and rights.

Data collection and management

An electrical database will be used for data collection and storage. All data will be de-identified with unique ID and input by the primary investigator or nominated investigators (less than two for each participating center) approved by the primary investigator, and a double check will be done by the research coordinator. Training for data entry will be performed by the provider of the electrical database. According to the schedule shown in Table 1, the investigator will collect data during the 2 months after enrollment or before discharge, which ever happen first, and follow up the patients at day 60 after enrollment for key clinical outcomes like AAP incidence, the length of hospital stay and 28-d mortality.

The principal investigator's center will be responsible for data safety, privacy and quality. The data will be monitored regularly for data quality control by a data management team at the principal investigator's center.

Outcome measurements

Primary outcome measure:

The incidence of asparaginase-associated pancreatitis within 60 days after enrollment measured using patient records.

Secondary outcome measures:

1. Incidence of elevated blood amylase within 30 days of enrollment: blood amylase levels are routinely monitored twice weekly for 30 days after the use of asparaginase. Any value above the normal range at any monitoring point is defined as elevated.
2. Length of Hospital Stay: the duration from enrollment to discharge measured using patient records.
3. 28-Day Mortality after enrollment measured using patient records
4. The vitamin A levels monitoring: the plasma vitamin A levels is monitored at enrollment, and 1, 2, 3, 4 and 6 weeks after enrollment.
5. Duration of Enteral Nutrition Interruption within 60 days of enrollment measured using patient records.
6. Prothrombin time, International Normalized Ratio, Activated partial thromboplastin time, fibrinogen, AT-III, albumin levels within 30 days after enrollment.
7. Cumulative use of plasma within 30 days after enrollment.

Planned Statistical Analysis

SAS statistical software is used for all statistical processing. The normality of continuous variables will be assessed by inspecting Q-Q plots. If the data follows a normal distribution, the results will be presented as mean ± standard deviation (SD). If the data does not follow a normal distribution, results will be expressed as median (interquartile range, IQR). Categorical variables will be reported as counts and percentages.

Due to the historical control involved in this study, considering that there is no

guarantee that the experimental group and the historical control group will be comparable, and some important clinical indicators of the two groups may be unbalanced between the two groups, such as the severity of disease or age distribution, we will use a multivariate logistic regression model to calculate the propensity score of each patient and match them. In addition, inverse probability weighting based on propensity scores will also be used for sensitivity analysis.

For the analysis of the primary indicator, we used the paired chi-square test (McNemar's test). For secondary outcomes, the paired t-test or signed-rank sum test will be used to test the between-group differences of continuous variables according to whether they were normally distributed, respectively, and the paired chi-square test was used for the categorical variables. For time-event variables, K-M survival curves were plotted, the Log-rank test was used to determine whether there was a difference between the two groups, and the Hazard ratio (HR) and 95% confidence interval were determined using a Cox proportional hazards regression model based on paired stratification.

For the two groups of cohorts weighted by inverse probability, we used the person chi-square test for the analysis of the main observation indicators. For secondary outcomes, continuous variables were compared using a t-test or rank-sum test according to whether they obeyed a normal distribution, respectively, and categorical variables were compared using a person's chi-square test.

The hypothesis test uses the two-sided test, and the test statistics and their corresponding P values are given, and the P values are directly given when the Fisher exact probability method is used, and the $P \leq 0.05$ is considered to be statistically significant

Comparability analysis of baseline metrics was based on the full analysis set (FAS) of intention-to-treat analysis. In addition to the full analysis set (FAS), the main efficacy indicators will also be analyzed according to the per-protocol set (PPS). Secondary efficacy measures were analyzed only in PPS set. Data management and statistical analysis are undertaken by a third-party organization with statistical qualifications.

Ethics approval and dissemination

This study was approved by the ethics committee of Children's Hospital of Soochow University. The ethical approval document ID is 2024016.

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Table 1. Schedule of enrollment, assessment and follow up.

	Study period			
	Enrollment	**Interventional period**		**Follow up**
Time point		Day0[a]	Day1-Day30	Day60
Enrollment:				
Eligibility screen	X			
Informed consent	X			
Demographic information	X			
Assessment:				
Blood amylase		X	←——→	
Vitamin A levels		X	←——→	

Interruption time for enteral nutrition		X	⟷	
Laboratory test		X	⟷	
Plasma support		X	⟷	
Follow up:				
28-d mortality			⟷	X
Hospital stays			⟷	X
The occurrence of AAP			⟷	X

a: Day0 is defined as the day from enrollment to 8 am the next day.