Effect of Vitamin D as Adjuvant Therapy in Preterm Infants With Neonatal Sepsis

Study Protocol and Statistical Analysis Plan

Version 1
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1 June 2025

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1. ADMINISTRATIVE INFORMATION

Protocol: Version 1 dated 1 June 2025
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1.1 Document Version History

Version Date	Version	Author	Signature	Change Description	Reason/Comment
	1.0			Initial release.	Not applicable.

2. STUDY SYNOPSIS

This will be a randomized, multi-centered clinical trial. Participants will include preterm neonates diagnosed with neonatal sepsis who are treated with antibiotics. All eligible patients with parental consent, who cannot tolerate enteral feed will be assigned as Group 1 (antibiotic only). All eligible patients with parental consent, who can tolerate enteral feed enrolled into the trial will be randomized into two treatment groups. Group 2 will receive vitamin D as adjuvant therapy (400 IU) for seven days. Group 3 will receive vitamin D as adjuvant therapy (800 IU) for seven days. Each patient will undergo clinical assessments and blood examination at the beginning and end of each treatment period.

Study assessments will include the sepsis score (Tollner score and sepsis prediction score) and C-reactive protein examination.

2.1. Primary Objective

1. To determine the effect on the modified Tollner sepsis score among groups of preterm neonates with sepsis receiving adjuvant Vitamin D therapy at doses of 400 IU/day and 800 IU/day, compared to those receiving antibiotics alone.

2. To determine the effect on the Sepsis Prediction Score among groups of preterm neonates with sepsis receiving adjuvant Vitamin D therapy at doses of 400 IU/day and 800 IU/day, compared to those receiving antibiotics alone.

2.2. Secondary Objectives

To determine the effect on C-reactive protein (CRP) levels among groups of preterm neonates with sepsis receiving adjuvant Vitamin D therapy at doses of 400 IU/day and 800 IU/day, compared to those receiving antibiotics alone.

2.3. Study Population

2.3.1 Number of Participants

We aim to recruit minimum of fifty-seven neonates (19 neonates of each group) aged between 0 and 28 days old or maximum post menstrual age 42 weeks with sepsis.

2.3.2 Eligibility Criteria

Participants will be assigned to a randomised study treatment only if they meet all inclusion criteria and none of the exclusion criteria.

2.3.3 Inclusion criteria

The inclusion criteria for the case group in this study comprise neonates (aged 0–28 days or a maximum post-menstrual age of 42 weeks) with a gestational age of 28–36 weeks who are admitted to the neonatal high care unit (NHCU) and neonatal intensive care unit (NICU) of Dr. Hasan Sadikin General Hospital, Bandung, and Bandung Kiwari Regional Hospital, and meet the following criteria:

- Patients diagnosed with neonatal sepsis confirmed by blood or cerebrospinal fluid (CSF) culture, OR patients meeting the criteria for suspected sepsis based on a modified Tollner sepsis score of ≥ 5, OR Sepsis Prediction Score ≥ 3.
- Parents or legal guardians are willing to participate in the study and have signed the informed consent form.

2.3.4 Exclusion criteria

Participants meeting any of the following criteria will be excluded from the study:

- Patients with major congenital malformations, including anencephaly, encephalocele, holoprosencephaly, hydrocephalus, meningomyelocele, spina bifida, omphalocele, gastroschisis, or congenital heart disease.
- Patients who were initially able to tolerate enteral feeding but developed a medical condition requiring fasting (NPO/Nil Per Os) during the 7-day monitoring period.
- Patients who initially required fasting (due to a medical condition) but became able to tolerate enteral feeding during the 7-day monitoring period.

2.4. Design and Intervention

This study is an analytical experimental study with a randomized controlled trial (RCT) design. All samples that meet the inclusion and exclusion criteria will be enrolled as study subjects. Subject characteristics data will be obtained from the patient's medical records. This was an open-label trial due to the nature of the nutritional intervention (NPO vs. enteral).

Subjects were subsequently allocated into three groups:

- Group 1: Subjects requiring medical fasting (NPO) due to their clinical condition, who received antibiotics alone.
- Group 2: Subjects able to tolerate enteral nutrition, who received antibiotics plus Vitamin D3 supplementation of 400 IU once daily for 7 days.
- Group 3: Subjects able to tolerate enteral nutrition, who received antibiotics plus Vitamin D3 supplementation of 800 IU once daily for 7 days.

2.5. Randomisation and Blinding

2.5.1 Randomisation Procedures

Randomization was performed using a computer-generated random number sequence and allocated by the author.

2.6. Sample Size

Samples will be drawn from study subjects who meet the inclusion criteria. The determination of the sample size is tailored to the study objectives and the type of data involved. This study utilizes a specific design, namely unpaired (non-paired) categorical-numeric analytical design. Therefore, the determination of the sample size is based on statistical calculation, setting a 95% confidence level and an 80% power of test (power). Thus, the minimum sample size for each group is 19 subjects. Therefore, the total minimum sample size for the three groups is 57 patients.

2.7. Study Procedures

Research Design and Data Source
The research will be conducted using an analytical experimental quantitative design. The data source used in this research is primary data, collected while neonates are hospitalized at Dr. Hasan Sadikin General Hospital in Bandung, Indonesia and Bandung Kiwari Regional Hospital, Bandung, Indonesia.

Study Protocol:

1. **Sampling Stage:** Sampling will be conducted in the NHCU and NICU wards of Dr. Hasan Sadikin General Hospital Bandung and Bandung Kiwari Regional Hospital Bandung.
2. **Informed Consent:** A verbal and written explanation of the entire study (informed consent) will be provided to the parents/guardians of the study subjects.
3. **Enrolment:** The parents/guardians of the study subjects who agree to the study procedures will then sign the consent form.
4. **Screening:** The researcher will conduct interviews to obtain general data, maternal history, and birth history to identify subjects who meet the inclusion and exclusion criteria.
5. **Blood Sample Collection:** Peripheral venous blood samples will be collected from the case and control subjects twice (on Day 0 and Day 7). Each collection will be approximately for the examination of Vitamin D levels (i.e., 25(OH)D), hematology, peripheral blood smear morphology, and CRP.
6. **Intervention:** The subject groups who can tolerate enteral feeding will receive Vitamin D3 supplementation of either 400 IU or 800 IU once daily for 7 days.
7. **Sample Processing:** Blood samples will be sent to the Clinical Pathology Laboratory at Dr. Hasan Sadikin General Hospital Bandung for sample preparation. The 25(OH)D level examination will utilize a stored biological material (SBM), which will be stored at a temperature of -80°C.

3 GENERAL STATISTICAL METHODOLOGY

3.1. Objectives of Analysis Plan

This analysis plan covers the analysis of all objectives.

3.2. Analysis Software

Analysis will be conducted using the Statistical Product and Service Solution (SPSS) for Windows, version 27.0 (IBM Corp., Armonk, NY, USA).

3.3. Definition of Baseline

The baseline is taken as the measures and assessments recorded at day-0.

3.4. Definition of analysis populations

Data from this study will be analysed as a per-protocol analysis. Data from all participants who are randomised will be included in the per-protocol analysis.

3.5. Definitions related to Adverse Events (AEs)

Other than standard adverse effects, the following specific adverse events will be reported:

- Gastrointestinal symptoms: diarrhoea, vomiting, bloating, increased abdominal circumference >2 cm
- Hypercalcemia

3.6. Adjustment for Multiplicity

There are no plans to adjust for multiplicity.

3.7. Interim Analyses

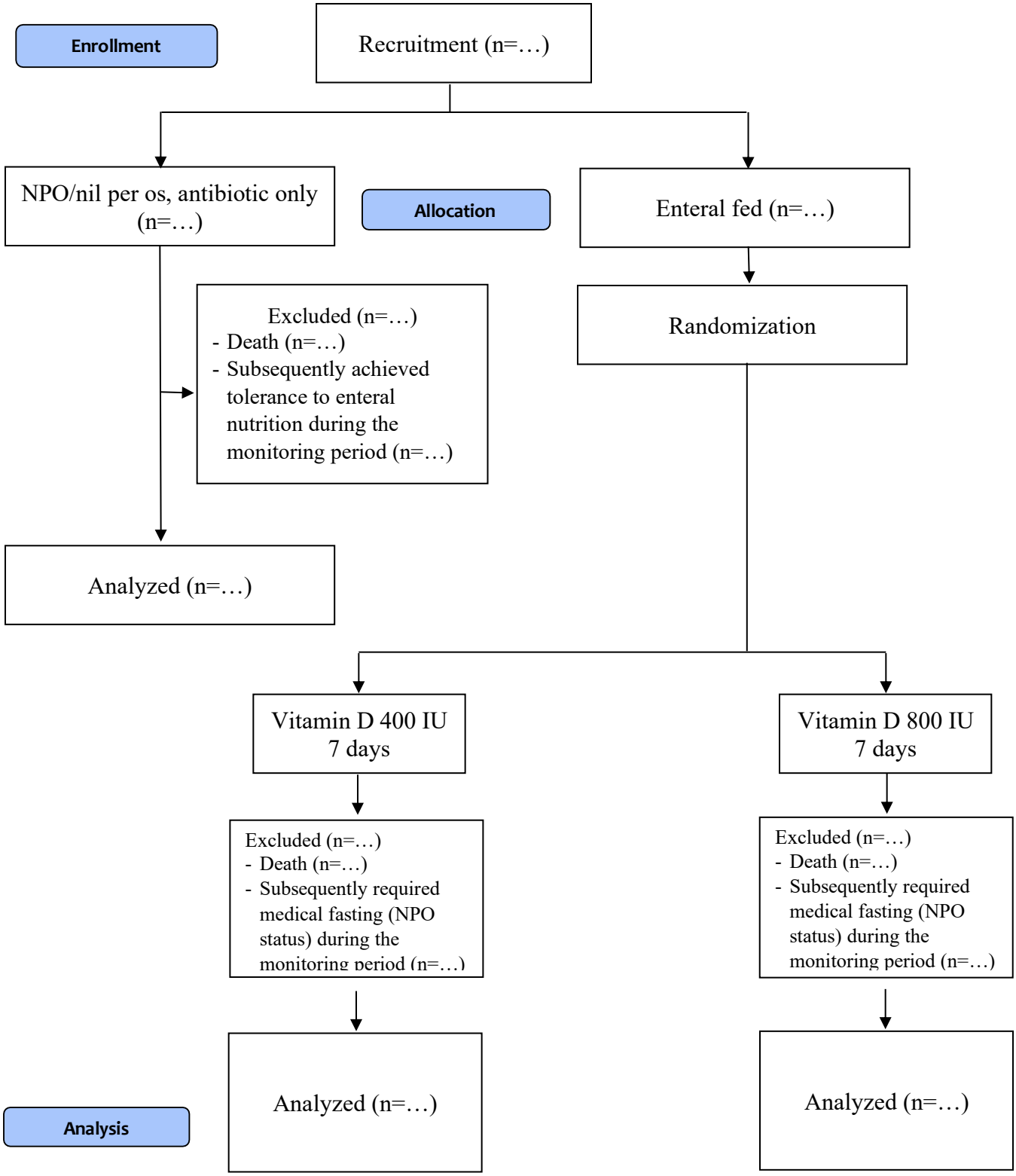
Interim analysis was not performed.

3.8. Handling of Missing Data

It is inevitable that some data will be missing. If less than ten percent of the total data is missing then no change to the analysis will be made.

4 DESCRIPTIVE STATISTICS

4.1. Recruitment and Follow-up



4.2. Baseline Characteristics

Baseline population will be summarised using mean and standard deviations or median and interquartile range.

Variable	Control (n=…)	Vitamin D 400 IU (n=…)	Vitamin D 800 IU (n=…)	p value
Gender, n (%)				
Male				
Female				
Age (days), Mean/Median (SD/IQR)				
Gestational age (weeks), Mean/Median (SD/IQR)				
Gestational age, n (%)				
32–<37 weeks				
28–<32 weeks				
Birth weight (gram), Mean/Median (SD/IQR)				
Birth weight, n (%)				
<1000 gram				
1000–<1500 gram				
1500–<2500 gram				
≥2500 gram				
Respiratory support, n (%)				
Low Flow				
CPAP/NIV				
Ventilator				
Vitamin D (ng/mL), Mean/Median (SD/IQR)				
Classification of vitamin D status				
Sufficient				
Insufficient				
Deficient				

5 REFERENCES

1. Fort P, Salas AA, Nicola T, Craig CM, Carlo WA, Ambalavanan N. A Comparison of 3 Vitamin D Dosing Regimens in Extremely Preterm Infants: A Randomized Controlled Trial. J Pediatr. 2016 Jul;174:132-138.e1. doi: 10.1016/j.jpeds.2016.03.028. Epub 2016 Apr 11.
2. Kamsiah K, Hasibuan BS, Arto KS. The relationship between vitamin D levels and clinical outcomes of neonatal sepsis in Haji Adam Malik Hospital Medan, Indonesia. Open Access Maced J Med Sci. 2021;9(B):698-703.
3. Cetinkaya M, Cekmez F, Buyukkale G, Erener-Ercan T, Demir F, Tunc T, Aydin FN, Aydemir G. Lower vitamin D levels are associated with increased risk of early-onset neonatal sepsis in term infants. J Perinatol. 2015 Jan;35(1):39-45. doi: 10.1038/jp.2014.146. Epub 2014 Aug 7.
4. Hagag AA, El Frargy MS, Houdeeb HA. Therapeutic Value of Vitamin D as an Adjuvant Therapy in Neonates with Sepsis. Infect Disord Drug Targets. 2020;20(4):440-447. doi: 10.2174/1871526519666190626141859.