Original Article

The metabolic effects of multi-trace elements on parenteral nutrition for critically ill pediatric patients: a randomized controlled trial and metabolomic research

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Background: We investigated the efficacy and metabolic dose-effect of multi-trace element injection I [MTEI-(I)] for severe pediatric patients via a parallel, randomized control study.

Methods: The inclusion criteria were as follows: (I) patients who required parenteral nutrition (PN) due to various diseases, and were expected to receive PN for >5 days; (II) patients aged <18 years; (III) patients with no serious cardiac, hepatic, renal, or pulmonary dysfunction; and (IV) patients with an established central venous pathway. Enrolled patients were randomly assigned into two groups using sequentially numbered, sealed, opaque envelopes: Group A (low-dose group) received MTEI-(I) at 1 mL/kg/d, and Group B (high-dose group) received MTEI-(I) at 2 mL/kg/d, up to a maximum dose of 15 mL/d. The concentrations of manganese (Mn), copper (Cu), zinc (Zn), and selenium (Se) were detected. The following indexes were measured after 5 days of treatment (T5): β-oxidation of very-long-chain fatty acids, arginine and proline metabolism, pentose phosphate metabolism, ketone body metabolism, citric acid cycle, purine metabolism, caffeine metabolism, and pyruvate metabolism. The participants, care givers, and data analysis staff were blinded to the group assignment.

Results: Overall, at T5, Mn and Cu levels were decreased, while Zn and Se levels were increased. The increase of Zn levels (A: 0.170±0.479 *vs.* B: 0.193±0.900) and decrease of Cu levels (A: −0.240±0.382 *vs.* B: −0.373±0.465) of patients in Group B (n=22) were significantly higher than those in Group A (n=18). At T5, the β-oxidation of very-long-chain fatty acids, arginine and proline metabolism, pentose phosphate metabolism, ketone body metabolism, citric acid cycle, purine metabolism, caffeine metabolism, and pyruvate metabolism were variably decreased (P<0.05) in Group B compared to Group A.

Conclusions: Our results suggested that the high-dose administration of MTEI-(I) is safe for severe pediatric patients, and may alleviate inflammation and antioxidation, relieve hyperactivity caused by stress, and improve tissues-based hypoxia and renal function.

Trial Registration: Chinese Clinical Trial Registry ChiCTR2100052198.

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Keywords: Multi-trace element injection I [MTEI-(I)]; pediatric; metabolomics; parenteral nutrition (PN); intensive care

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Introduction

Optimal nutritional support is considered to be important for critically ill pediatric patients in the intensive care unit (ICU), since malnutrition and inadequate nutrient supply are related to worse clinical outcomes (1). To improve nutritional status and cure rates of associated conditions, clinical guidelines (2) have recommended that total or supplementary parenteral nutrition (PN) should be administered to provide calories, amino acids, and other nutrients in situations where there is unavailable or insufficient enteral nutrition (EN).

Patients receiving PN should also receive trace elements (TEs), since failure to receive adequate TEs may lead to clinical symptoms of deficiencies (3). Low circular TEs are associated with the application of continuous renal replacement therapy, systemic inflammatory response, and cardiac surgery (4). Even subclinical TE deficiencies can be theoretically detrimental to a patient's recovery (5). However, there are few randomized clinical trials investigating the effects of nutritional supply on the clinical outcomes of critically ill pediatric patients (4). Well-designed clinical studies are urgently needed to further investigate the pediatric micronutrient requirements among pediatric critically ill patients.

Metabolomics is an emerging high-throughput research method that reflects body health or disease status (6). It provides an important area of science, namely, that exploring the effects of biometals in biological systems, with extremely high sensitivity (7). Moreover, since numerous metals are linked to biomolecules (proteins or metabolites), techniques for separation, such as liquid chromatography and capillary electrophoresis, are required in metabolomics studies (7). Using these technologies, we explored the TE deficiencies in ICU pediatric patients, and analyzed the efficacy and safety of different multi-trace element injection [MTEI-(I)] doses on the nutritional metabolism of these patients. We present the following article in accordance with the CONSORT reporting checklist (available at https://dx.doi.org/10.21037/tp-21-456).

Methods

Patients

In total, 40 critical ill patients requiring PN who were hospitalized at the Chengdu Women's and Children's Central Hospital from November 2017 to March 2018 were enrolled. The research protocol was approved by the ethics committee of Chengdu Women's and Children's Central Hospital (No. 2017 [21]). The study was conducted according to the guidelines of the Declaration of Helsinki (as revised in 2013).

Inclusion criteria: (I) patients requiring PN due to gastrointestinal failure, congenital malformation surgery of the digestive tract, other congenital malformation surgeries, gastrointestinal bleeding, and were expected to receive PN for >5 days; (II) patients aged <18 years; (III) patients with no serious cardiac, hepatic (hepatic function index exceeding twice the normal upper limit), renal [below chronic kidney disease (CKD) IV stage], or pulmonary dysfunction; (IV) patients where the central venous pathway (subclavian, internal jugular, femoral vein, or peripherally inserted central catheter) was established.

Exclusion criteria: (I) patients with allergies to PN components; (II) patients with diabetes; (IIII) patients with allergies or adverse reactions to known TEs; (IV) patients with obvious trace element deficiencies [iron (Fe), zinc (Zn), copper (Cu), etc.]; (V) patients with congenital metabolic abnormalities; (VI) patients with Fe deficient anemia.

General information, such as gender, weight, main diagnosis, vital signs, length of hospital stay, and hospitalization expenses were collected. At the same time, we also collected routine blood and biochemical data, as well as blood samples from the patients before PN, and after 1, 3, and 5 days of treatment, for TE and metabolomics studies.

Study design

This is a prospective, paralleled, randomized controlled trial. Patients were randomly assigned to low-dose MTEI-(I) group or high-dose MTEI-(I) group (1:1 ratio), using a

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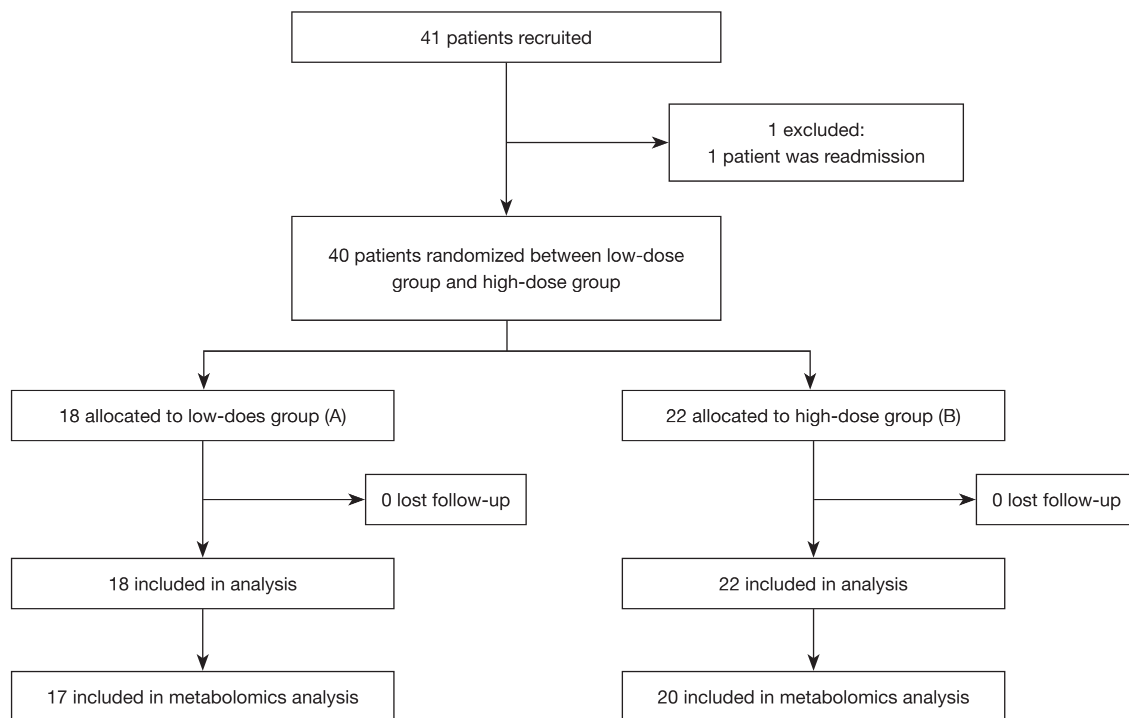

Figure 1 Patient recruitment into groups.

computerized random number generator and sequentially numbered, sealed, opaque envelopes. MTEI-(I) contains Zn (249 µg/mL), Cu (20.1 µg/mL), Mn (1 µg/mL), Se (2 µg/mL), fluorine (F, 57 µg/mL) and iodine (I, 1 µg/mL). The random allocation sequence, participant inclusion, and intervention assignation were carried out by different staff. As for the sample size, all patients meeting the inclusion criteria in our hospital were included. Patients assigned to Group A were administrated MTEI-(I) at 1 mL/kg/d, while those assigned to Group B were administrated MTEI-(I) at 2 mL/kg/d, up to a maximum dose of 15 mL/d. The dosage standards met the requirements of the guidelines (8,9) and did not exceed the maximum dose specified in the drug label. The two interventions were similar in appearance and color. Patients in both groups received basic standard of care treatment according to their clinical needs, and were given PN support for at least 5 consecutive days (as shown in *Figure 1*). The following indexes were measured after 5 days of treatment: β-oxidation of very-long-chain fatty acids, arginine and proline metabolism, pentose phosphate metabolism, ketone body metabolism, citric acid cycle, purine metabolism, caffeine metabolism, and pyruvate metabolism. Metabolic changes were the primary endpoints. The concentrations of Mn, Cu, Zn, and Se were detected and as the secondary. The participants, care givers, and data analysis staff were blinded to the group assignment. All patients or statutory guardians provided informed consent.

Sample collection

After enrolment, bloods were drawn prior to PN treatment, and after 1, 3, and 5 days of PN treatment. Approximately 2×2 mL samples were taken each time, with one sample taken in a non-anticoagulant blood tube for trace element detection, and the other into an anticoagulant tube for proton nuclear magnetic resonance (^{1}H-NMR) analysis. Samples were centrifuged at 4,000 rpm for 10 min, and the upper plasma layer was subsequently removed and stored at −80 ℃.

Analytical method of TE detection

Samples were sent to the Beijing Qingxi Technology Research Institute for TE detection.

Methodology

Instruments and reagents

Inductively Coupled Plasma-Mass Spectrometry (ICP-

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MS), NexION 300 (PerkinElmer, USA); direct injection system (PerkinElmer); multi-element mixed standard solution: 10 mg/L, CAS#: Nitric acid (HNO_3) (7697-37-2) (PerkinElmer). Nitric acid: Suprapu (Merck KGaA, Germany). Oxygen: 99.999%. Water: ultrapure water obtained after double treatment by UPR pure water system.

Instrument conditions

Power of RF generator: 1,600 W; plasma gas flow rate: 18 L/min; auxiliary gas flow rate (Ar): 1.2 L/min; carrier gas flow (Ar): 0.96 L/min; chamber vacuum: 1×10^{-6}; analog voltage: –1,700 V; pulse voltage: 850 V; measurement method: quantitative analysis; organic sampling system conditions: oxygen flow rate of 8 mL/min; temperature of 4 ℃.

Sample pretreatment and preparation of standard solution

Pre-treatment: 0.25 mL serum sample, added with 0.8 mL of 65% HNO_3 and 0.2 mL of 29–32% H_2O_2, heated and digested on a heating plate at 90 ℃ for 3 h, then diluted to 10 mL with deionized water.

Preparation of standard solution: the 10 mg/L standard mixture solution diluted to 1 mg/L for future use.

Calibration curve

The multi-element mixed standard solution was accurately pipetted, the standard curve solution was prepared, and the final concentrations were 1, 5, 10, 20, 50, and 100 μg/L. The prepared standard solution was tested using the organic injection system to obtain the standard curve.

In the experiment, parallel samples and spike recovery were used as the means of sample quality control to ensure the accuracy of the results.

Metabolomics

Preparation of plasma samples

After thawing at room temperature, plasma samples were centrifuged at 16,000 rpm for 10 min. Next, 50 μL of deuterated heavy water (D_2O) was added to a nuclear magnetic resonance (NMR) tube, to which 450 μL of plasma was added. The sample was shaken for 2 min and incubated at room temperature for 10 min until ^{1}H-NMR (600 MHz) analysis.

^{1}H-NMR data collection

A one-dimensional hydrogen spectrum was obtained after sample processing. In this study, ^{1}H-NMR analysis was performed using a Bruker Avance DR ×600 MHz model (BRUKER, Germany), with a working frequency of 600.13 MHz, equipped with a Bruker inverse broad band probe (rIBB). The addition of 10% D_2O inhibited the solvent peak during sample preparation, and the pulse sequence (zgp) was used to inhibit the water peak during pre-saturation. All spectra were collected at room temperature [i.e., 300 Kelvin (K)], with a spectrum width of 20 ppm, sampling points of 32 K, and a cumulative frequency of 256 times.

^{1}H-NMR spectrum processing

In plasma samples, the molecular nucleus of a compound resonates in a high magnetic field, and its frequency is gradually decreased. The original decay signal of the tested sample referred to our raw data (free induction decay; FID). The FID signal was imported into MestReNova software (MestreLab Research, Spain) for Fourier transformation, to generate one-dimensional ^{1}H-NMR spectra. These were processed for chemical shift and automatic baseline adjustment. The convolution technique was used to minimize changes in peaks, and to ensure that larger peaks did not cover up smaller ones. Subsequently, for all plasma samples, 0–9 parts per million (ppm) segments of one-dimensional hydrogen spectra were divided into 0.04 ppm sections, and 223 chemical shift value segments were integrated to finally obtain corresponding integral values. The two-dimensional matrix was then exported in CSV format for analysis.

Data preprocessing

All data matrices were preprocessed - line normalization and standardization. Due to differences in the plasma sample dilution, concentration, test temperature, instrument working stability, and other factors during processing and measurement, ^{1}H-NMR spectra from the same types of plasma samples in different batches were not completely consistent. Therefore, line normalization of data matrices was required (it was assumed that the highest peak in each ^{1}H-NMR spectrum referred to the same substance with very similar content). The line normalization formula was represented as:

$$X^* = \frac{X - \min}{\max - \min} \quad [1]$$

Spectrum data analysis

We used the supervised pattern recognition method, Partial Least Squares-Discriminant Analysis (PLS-DA) to perform data dimension reduction. The variable importance in the projection (VIP) of the PLS-DA model, with corresponding chemical shifts, was calculated. Chemical shifts with VIP values >1 and P<0.05 were selected, and corresponding

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metabolites were investigated using the human metabolome database (HMDB, https://hmdb.ca/).

Statistical analysis

All clinical data were statistically analyzed using SPSS Version 21.0 software (International Business Machines Corporation, the United States of America), and described by median (interquartile range) or mean values ± standard deviation (SD) according to distribution type. Measurement data were first tested for data distribution type. Student's *t*-test was used for normally distributed data, and the rank-sum test was used for non-normally distributed data. The chi-square test was used for enumeration data. The statistical significance level was set at $P<0.05$. The mean substitution method was used for missing clinical data.

Results

Patients' status

In total, 40 patients (ranging from 29 days to 10 years old) were enrolled into the study from November 2017 to March 2018, including 18 patients in Group A and 22 patients in Group B (as shown in *Figure 1*). The principal diagnoses were as follows: (I) ten patients had received fistulation surgery; (II) six patients had acute upper gastrointestinal bleeding; (III) four patients had congenital mega-colon; (IV) three patients had acute gangrenous appendicitis with perforation; (V) two patients had congenital hypertrophic pyloric stenosis; (VI) two patients had adhesive intestinal obstruction; and (VII) two patients had acute intussusception. The remaining diagnoses included the following: one patient each with Merkel diverticulitis with bleeding, necrotizing enterocolitis, acute descending colon perforation, small intestine torsion, portal hypertension syndrome, acute severe myocarditis, toxic intestinal paralysis, autotransplantation after splenectomy, congenital anal atresia and traumatic splenic rupture, and oesophageal atresia surgery.

The patients' information at admission is shown in *Table 1*. No significant differences were observed between the groups in terms of gender, weight, pediatric critical illness score, vital signs, length of hospital stay, and hospitalization expenses. Similarly, we observed no significant differences in the routine blood and biochemical tests, except hemoglobin levels.

Economic aspects were also considered in this study. After comparing the length of hospital stay and hospitalization expenses between the groups, we observed that the high-dose administration of MTEI-(I) did not significantly prolong these factors for patients, suggesting minimal impact and burden on patients and their families.

Changes in the general condition of patients before and after treatment

According to TE detection results, there were no significant differences observed in patients after 1, 3, and 5 days of treatment in each group, and therefore, we focused on and analyzed the pre-treatment data (T0) as well as that after 5 days of treatment (T5). The general condition, routine bloods, and biochemistry of both groups after 5 days of treatment are shown in *Table 2*; no significant differences were observed between the two groups. Routine blood and biochemistry data before and after treatment were compared between the groups (*Table 3*). After treatment, the white blood cells (WBC), neutrophil (N), creatinine (Cr), total bilirubin (TB), direct bilirubin (DB), and albumin (ALB) decreased in both groups, of which WBC and Cr in Group B were significantly lower after 5 days of treatment.

TE data in patients before and after treatment

The patients' TE data before and after treatment are shown in *Table 4*. After 5 days of treatment, Mn and Cu decreased to different extents, whereas Zn and Se increased in both groups. For both Zn and Cu, we observed significant differences compared to the pre-treatment levels. We also compared TE data in both groups before and after treatment (*Table 5*). After treatment, Mn and Cu decreased in both two groups, while the levels of Zn and Se increased. Compared to Group A, Zn levels in Group B increased significantly, and Cu in Group B decreased markedly.

Differences in patient metabolomics before and after treatment

Qualified metabonomic samples were obtained from 37 patients in both groups; 17 from Group A and 20 from Group B. We used ^{1}H-NMR metabolic fingerprinting of patient plasma to distinguish patient metabolomics before treatment (T0) and after treatment (T5) (*Figure 2*). The VIP of chemical shift values of the metabolites at T0 and T5 are shown in *Figure 3*, of which those with a VIP ≥1 and $P<0.05$ were taken as characteristic metabolites (*Tables 6,7*). According to *Tables 6,7*, after 5 days of treatment, valine, leucine,

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Table 1 General condition of patients before treatment

Characteristic	Group A (n=18)	Group B (n=22)	P value
Gender, n			0.761
Male	10	11	
Female	8	11	
Height (cm, mean ± SD)	73.50±25.91	86.18±27.81	0.147
Weight (kg, mean ± SD)	9.11±6.48	11.90±7.22	0.211
Pediatric critical illness score (mean ± SD)	95.67±4.13	94.36±5.19	0.393
Body temperature (℃, mean ± SD)	36.81±0.38	36.69±0.48	0.413
Respiratory rate (breaths/min, mean ± SD)	33.56±10.70	29.86±7.36	0.205
Heart rate (beats/min, mean ± SD)	130.05±24.08	123.27±22.71	0.366
Testing indices			
WBC ($\times 10^9$/L, mean ± SD)	11.24±5.04	12.93±6.69	0.380
N (%, mean ± SD)	51.59±23.65	55.64±26.86	0.620
LY (%, mean ± SD)	41.51±22.16	35.05±24.86	0.397
HGB (g/L, mean ± SD)	112.67±25.30	94.55±17.37	0.011
PLT ($\times 10^9$/L, mean ± SD)	401.33±166.70	361.14±179.54	0.472
ALT (IU/L, mean ± SD)	27.02±14.70	32.51±20.72	0.355
Cr (mmol/L, mean ± SD)	23.87±11.50	24.48±10.41	0.860
TB [μmol/L, median (IQR)]	6.65 (7.0)	8.45 (6.9)	0.693
DB (μmol/L, mean ± SD)	3.93±5.09	3.12±2.12	0.495
ALB (g/L, mean ± SD)	42.52±5.17	39.10±7.01	0.093
Received surgery			0.747
Yes	12	13	
No	6	9	
Transferred to ICU			1.000
Yes	18	21	
No	0	1	
Length of hospital stay (days, mean ± SD)	19.28±7.84	21.5±21.17	0.676
Hospitalization expenses [RMB, median (IQR)]	25,739.62 (18,220.26)	27,938.09 (128,896.89)	0.892

SD, standard deviation; IQR, interquartile range; WBC, white blood cell; N, neutrophile granulocyte; LY, lymphocyte; HGB, hemoglobin; PLT, platelets; ALT, alanine aminotransferase; Cr, creatinine; TB, total bilirubin; DB, direct bilirubin; ALB, albumin; ICU, intensive care unit; RMB, renminbi.

isoleucine (α-ketoisovaleric acid), taurine and hypotaurine metabolism (hypotaurine), arginine and proline metabolism (phosphocreatine and glycocyamine), ketone body metabolism (acetoacetic acid and acetone), and other metabolic processes were significantly decreased.

Figure 4 shows PLS-DA analyses based on patient metabolic fingerprint spectra. From these data, we observed metabolic differences between patients in both groups. The VIP metabolite data are shown in *Figure 5*, for which the characteristic metabolites with a VIP >1 and $P<0.05$

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Table 2 General patient data after 5 days of treatment

Characteristic	Group A (n=18)	Group B (n=22)	P value
Body temperature (℃, mean ± SD)	36.6±0.2	36.6±0.3	0.447
Respiratory rate (breaths/min, mean ± SD)	32.59±8.43	29.05±7.22	0.172
Heart rate (beats/min, mean ± SD)	122.18±14.01	111.95±21.39	0.099
Testing index			
WBC ($\times 10^9$/L, mean ± SD)	8.34±3.39	8.70±4.63	0.781
N (%, mean ± SD)	46.35±17.59	54.77±16.43	0.127
LY (%, mean ± SD)	42.08±14.62	33.49±17.35	0.103
HGB (g/L, mean ± SD)	95.98±9.79	100.80±15.93	0.269
PLT ($\times 10^9$/L, mean ± SD)	392.61±94.13	380.50±168.32	0.776
ALT (IU/L, mean ± SD)	34.06±24.73	36.83±21.57	0.707
Cr (mmol/L, mean ± SD)	19.76±5.23	18.10±4.94	0.308
TB (μmol/L, mean ± SD)	7.75±8.18	7.38±3.98	0.852
DB (μmol/L, mean ± SD)	2.53±2.25	2.59±1.45	0.923
ALB (g/L, mean ± SD)	35.86±4.42	36.55±4.89	0.644

SD, standard deviation; WBC, white blood cell; N, neutrophile granulocyte; LY, lymphocyte; HGB, hemoglobin; PLT, platelets; ALT, alanine aminotransferase; Cr, creatinine; TB, total bilirubin; DB, direct bilirubin; ALB, albumin.

Table 3 Routine test data in both groups before and after treatment

Testing index	Group A (n=18)			Group B (n=22)		
	T0	T5	P value	T0	T5	P value
WBC ($\times 10^9$/L, mean ± SD)	11.24±5.04	8.34±3.39	0.085	12.93±6.69	8.70±4.63	0.011
N (%, mean ± SD)	51.59±23.65	46.35±17.59	0.267	55.64±26.86	54.77±16.43	0.873
LY (%, mean ± SD)	41.51±22.16	42.08±14.62	0.901	35.05±24.86	33.49±17.35	0.754
HGB (g/L, mean ± SD)	112.67±25.30	95.98±9.79	0.015	94.55±17.37	100.80±15.93	0.272
PLT ($\times 10^9$/L, mean ± SD)	401.33±166.70	392.61±94.13	0.838	361.14±179.54	380.50±168.32	0.642
ALT (IU/L, mean ± SD)	27.02±14.70	34.06±24.73	0.335	32.51±20.72	36.83±21.57	0.505
Cr (mmol/L, mean ± SD)	23.87±11.50	19.76±5.23	0.056	24.48±10.41	18.10±4.94	0.007
TB (μmol/L, median (IQR))	6.65 (4.15–11.28)	4.48 (3.53–7.96)	0.005	8.45 (4.75–11.68)	6.55 (4.8–9.9)	0.112
DB (μmol/L, mean ± SD)	3.93±5.09	2.53±2.25	0.076	3.12±2.12	2.59±1.45	0.296
ALB (g/L, mean ± SD)	42.52±5.17	35.86±4.42	0.000	39.10±7.01	36.55±4.89	0.106

SD, standard deviation; IQR, interquartile range; WBC, white blood cell; N, neutrophile granulocyte; LY, lymphocyte; HGB, hemoglobin; PLT, platelets; ALT, alanine aminotransferase; Cr, creatinine; TB, total bilirubin; DB, direct bilirubin; ALB, albumin.

are shown in *Table 8*. For Group B, β-oxidation of very-long-chain fatty acids (hexacosanoic acid), arginine and proline metabolism (phosphocreatine), pentose phosphate metabolism (D-ribose), ketone body metabolism (acetone), citric acid cycle (succinic acid), purine metabolism (adenine), caffeine metabolism (dimethylxanthine), and pyruvate metabolism (acetyl phosphate) were all decreased compared to Group A at T5.

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Table 4 Trace element data in 40 patients before and after treatment (mg/L)

Testing index	T0	T5	P value
Mn (mean ± SD)	0.044±0.028	0.035±0.053	0.303
Zn [median (IQR)]	1.028 (1.050)	1.052 (1.101)	0.013
Cu (mean ± SD)	1.700±0.601	1.387±0.549	0.000
Se [median (IQR)]	0.086 (0.039)	0.095 (0.050)	0.089

SD, standard deviation; IQR, interquartile range; Mn, manganese; Zn, zinc; Cu, copper; Se, selenium.

Table 5 Patients data in both groups before and after treatment* (mg/L)

Testing index	Group A (n=18)				Group B (n=22)			
	T0	T5	Difference value	P	T0	T5	Difference value	P
Mn (mean ± SD)	0.046±0.031	0.029±0.042	−0.017±0.036	0.055	0.042±0.026	0.041±0.062	−0.001±0.062	0.930
Zn [median (IQR)]	0.856 (1.292)	0.908 (1.312)	0.170±0.479	0.025	1.027 (0.808)	1.231 (1.017)	0.193±0.900	0.223
Cu (mean ± SD)	1.690±0.631	1.450±0.537	−0.240±0.382	0.016	1.709±0.590	1.336±0.566	−0.373±0.465	0.001
Se (mean ± SD)	0.091±0.036	0.148±0.221	0.057±0.232	0.311	0.083±0.025	0.095±0.029	0.012±0.029	0.065

*, difference = post-treatment value – pre-treatment value. SD, standard deviation; IQR, interquartile range; Mn, manganese; Zn, zinc; Cu, copper; Se, selenium.

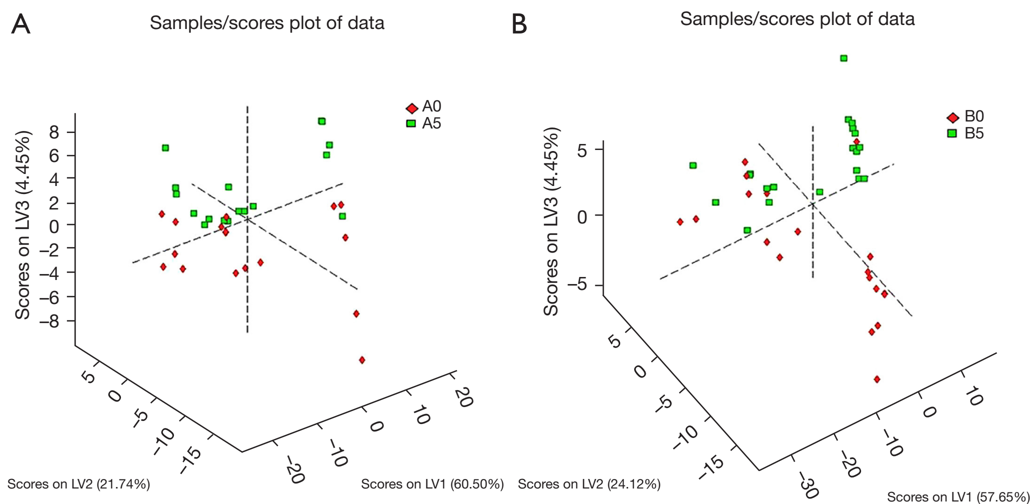

Figure 2 PLS-DA of patients at T0 and T5 (A: 1 mL/kg; B: 2 mL/kg). PLS-DA, partial least squares-discriminant analysis.

Discussion

Critically ill children in pediatric intensive care unit (PICU) often fail to meet caloric target through the preferred enteral route. The result of a systematic review showed (9) that nitrogen balances and inflammation markers, which appeared to be beneficially affected by providing more or altered parenteral nutrition early during critical illness. However, there are few studies on the early use of parenteral nutrition in PICU. Large RCTs with clinically relevant outcome measures are urgently needed to support the current nutritional guidelines that advise the use of parenteral nutrition in the PICU. Besides, nutrition support via the parenteral route has shown to increase the risk of metabolic disturbances such as hyperglycemia and dyslipidemia and

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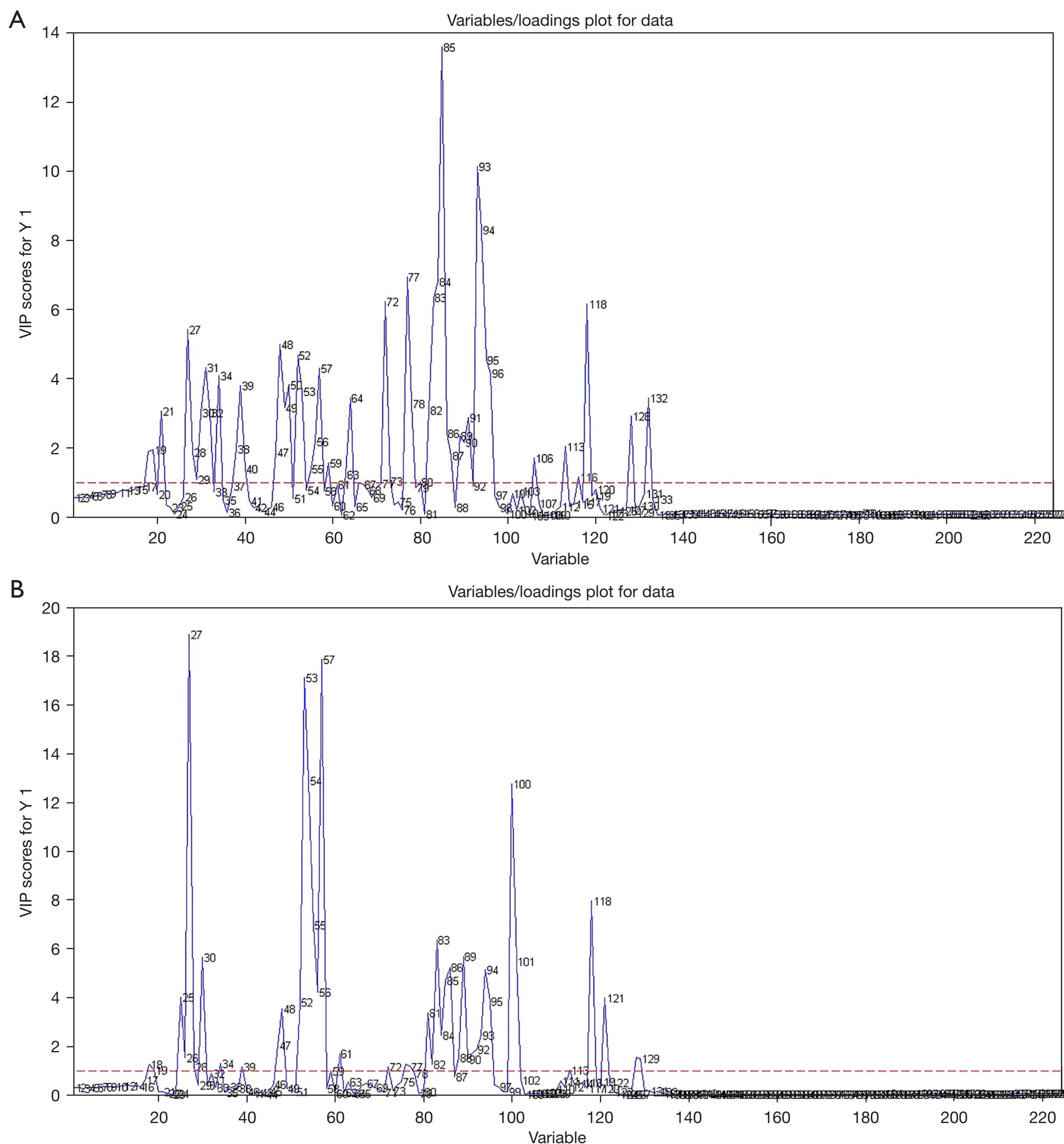

Figure 3 PLS-DA VIP values of chemical shifts between T0 and T5 (A: 1 mL/kg; B: 2 mL/kg). PLS-DA, partial least squares-discriminant analysis; VIP, variable importance in the projection.

to be associated with more nosocomial infections (10). According to Guidelines for pediatric clinical application of enteral and parenteral nutritional support in China (2), the route of parenteral nutrition should be established by qualified medical personnel and catheter-related blood stream infections (CRBSI) should be prevented. In addition, pediatric patients receiving long-term parenteral nutrition should supplement with trace elements. Moreover, body composition, blood and biochemical detection should be monitored regularly.

Although TE levels in human tissue account for <0.01% of total organism mass, these components are vital for human

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Table 6 Small metabolite differences during patient treatment in Group A (P<0.05)

Metabolic pathway	Chemical shift (PPM)	Metabolite	HMDB	Trend
Valine, leucine, and isoleucine degradation	1.12	α-ketoisovaleric acid	HMDB0000019	↓
Taurine and hypotaurine metabolism	3.36	Hypotaurine	HMDB0000965	↓
Arginine and proline metabolism	3.40	Phosphocreatine	HMDB0001511	↓
	3.76	Glycocyamine	HMDB0000128	↓
Ketone body metabolism	3.44	Acetoacetic acid	HMDB0000060	↓
N/A	3.80	Acetyl phosphate	HMDB0031419	↓

PPM, parts per million; HMDB, human metabolome database; N/A, not applicable.

Table 7 Small metabolite differences during patient treatment in Group B (P<0.05)

Metabolic pathway	Chemical shift (PPM)	Metabolite	HMDB	Trend
Valine, leucine and isoleucine degradation	1.12	α-ketoisovaleric acid	HMDB0000019	↓
Ketone body metabolism	2.16	Acetone	HMDB0001659	↓
Pentose phosphate pathway	2.20	D-ribose	HMDB0000283	↓
N/A	2.32	Dimethylglyoxal	HMDB0003407	↓
Caffeine metabolism	4.03	7-dimethylxanthine	HMDB0001860	↓

PPM, parts per million; HMDB, human metabolome database; N/A, not applicable.

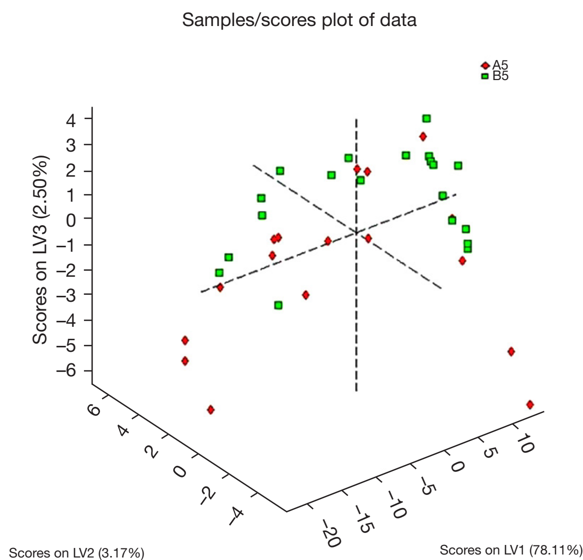

Figure 4 PLS-DA for patients at T5 in Group A and Group B. PLS-DA, partial least squares-discriminant analysis.

growth and development (11). During enteral feeding, patients receive adequate TEs through diversified diets, enteral nutrition products, or oral supplement products. For PN, due to chemical molecule stability, a variety of complex drug products containing multi-TEs are required to meet clinical needs (12). MTEI-(I) is a complex drug product containing multi-TEs specially developed for children. It supplements six trace elements, including Zn, Cu, Mn, Se, F, and I, but not Fe or chromium, to meet guideline requirements for the addition of TEs during PN (9,13). Guidelines for clinical application of neonatal nutrition support in China (8) recommended that Zn should be provided with PN at a dose of 400–500 µg/kg/d in preterm infants, 250 µg/kg/d in infants from term to 3 months, 100 µg/kg per day for infants >3 months of age; Cu should be provided with PN at a dose of 20 µg/kg/day in infants and children; Iodine should be provided with PN at a daily dose of 1µg/kg daily in infants and children; Se should be provided with PN at a dose of 2–3 µg/kg/day in infants and children; Mn should be supplied in long term PN at a dose of no more than 1 µg/kg/day. European guidelines raise dosage standards for preterm infants (9).

Inflammatory mechanisms generated by inflammation and oxidative stress responses from free radical accumulation often cause normal proteins, lipids, and nucleic acids to attack, undermine, and destroy normal physiological functions. TEs are required for the regulation

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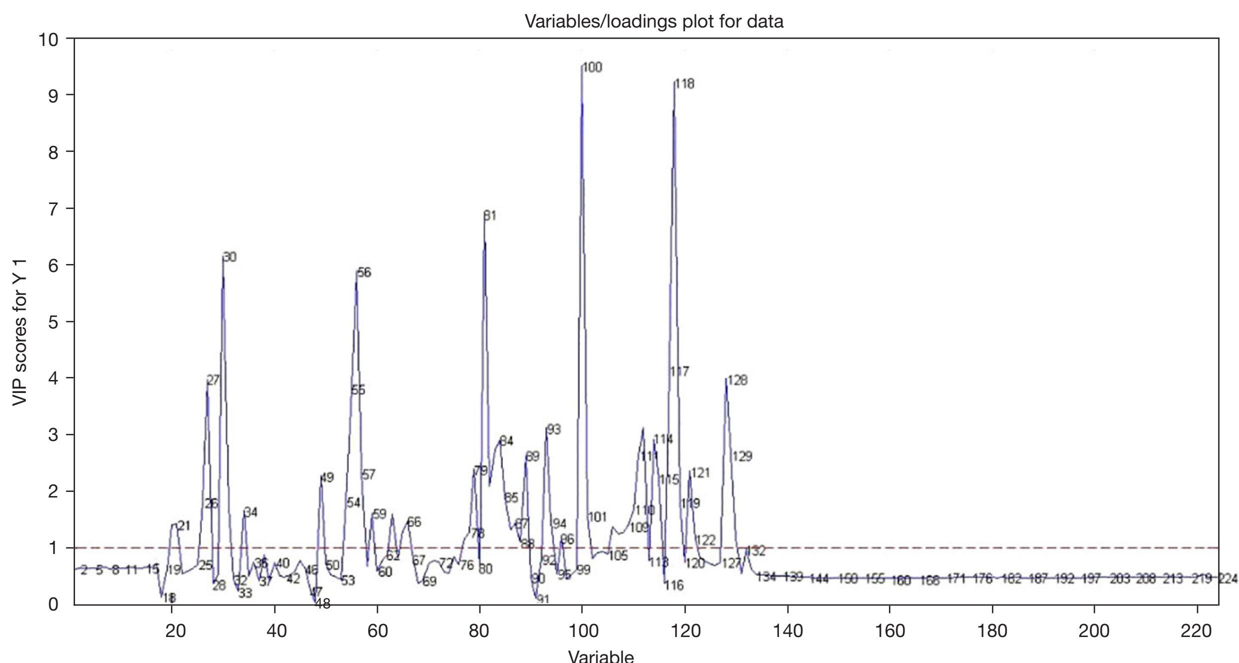

Figure 5 VIP chemical shift values between T5 metabolic differences in Group A and Group B. VIP, variable importance in the projection.

Table 8 Small metabolite differences at T5 treatment in Groups A and B

Metabolic pathway	Chemical shift (PPM)	Metabolite	HMDB	Trend
β-oxidation of very-long-chain fatty acids	0.88	Hexacosanoic acid	HMDB0002356	↓
Arginine and proline metabolism	1.08	Phosphocreatine	HMDB0001511	↓
Pentose phosphate metabolism	2.20	D-ribose	HMDB0000283	↓
Ketone body metabolism	2.24	Acetone	HMDB0001659	↓
N/A	2.32	Dimethylglyoxal	HMDB0003407	↓
Citric acid cycle	2.40	Succinic acid	HMDB0000254	↓
Purine metabolism	3.20	Adenine	HMDB0000034	↓
Caffeine metabolism	4.08	Dimethylxanthine	HMDB0001860	↓
N/A	4.40	Dihydroxy acetone	HMDB0001882	↓
Pyruvate metabolism	4.44	Acetyl phosphate	HMDB0001494	↓

PPM, parts per million; HMDB, human metabolome database; N/A, not applicable.

of substance metabolism, enzyme catalytic activity, etc., and thus affect inflammation and oxidative stress mediators. For example, during oxidative stress and inflammation, TE distribution will be altered, and thus, a reasonable intake of these elements will exert positive effects towards inflammation control, and slow or reduce oxidative stress responses (14). Previous studies have demonstrated that appropriate Cu, Zn, and Se levels reduce free radicals, enhance antioxidant capacity, and regulate inflammatory reactions (15-17). Meanwhile, Cu is positively correlated with bacterial levels and inflammatory markers (18,19), while Zn and Se are negatively correlated with inflammation and oxidative stress (20,21). Supplementation with Zn has been shown to improve high Zn-Cu ratios in the blood,

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reduce oxidative stress, improve inflammatory conditions, and maintain immune functions (22). These previous reports are consistent with our findings, which indicated that Cu was decreased, and Zn and Se were increased after PN treatment, with TE differences in Group B being more significant. Equally, we observed that WBC levels in both groups were decreased after PN treatment, with levels in Group B being significantly decreased after PN treatment ($P=0.011$). This observation suggests that the appropriate high-dose administration of MTEI-(I) was effectively controlling inflammation and antioxidation.

Hexacosanoic acid is a very-long-chain fatty acid, and is an important component of phospholipid molecules. A previous study (23) found that these molecules play important roles in cellular biochemical reactions, nutrient storage, and intercellular communications. Due to homeostatic imbalances between molecular transport and utilization, excessive fatty acid accumulation may cause toxicity in some tissues, which becomes manifested as oxidative stress and inflammation, potentially culminating in cell apoptosis (24). Several studies have reported that very-long-chain fatty acids induce the production of reactive oxygen species in the SK-N-BE neuroblastoma cell line, and enhance oxidative stress (25). Dhaunsi *et al.* (26) observed these molecules activate nicotinamide adenine dinucleotide phosphate oxidase activity, and enhance superoxide anion-mediated lipid peroxidation in skin fibroblasts. In this study, we observed that the β-oxidation of very-long-chain fatty acids (hexacosanoic acid) was significantly reduced in Group B ($P<0.05$), indicating patients were less prone to oxidative damage caused by lipid peroxidation. Therefore, appropriate high-dose administration of MTEI-(I) exerted positive antioxidation effects in this group (27).

Stress has an important impact on various metabolic pathways. Under stress conditions, the following metabolic characteristics are often observed: high metabolic rate, increased catabolism, and reduced anabolic metabolism, resulting in a negative balance in overall metabolism. In this study, 37 children were under acute stress after surgery or disease. Chen *et al.* (28) observed that stress induces the loss of Zn from the body, and Zn supplementation exerts protective effects. In this study, after supplementing MTEI-(I), we observed that valine, leucine, isoleucine degradation, taurine and hypotaurine metabolism, arginine and proline metabolism, and other amino acid metabolism were all reduced, suggesting a benefit to disease recovery. At the same time, ketone metabolism was also reduced, suggesting that the high metabolic rate had been relieved. Of these components, β-oxidation of very-long-chain fatty acids, pentose phosphate metabolism, ketone body metabolism, citric acid cycle, and pyruvate metabolism were all significantly reduced in Group B. These factors were related to energy metabolism (29,30), indicating that appropriate high-dose administration of MTEI-(I) was helpful in relieving stress-induced elevated metabolism.

Hypoxia is a basic pathological process that is implicated in several diseases (31). Severe hypoxia induces considerable cellular harm, and often leads to death. Kim *et al.* (32) observed that Zn ameliorates hypoxic neuronal death induced by deferoxamine (DFX) and sodium azide (NaN_3). Yu *et al.* (33) reported that Zn chelating agents had protective effects towards hypoxic ischemic brain damage in zebrafish. Xie *et al.* (34) proposed that exogenous Zn had protective effects towards hypoxic neurons. Hypoxanthine is a naturally occurring purine derivative, and is the major catabolite of adenosine triphosphate (ATP) in hypoxic or ischemic tissue (35). In general terms, a large increase in hypoxanthine levels in bodily fluids indicates adenosine triphosphate (ATP) depletion (36). In a trial of patients with critical illness, burns, and burn-induced sepsis (36), the evidence suggested that elevated ATP-associated degradation products i.e., adenosine, inosine, hypoxanthine, and xanthine were associated with tissue hypo-perfusion and hypoxia levels. Therefore, it was suggested that purine metabolites, such as xanthine and hypoxanthine, are potential markers of tissue hypoxia (37). In our study, the administration of MTEI-(I) in Group B significantly increased plasma Zn levels. In our metabolomics study, we observed that purine metabolism in Group B was significantly reduced, and related metabolites were similarly reduced, indicating that appropriate high-dose administration of MTEI-(I) improved hypoxic conditions in these patients.

There were several limitations in this study that should be noted. Firstly, the sample size was relatively small, especially the disease spectrum is relatively single. It is difficult to reveal the effect of parenteral nutrition on serum free trace elements in patients with different diseases in this study. More samples with different diseases should be included in future studies. Also, this was a single center study, and thus, multi-center studies are needed to verify our conclusions.

Conclusions

In summary, the high-dose administration of MTEI-(I) is beneficial for pediatric patients. Such administration does

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not increase the burden on visceral organs, and appears to exert protective effects on liver and kidney functions. Several studies have shown that Se protects the kidney from oxidative damage, and reduces oxidative damage to the kidney (38-40). The high-dose administration of MTEI-(I) also reduces serum glutamic pyruvic transaminase, total and direct bilirubin, and has been shown to reduce ultra-structure liver cell damage in rats (41). Supplementation with Zn also delays the progression of chronic kidney disease damage and relieves its complications (42,43). In this study, Zn and Se plasma levels were increased by MTEI-(I) administration. Also, liver and kidney functional analyses of our patients indicated that Cr, TB, DB, and ALB levels were decreased after supplementation with MTEI-(I), and Cr was significantly decreased in Group B, suggesting that appropriate high-dose supplementation of MTEI-(I) was beneficial in improving renal function. Finally, the economic aspects and duration in hospital were also considered in this study, and administration at a high dose did not increase expenditure and duration dramatically.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/tp-21-456). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted according to the guidelines of the Declaration of Helsinki (as revised in 2013) and approved by the ethics committee of Chengdu Women's and Children's Central Hospital (No.2017 [21]). All patients or statutory guardians provided informed consent.

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