Effect of preoperative intervention with folic acid and vitamin B12 on postoperative neurobehavioral changes in children

Clinical trial protocol

Version number: V2.0 **Version time: May 7, 2020**

1、Research background

Delirium (ED) and long-term cognitive dysfunction are common complications after children undergoing surgery under general anesthesia. The prevalence of delirium (ED) in pre-school children is about 38.8% [1]. Childhood delirium is defined as "the disorientation and cognitive changes that occur within a short period of time after the child is anesthetized and awakened, manifested as high responsiveness to stimulation and hyperactivity" [2]. Delirium (ED) during the recovery period usually appears within 30 minutes after anesthesia resuscitation. It is a self-limiting process and subsides in about 5 to 15 minutes [3][4]. Restlessness and delirium during postoperative recovery can lead to wound cracking and hemorrhage, and even cause respiratory tract obstruction or other pulmonary complications. It could delay the children's postoperative healing and prolong the hospital stays [6].

The long-term neurobehavioral changes caused by general anesthesia in children are mainly fine motor injuries. The Mayo Clinic's MASK research find that after multiple anesthesia, infants and young children's processing speed, motor coordination and visual motor integration related scores have significant statistical reductions by accurate factor analysis and cluster analysis statistical methods [5]. The integrity of the myelin sheath is a key structure to ensure the processing speed and motor coordination function of the brain. The clinical phenomenon observed by the MASK research and the basic research of Professor Jiang Hong get the same conclusion. In June 2019, a clinical research on the effects of general anesthesia drugs on the development of myelin sheath in children was published again in the top journal JAMA Oncology [6]. The research found that the greater exposure dose of the general anesthetic drug or longer anesthesia time cause lower integrity of the corpus callosum white matter, which

is related to the neurocognitive impairment. It shows that general anesthetic drugs may affect the white matter connection between the cerebral hemispheres, disrupt the effective communication between neurons, and cause impaired processing speed and attention.

Folic acid, a one-carbon unit transferase coenzyme, participates in the synthesis of purine and thymine, is an important element of the nervous system. Vitamin B12 participates in methyl conversion and folic acid metabolism in vivo, promoting the conversion of 5-methyltetrahydrofolate to tetrahydrofolate [7]. It has been reported that the lack of serum folic acid and B12 is associated with an increased risk of cognitive impairment [8]. Folic acid deficiency leads to impaired central nervous system methylation and results in insufficient methyl synthesis of myelin sheaths, neurotransmitters, membrane phospholipids and deoxyribonucleic acid [9]. Our previous studies showed that preoperative folic acid supplementation can alleviate myelin damage and cognitive impairment in young mice caused by sevoflurane [10]. Therefore, this research further explored the effect of preoperative folic acid and coenzyme B12 supplementation on children's delirium and long-term neurobehavioral changes after general anesthesia.

references：

1. Gomezpesquera E, Povesalvarez R, Martinezrafael B, et al. Cerebral Oxygen Saturation and Negative Postoperative Behavioral Changes in Pediatric Surgery: A Prospective Observational Research[J]. The Journal of Pediatrics, 2019.

2. Sikich N, Lerman J. Development and psychometric evaluation of the pediatric anesthesia emergence delirium scale. Anesthesiology 2004;100:1138–45.

3. Olympio MA. Postanesthetic delirium: historical perspectives. J Clin Anesth 1991;3:60–3.

4. Moore JK, Moore EW, Elliott RA, et al. Propofol and halothane versus sevoflurane in paediatric day-case surgery: induction and recovery characteristics. Br J Anaesth 2003;90:461–6.

5. Zaccariello MJ, Frank RD, Lee M, et al. Patterns of neuropsychological changes after general anaesthesia in young children: secondary analysis of the Mayo Anesthesia Safety in Kids research. British journal of anaesthesia 2019; 122: 671-81

6. Banerjee P, Rossi MG, Anghelescu DL, et al. Association Between Anesthesia Exposure and Neurocognitive and Neuroimaging Outcomes in Long-term Survivors of Childhood Acute Lymphoblastic Leukemia. JAMA Oncol. 2019 Jun 20.

7. Dolatabadi HRD, Reisi P, Alaei H, Malekabadi HA, Pilehvarian AA. Folic acid and coenzyme Q10 ameliorate cognitive dysfunction in the rats with intracerebroventricular injection of streptozotocin. Iran J Basic Med Sci(2012) 15:719.

8. Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, Porcellini E, Licastro F. Homocysteine and folate as risk factors for dementia and Alzheimer disease. Am J Clin Nutr (2005)82:636–643.

9. Calvaresi E, Bryan J. B vitamins, cognition, and aging: a review. J Gerontol B Psychol Sci Soc Sci (2001) 56:P327–PP39.

10. Zhang L, Xue Z. Disrupted folate metabolism with anesthesia leads to myelination deficits mediated by epigenetic regulation of ERMN.EBioMedicine. 2019 May;43:473-486.

2、Research Purpose

Comparison of preoperative folic acid, VitB12 and placebo intervention on postoperative delirium and long-term neurobehavioral changes in children under general anesthesia.

3、Experimental design

3.1 Experiment type

Randomized, double-blind, placebo-controlled, prospective research

3.2 Randomized grouping：

The block randomization method was used to randomize the participants. The SAS software was used to design the block randomization program. The participants were randomly grouped according to the random digital seeds generated by the software. The block length was 8. The patients were randomly divided into two groups, namely the intervention group (continuously taking folic acid and VitB12 for 3 days before surgery) and placebo group (continuously taking placebo for 3 days before surgery), and completed the preparation of drug blinding and emergency letters. Emergency letters are prepared at the same time as the drug is blinded. Each blind number is set with an

emergency envelope, which contains the case drug number and drug name, so that in case of an emergency, the individual cases are blinded and rescued. After unblinding, the corresponding case is treated as a shedding case. The emergency letter was sent to the researchers along with the drugs that had been blinded. The blind law is supervised by a supervisor who is responsible for coordinating and supervising the entire research work, to ensure the safety of the test participants and the reliability of the results, to distribute emergency letters, keep the blind base, and unblind the end of the test.

3.3 Blind method：

Before the operation, inform the patient and the family of the patient that the randomly assigned drugs may be folic acid and VitB12, or may be a placebo. Due to the pale yellow color after folic acid dissolution, it is planned to dissolve folic acid tablets and VitB12 in 20ml of brown sugar water to ensure the consistency of the drug properties in the intervention group and placebo group, to avoid the risk of blindness. All participants will be randomly divided into intervention group and placebo group. After randomization, the intervention group: patients took folic acid and VitB12 tablets for 3 days before surgery (2 years old children 0.4mg/d + VitB12 1.2μg/d, dissolved in 20ml brown sugar water once a day.). Placebo group: Patients took the same dose of brown sugar water for 3 consecutive days before surgery. The choice of folic acid and VitB12 dosage is based on the children's tolerable upper intake levels (UL). This dosage is widely verified by toxicology experiments and has no side effects and average risk for almost all individuals. The maximum daily intake of nutrients, the use of this dose can avoid the adverse reactions caused by folic acid intervention to the greatest extent. Folic acid, VitB12 and placebo are all in light-shielding bottles, and the researchers distribute the medicine to the children every day. Patients and their families, anesthesiologists, surgeons, research recorders, and evaluators were unaware of the grouping and the composition of the drugs issued.

3.4 Experimental grouping

According to the results of random grouping, the children were randomly divided into intervention group and placebo group.

Intervention group: The patient took 20ml of brown sugar aqueous solution containing folic acid and VitB12 for 3 days before operation (the concentration of folic acid was 0.4mg/d + VitB12 1.2μg/d for 2 year old children, dissolved in 20ml brown sugar water once a day) Postoperatively, PAED scores were performed at the time of awakening, extubation, and every 10min within 30min after extubation, and all children's PAED scores were performed by the same measurer (total score 0-20, score ≥ 10 points is defined as delirium during the recovery period). Long-term neurobehavioral changes were evaluated using the Gesell scale and followed up every six months until the age of three.

Placebo group: The patients in the placebo group took 20 ml of brown sugar aqueous solution with the same concentration as the intervention group 3 days before the operation. Postoperatively, PAED scores were performed at the time of recovery, extubation, and every 10 minutes within 30 minutes after extubation. The PAED scores of all children were measured by the same person. (The total score is 0-20, and the score ≥10 is defined as delirium during the recovery period). Long-term neurobehavioral changes were evaluated using the Gesell scale and followed up every six months until the age of three.

4 **Experiment object:**

4.1 Recruitment and sample size calculation:

According to the literature review, the incidence of delirium in children during recovery is about 38.8% (Reference 1). The sample size was calculated based on the incidence of delirium in children in the placebo group at 38%, assuming a type of error probability α=0.05, test efficacy 1-β=0.8, considering folic acid and vitamin B12 supplementation for 3 days can reduce the incidence of delirium to 23%. A chi-square test is planned to assess whether there is a difference in the incidence of delirium among the groups. Through the calculation of the PASS software, the required sample size is 300; considering the 20% drop-out rate, a total of 360 hospitalized children are planned to be divided into 2 groups, 180 cases in each group.

4.2 Inclusion criteria:

(1) ASA grade is Ⅰ ~ Ⅱ;

(2) Children aged 6 months to 2 years old;

(3) It is planned to undergo head, neck and maxillofacial surgery under general anesthesia, and the anesthesia time is less than 6 hours;

4.3 Exclusion criteria:

(1) Children with a history of respiratory tract infection within 1 week;

(2) Children with congenital malformations such as congenital heart disease;

(3) Children with central nervous system diseases or mental disorders or mental disorders;

(4) Children with long-term use of sedative or analgesic drugs;

(5) Children with severe liver and kidney dysfunction;

(6) Received folic acid and VitB12 supplement treatment or taken related derivatives;

(7) Have taken drugs that affect absorption within the past month, such as sulfonamides, aspirin, etc.;

(8) Those who have participated in other relevant clinical research in the past 3 months;

(9) Children with stunting

4.4 Shedding cases

After signing the informed consent and entering the clinical trial after screening, there were no cases that completed the entire clinical research. If an adverse event occurs, the patient or family member voluntarily requests to quit, open an emergency letter to uncover the blind, etc.

4.5 experiment aborted

(1) Those who are allergic to drugs;

(2) Those who have serious adverse events or complications should not be accepted for the test;

(3) Emergency blinding cases;

(4) Patients or family members voluntarily request to withdraw;

5、Elimination criteria

(1) Those who do not meet the selection criteria after selection;

(2) Violation of the regulations on the blind use of drugs;

(3) Failure to take the medicine at the prescribed dose affects the judgment of drug efficacy;

(4) Incomplete information;

6、experiment termination

(1) Blind leaks;

(2) The ethics committee terminates or suspends the experiment;

(3) The rate of dismantling emergency letters exceeds 20%;

(4) The incidence of complications or adverse events in the intervention group was significantly higher than that in the control group in the mid-term;

7、experiment content

According to the results of randomization, three groups of participants took folic acid and VitB12 or placebo daily three days before the operation. The children were fasted for 6 hours and fasted for 2 hours before surgery. After entering the operating room, they monitored breathing, heart rate (HR), electrocardiogram, blood pressure, mean blood pressure, and oxygen saturation (SpO2). Through the peripheral venous system, 1-2 ml of blood was drawn according to different ages to measure the concentration of IL-6, TNF-a cytokines, folic acid and vitamin B12. Preoperative medication (atropine 0.02mg/kg, intravenous injection; dexamethasone 0.2mg/kg, intravenous injection). After routine induction (intravenous midazolam: 0.1 mg/kg, fentanyl 2 μg/ kg, propofol 3 mg/kg, rocuronium at 0.6 mg/kg), tracheal intubation was performed under oral vision . After intubation, adjust the sevoflurane volatile tank to 2-3 vol%, the oxygen flow rate to 2 L•min-1, the tidal volume VT 8~10ml/kg, the respiratory rate 18~25 times/min, adjust the ventilator parameters Maintain end-tidal carbon dioxide (EtCO2) at 35~45mmHg. Start the anesthesia maintenance process. Intraoperative routine monitoring of heart rate, noninvasive blood pressure, oxygen saturation, and end-expiratory carbon dioxide. All anesthetic drugs were stopped 5 to 10 minutes before the end of the operation. After the operation, 1-2 ml of blood was drawn through the peripheral venous system according to age to measu

re the concentration of IL-6, TNF-a cytokines, folic acid and vitamin B12. Aft er pressing, there is no bleeding and edema. The child was sent to the post-an esthesia care unit (PACU) to continue monitoring and oxygen inhalation. After spontaneous breathing is restored and normal SpO2 and ETCO2 can be maintai ned, the protective reflex is restored, and the tracheal tube is removed after th e secretions are fully aspirated. After reaching the PACU standard, the child is safely returned to the ward.

8、Evaluation index

8.1 Main observation indicators:

PAED scores were performed at the time of awakening, extubation, and every 10min within 30min after extubation (total score 0~20, score ≥10 is defined as delirium in the awakening period). Long-term neurobehavioral changes were assessed using Gesell scale.

8.2 Secondary observation index：

(1) Changes in the induction period of anesthesia, intubation, and the operation center rate (HR) and mean blood pressure (MBP);

(2) Changes in HR and MBP after entering the resuscitation room (T1), 5 minutes before extubation (T2), during extubation (T3), and 2 minutes after extubation (T4);

(3) Extubation time and recovery time;

(4) Ramsay sedation score after recovery, extubation and every 10min within 30min after extubation;

(5) The postoperative pain CHEOPs scores were taken at the time of extubation and every 10 minutes within 30 minutes after extubation (the total score was less than 6 points, there was no pain, and ≥10 points for corresponding analgesia treatment);

(6) The use of narcotic drugs (eg pentazocine, propofol);

(7) Changes in serum IL-6, TNF-α, folic acid and vitamin B12 levels before and after operation. Blood sequencing was used to detect metabolomics before and after surgery.

(8) Other adverse events during the recovery period (eg nausea and vomiting, bronchospasm, respiratory depression, etc.);

(9) Gesell scale score changes every six months before the age of three.

(10) Postoperative child scale score (PHBQ).

(11) PAED scale score.

9、 Adverse event

9.1 Definition of adverse events

Adverse event (Adverse Event, AE) refers to all adverse medical events that occur in clinical trial participants after receiving the test drug, which can be manifested as symptoms, signs, diseases, or abnormal laboratory tests, but may not be inferred from the test drug There is a clear cause and effect relationship.

9.2 Adverse event records

Any adverse events that occur during the test should be recorded in the participant's research medical record and signed by the investigator, including the type, degree, appearance time, duration, treatment measures, and treatment process.

9.3 Adverse event handling

If any adverse event occurs in the clinical trial, regardless of whether there is a causal relationship with the test drug, the researcher should take the necessary measures to give treatment and rescue, and follow up the adverse event until the adverse event is relieved, or the participant's condition is stable , Or return to the baseline value, or the participant lost to follow-up.

If the follow-up cannot be performed for some reason, it must be explained in the research medical record.

In the course of follow-up of adverse events, the disease progresses to cases of serious adverse events, and the investigator should report according to the serious adverse event process after being informed.

9.4 Assessment of adverse events

(1) Grading of the severity of adverse events

The severity of the adverse events during the trial should be graded. As a unified standard, the adverse events will be based on the Common Terminology Standards for Adverse Events (NCI-CTCAE V4.03) developed by the National Cancer Institute of the National Institutes of Health in the US Department of Health and Human Services. The severity of the incident is graded. According to the severity of adverse events, it is

divided into:

Level 1: Mild; asymptomatic or mild symptoms; only clinical or diagnostic findings; no treatment required;

Level 2: Moderate; minimal, local or non-invasive indications for treatment; age-related instrumental daily life activities are limited*;

Level 3: Severe or important medical significance, but not immediately life-threatening; indications for hospitalization or prolonged hospitalization; disability; limited self-reasoning activities of daily life**;

Level 4: Life-threatening and requires emergency treatment;

Level 5: Death.

Daily life activities (ADL)

*Instrumental daily life activities include cooking, buying groceries or clothes, using the phone, managing money, etc.

**Self-rational activities of daily living refer to bathing, dressing and undressing, eating, toileting, and taking medicine, rather than being bedridden.

(1) Evaluation of the association between adverse events and test drugs

A. The causal analysis of the relationship between all adverse events and the test drug is judged according to five levels: positively related, likely related, likely related, possibly unrelated, and positively unrelated, and the first three are regarded as adverse reactions of the drug. The considerations for causal analysis include the following five aspects:

B. Is there a reasonable order of time between the start of the medication and the time when the suspected Adverse Drug Reaction (ADR) appears (medication appears);

C. Whether the suspected ADR complies with the drug's known ADR (in accordance with the literature);

D. Can the suspected ADR be explained by combined medication, previous medication, the patient's clinical situation, or the effects of other therapies (other interpretations);

E. Whether the suspicious ADR disappeared or reduced after drug withdrawal or

reduction (drug withdrawal reaction);

F. Whether the suspicious ADR reappears after re-exposure to the same drug (reuse and reproduce); The investigator should evaluate the possible associations between adverse events and the trial drug and co-administration, refer to the table below.

(2) Judgment table of correlation of adverse events

Considerations	Medication appears	In line with literature	Other explanations	Withdrawal disappears	Reuse reproduction
Definitely related	＋	＋	－	＋	＋
Probably related	＋	＋	－	＋	?
May be relevant	＋	＋	±	±	?
May be irrelevant	＋	－	±	±	?
Definitely irrelevant	－	－	＋	－	－

Remark：“＋”certainty；“－”negative；“±”Difficult to affirm or deny：“？”Unknown；

(3) The outcome of adverse events

The following categories are used to evaluate the progress of adverse events:

A. A. Recovery: The signs and symptoms disappear or the participant recovers; the test value returns to normal or returns to baseline;

B. B. Improvement: the severity is reduced by one grade or more, or the mild symptoms and findings disappear, or the indicators return to the level before the trial treatment;

C. C. Not recovered: symptoms, findings, and various indicators have not improved; according to the data of the last visit during the observation period, the severity has deteriorated relative to when it occurred, causing irreversible abnormalities;

D. D. Recovery but with sequelae: some symptoms and findings are recovered, and some findings are transformed into sequelae;

E. E. Death: There is a direct causal relationship with the adverse event discussed. "There is a direct cause and effect relationship" means that the adverse event in question is the cause of death or that the adverse event is indeed related to death;

F. F. Unknown: During the administration of the test drug or immediately after the

trial protocol, the visit cannot be conducted due to the transfer or discharge of the participant.

9.5 Serious adverse event

9.5.1 Definition of serious adverse events

A serious adverse event (Serious Adverse Event, SAE) refers to an adverse event occurring during the research phase that meets one or more of the following criteria:

A. Cause death;

B. Life-threatening;

C. result in hospitalization or prolonged hospitalization;

D. Permanent or severe disability/disability;

E. Congenital malformations/birth defects.

Some medical events that have not yet resulted in death, life risk, or need for hospitalization should be considered serious when they are deemed to be harmful to the participant or require medical or surgical treatment to avoid the above-mentioned conditions after proper medical judgment. Adverse events.

9.6 Recording and reporting of serious adverse events

The participants collected serious adverse events (SAE) during the last follow-up period after signing the informed consent form.

If a serious adverse event occurs during the trial, regardless of whether it is related to the test drug, the investigator should immediately take first aid measures, fill out the serious adverse event report form, and report to the Anesthesiology Department of the Ninth People's Hospital and the Ethics of the Ninth People's Hospital within 24 hours after being informed Committee.

10、Data management

The entire experiment process strictly implements the clinical trial quality management regulations. All paper CRF records are complete, true, clear, and objective. After entering the test data into the computer, the data is locked and stored.

10.1 Establishment of a case report form (CRF)

The case report form (CRF) is filled in truthfully by the investigator, and each selected case must complete the case report form. The investigator is responsible for

the accuracy and authenticity of the CRF information of each participant. Each completed case report form is checked by the inspector for consistency, and if there is any missing or mis-filled data, the questionnaire is passed online to the researcher who filled out the case report form answer. If you need to modify it, follow the CFR instructions and make changes.

10.2 Data entry and modification

After the consistency review is completed, the data administrator performs data entry and management, and checks the logic of the entered data. The data found in the verification process that are missing, logically contradictory, and uncertain can be passed to the researcher who filled out the case report form in the form of an online question, and the researcher should answer and return as soon as possible. The data administrator revises, confirms, and enters the data based on the researchers' answers. In order to ensure the accuracy of the data, two data entry staff should independently enter and proofread double copies. All online questions, feedback and modifications have left traces.

10.3 Data review, lock, transfer

After the data entry, questioning and modification are completed, the data management personnel, main researchers, statistical analysts, sponsors, and supervisory management personnel jointly participate in the data (blind) review meeting, and perform blind review on the data. Sign a blind review resolution. The data administrator then locks the database. Normally, the locked database or file cannot be changed. After the database is locked, submit it to a statistical analyst for statistical analysis.

11、Statistical analysis

11.1 General principles

Statistical analysis was completed using SAS and SPSS software. All statistical tests use a two-sided test, and a P value of less than or equal to 0.05 will be considered statistically significant.

Statistical description: Quantitative data is described by mean, standard deviation, median, P25, P75, minimum and maximum values, and qualitative data is calculated by frequency percentage for statistical description.

Statistical assumptions: To show that the folic acid + VitB12 intervention group and the placebo group of four groups of participants had a 90% confidence level difference in the rate of delirium during the recovery period, you need to statistically test the following hypotheses:

H_0： $\pi_0=\pi_1$， That is, the two groups of participants had the same incidence of delirium during the recovery period

H_1： $\pi_0\neq\pi_1$ The incidence of delirium during awakening was not equal between the two groups of participants ($\alpha=0.05$, bilateral)

If the above hypothesis test results accept H1, reject H0. Then, you need to compare the groups to determine whether there is a statistical difference between the groups, and whether there is a statistical difference between the groups.

Hypothetical test: Quantitative indicators were compared using analysis of variance test or non-parametric test (Mann-Whitney U test); classification indicators were used chi-square test or Fisher's exact probability method.

11.3 Homogeneity analysis

The demographics and relevant baseline characteristics of the selected cases in each group were compared using appropriate statistical methods to test whether the two groups were balanced and comparable.

11.4 Index evaluation and analysis

Chi-square test or exact probability method was used to test the incidence of delirium in children during the recovery period. For other secondary observation indicators, based on the type of data, use appropriate statistical methods for comparison.

11.5 Security analysis

Safety analysis is given to the safety data set (SS). List the frequency and number of adverse events and adverse reactions in each group, calculate the incidence and corresponding severity of adverse events and adverse reactions in each group, and use chi-square test or Fisher exact probability method for comparison.

11.6 Compliance analysis

According to the data recorded in the drug return form, ≥80% indicates good compliance.