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Serum Calcium Level as a Prognostic Factor in Full-Term Infants with Neonatal Sepsis

Ika Kurnia Faizin[1*], Ema Alasiry[1,2], A. Dwi Bahagia Febriani[1,2], Hadia Angriani[1,2], Aidah Juliaty A. Baso[1,2], Urfianty[1]

[1]Department of Pediatrics, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[2]Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia
Corresponding Author*: Ika Kurnia Faizin, MD. Department of Pediatrics, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia. Perintis Kemerdekaan Street No. Km 10, Tamalanrea Indah, Tamalanrea District, Makassar, South Sulawesi 90245, Indonesia. Email ID: ikfaizin89@gmail.com.

ABSTRACT: Neonatal sepsis is a major cause of neonatal morbidity and mortality, and early prognostic markers remain needed. This study aimed to evaluate the association between serum calcium levels and clinical outcomes and to determine their potential prognostic relevance in full-term infants with neonatal sepsis. Full-term infants with neonatal sepsis in the intensive care unit of a tertiary hospital were enrolled in prospective cohort study; Sixty neonates were recruited. Serum calcium was measured at sepsis diagnosis by a colorimetric method and categorized as normocalcemia (≥8 mg/dL) or hypocalcemia (<8 mg/dL). They were followed until improvement or death. Calcium concentrations and outcomes were analyzed using Mann–Whitney U and categorical analyses. Normocalcemia was observed more frequently than hypocalcemia. Hypocalcemia was more frequent among infants who died than among those who improved. Median serum calcium was significantly lower in infants who died than in those who improved (8.30 vs. 8.70 mg/dL, p = 0.045). However, the categorical association between hypocalcemia and clinical outcome did not reach statistical significance. Lower serum calcium concentrations were associated with poorer clinical outcomes in full-term infants with neonatal sepsis, although categorical hypocalcemia was not independently associated with mortality. Serum calcium may serve as an adjunctive marker for early risk stratification, but its independent prognostic value requires confirmation in larger studies using serial and ionized calcium measurements.

1. INTRODUCTION

Neonatal sepsis is a life-threatening condition characterized by a dysregulated host response to infection that can result in organ dysfunction and death. It remains a major cause of neonatal morbidity and mortality, with a global incidence estimated at approximately 28.2 cases per 1,000 live births and a case-fatality rate of 17.6%.[1] The burden is disproportionately high in low- and middle-income countries, where limited diagnostic capacity, antimicrobial resistance, and delayed recognition contribute to poor outcomes.[2] Indonesia continues to face a substantial burden of neonatal sepsis, with recent multicenter data demonstrating considerable pathogen diversity and high levels of antimicrobial resistance.[3] Therefore, early identification of neonates at increased risk of mortality remains an important clinical priority, particularly in resource-constrained neonatal intensive care settings.[4]

Sepsis is accompanied not only by systemic inflammation and organ dysfunction but also by profound metabolic disturbances, including abnormalities in calcium homeostasis.[5] Calcium is essential for myocardial and skeletal muscle contraction, neuromuscular transmission, coagulation, and cellular signaling, while its extracellular concentration is tightly regulated through the parathyroid hormone–vitamin D axis and calcium-sensing receptor.[6] Hypocalcemia has been repeatedly observed in

neonates with infection, and studies of early-onset neonatal infection have demonstrated an association between calcium concentrations and inflammatory markers.[7] In sepsis, hypocalcemia may arise from impaired parathyroid function, altered vitamin D metabolism, reduced renal activation of vitamin D, and inflammatory-mediated disruption of calcium homeostasis.[8] More broadly, hypocalcemia in critically ill patients has been associated with greater disease severity and increased mortality, although the clinical significance of calcium supplementation remains uncertain.[9] These findings suggest that serum calcium may represent a readily available biochemical marker for early risk stratification in neonatal sepsis.

Despite growing evidence linking calcium disturbances with adverse outcomes in critical illness, evidence specifically addressing the prognostic value of calcium in neonatal sepsis remains limited and heterogeneous.[10] A previous study in very-low-birth-weight infants suggested that ionized calcium may have prognostic value in neonatal sepsis, but its findings cannot be directly extrapolated to full-term infants because prematurity and low birth weight substantially influence calcium physiology and sepsis outcomes. This distinction is clinically relevant because full-term infants constitute an important subgroup of neonates with sepsis, yet their calcium status as a prognostic marker has not been adequately characterized.[11] In addition, evidence from Indonesia, particularly from tertiary neonatal intensive care settings in Eastern Indonesia, remains scarce. Accordingly, this study aimed to evaluate whether serum calcium levels are associated with clinical outcomes and can serve as a prognostic factor in full-term infants with neonatal sepsis admitted to the neonatal intensive care unit of a tertiary referral hospital.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This prospective cohort study was conducted to evaluate the prognostic value of serum calcium levels for clinical outcomes in full-term infants with neonatal sepsis. The study was conducted in the neonatal intensive care unit (NICU) of a tertiary referral hospital from September 2025 until the required sample size was achieved. Blood samples were analyzed at the hospital's clinical laboratory.

2.2 Study Population and Participants

The study population comprised full-term neonates diagnosed with neonatal sepsis and admitted to the NICU during the study period. Eligible participants were recruited consecutively according to predefined inclusion and exclusion criteria. Full-term neonates were defined as infants born at a gestational age of 37 weeks 0 days to 41 weeks 6 days and aged less than 28 days at enrollment. Neonatal sepsis was diagnosed using the EMA Score criteria. Eligible neonates were required to fulfill at least two clinical criteria and at least two laboratory criteria consistent with neonatal sepsis. Clinical criteria included temperature instability, cardiovascular instability, skin or subcutaneous lesions, respiratory instability, gastrointestinal manifestations, and nonspecific clinical manifestations. Laboratory criteria included abnormal leukocyte count, an elevated immature-to-total neutrophil ratio, thrombocytopenia, elevated inflammatory markers, abnormal blood glucose concentrations, and metabolic acidosis.

2.3 Eligibility Criteria

Infants were eligible if they were full-term neonates aged less than 28 days, fulfilled the predefined criteria for neonatal sepsis, had not received enteral or parenteral nutrition before the initial calcium measurement, and had parental consent for participation. Neonates with major congenital anomalies or severe perinatal asphyxia were excluded.

2.4 Sample Size and Sampling Procedure

The minimum sample size was determined based on a comparison of the expected mortality proportions between infants with hypocalcemia and those with normal calcium concentrations. Assuming a mortality proportion of 70% in the hypocalcemic group and 35% in the normocalcemic group, with a two-sided 95% confidence level and 80% statistical power, the required sample size was estimated at 60 participants. Participants were recruited using consecutive sampling, whereby all eligible neonates admitted during the study period were enrolled sequentially until the target sample size was reached.

2.5 Data Collection and Clinical Assessment

At enrollment, demographic and clinical data were recorded, including age, sex, birth weight, nutritional status, body temperature, heart rate, respiratory rate, level of consciousness, and relevant clinical manifestations. The diagnosis of neonatal

sepsis was established according to the predefined EMA Score criteria. Baseline venous blood was subsequently collected for serum calcium measurement and other laboratory investigations performed as part of routine clinical care.

Participants were prospectively followed throughout their hospitalization. Clinical status was monitored until the occurrence of the predefined clinical outcome. The primary outcome was categorized as clinical improvement or death during hospitalization.

2.6 Serum Calcium Measurement

Serum calcium was measured once at the time of neonatal sepsis diagnosis and before the initiation of enteral or parenteral nutrition. Venous blood was collected using standard hospital laboratory procedures, and total serum calcium was determined using an enzymatic colorimetric method in the hospital laboratory. For prognostic classification, total serum calcium of 8 mg/dL or higher was considered within the normal range, whereas hypocalcemia was defined as a total serum calcium concentration below 8 mg/dL. Hypercalcemia was defined as a total serum calcium concentration above 12 mg/dL (>3 mmol/L). The primary prognostic analysis focused on the comparison between the hypocalcemic and normocalcemic groups.

2.7 EMA Score Assessment

The EMA Score was used to establish the diagnosis of neonatal sepsis based on clinical and laboratory abnormalities. Clinical assessment required at least two qualifying manifestations. Temperature instability was defined by a temperature above 38.5°C or below 36°C and/or unexplained temperature fluctuation. Cardiovascular instability included bradycardia or tachycardia, irregular cardiac rhythm, urine output below 1 mL/kg/h, hypotension, or impaired peripheral perfusion characterized by pallor, mottling, or prolonged capillary refill.

Skin and subcutaneous abnormalities were defined by the presence of petechiae or sclerema. Respiratory instability included apnea, tachypnea, increased oxygen requirements, or increased need for mechanical ventilation. Gastrointestinal manifestations included feeding intolerance, increased gastric residual volume, vomiting, poor tolerance of breast milk, or abdominal distension. Nonspecific manifestations included irritability, lethargy, or hypotonia.

Laboratory abnormalities were assessed using leukocyte count, immature-to-total neutrophil ratio, platelet count, inflammatory markers, blood glucose, and metabolic parameters. An abnormal leukocyte count was defined as below 4,000/mm³ or above 20,000/mm³, while an immature-to-total neutrophil ratio of ≥0.20 was considered abnormal. Thrombocytopenia was defined as a platelet count below 100,000/mm³. An abnormal inflammatory response was defined by a C-reactive protein concentration above 15 mg/L or a procalcitonin concentration of ≥2 ng/mL. Abnormal glucose status was defined as hyperglycemia above 180 mg/dL or hypoglycemia below 45 mg/dL based on at least two measurements. Metabolic acidosis was defined by a base excess below −10 mEq/L or a serum lactate concentration above 2 mmol/L.

2.8 Study Variables and Outcomes

The primary independent variable was serum calcium concentration, categorized according to the predefined calcium thresholds. The dependent variable was clinical outcome, categorized as improvement or death during hospitalization. Potential control variables included major congenital abnormalities and antibiotic therapy. Birth weight was considered a potentially random variable, while inflammatory mediators and neuroendocrine responses, including proinflammatory mediators, corticotropin-releasing hormone, adrenocorticotropic hormone, and glucocorticoids, were considered intermediate factors in the proposed causal pathway between sepsis, calcium homeostasis, and clinical outcome.

2.9 Statistical Analysis

Continuous variables were summarized as means ± standard deviations or medians with ranges, as appropriate, and categorical variables as frequencies and percentages. Normality was assessed using the Kolmogorov–Smirnov or Shapiro–Wilk test. Between-group comparisons used the Student's t test or Mann–Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical variables. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the association between serum calcium status and clinical outcomes. Statistical significance was set at a two-sided $p \leq 0.05$. Multivariable logistic regression with forward stepwise selection was performed to identify independent prognostic factors, with $p < 0.15$ for entry and $p < 0.20$ for removal. Adjusted ORs with 95% CIs were reported.

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3. RESULTS AND DISCUSSION

3.1 Participant Characteristics

A total of 60 full-term neonates diagnosed with neonatal sepsis according to the European Medicines Agency (EMA) criteria were included in the study. The baseline demographic, clinical, and laboratory characteristics of the participants are presented in Table 1. The study population comprised neonates with varying clinical and laboratory manifestations of sepsis, including abnormalities in vital signs and inflammatory parameters.

Table 1. Characteristics of the study subjects

Characteristic	Subcategory	Improved (n=46)	Died (n=14)	Total (n=60)	*p* value
Sex	Male	25 (54.3)	7 (50.0)	32 (53.3)	0.775
	Female	21 (45.7)	7 (50.0)	28 (46.7)	
Gestational age	37–38 weeks	37 (80.4)	8 (57.1)	45 (75.0)	0.092
	39–41 weeks	9 (19.6)	6 (42.9)	15 (25.0)	
Birth weight	Low birth weight (<2.500 g)	13 (28.3)	3 (21.4)	16 (26.7)	0.740
	Normal (≥2.500 g)	33 (71.7)	11 (78.6)	44 (73.3)	

Values are presented as n (%). A p value <0.05 was considered statistically significant.

3.2 Distribution of Serum Calcium Levels

Among the 60 full-term neonates with neonatal sepsis, 46 (76.7%) had serum calcium concentrations within the normal range (normocalcemia), whereas 14 (23.3%) had hypocalcemia, defined as a total serum calcium concentration below 8 mg/dL (Table 2). Thus, approximately one-quarter of the study population presented with hypocalcemia at the time of sepsis diagnosis.

Table 2. Comparison of hypocalcemia and normocalcemia according to clinical outcome in full-term infants with neonatal sepsis

Serum Calcium Status	Died, n (%)	Improved, n (%)	*p* value	OR (95% CI)
Hypocalcemia (<8 mg/dL)	6 (42.9)	8 (57.1)	0.071	3.56
Normocalcemia (≥8 mg/dL)	8 (17.4)	38 (82.6)		(0.97–13.13)
Total	14 (23.3)	46 (76.7)		

Values are presented as n (%). Fisher's exact test was used. OR, odds ratio; CI, confidence interval. $p < 0.05$ was considered statistically significant.

3.3 Comparison of Hypocalcemia and Normocalcemia According to Clinical Outcome

The distribution of serum calcium status according to clinical outcome is presented in Table 2. Hypocalcemia was more frequent among neonates who died than among those who clinically improved, occurring in 6 of 14 neonates (42.9%) and 8 of 46 neonates (17.4%), respectively. In contrast, normocalcemia was more common among neonates who improved, accounting for 38 cases (82.6%), compared with 8 cases (57.1%) among those who died.

3.4 Comparison of Serum Calcium Levels According to Clinical Outcome

As shown in Table 3, the median serum calcium concentration was higher among neonates who clinically improved than among those who died (8.70 vs. 8.30 mg/dL). Because the distribution of serum calcium was non-normal, the Mann–Whitney *U* test was used for between-group comparison. Serum calcium concentrations differed significantly between the two outcome groups ($p = 0.045$).

Table 3. Comparison of serum calcium levels between full-term infants with neonatal sepsis who improved and those who died

Clinical Outcome	n	Median (Min–Max) (mg/dL)	*p* value
Improved	46	8.70 (6.10–12.80)	0.045
Died	14	8.30 (6.50–9.50)	
Total	60		

Values are presented as median (minimum–maximum). Mann–Whitney U test was used for comparison between clinical outcome groups.

3.5 Association Between Serum Calcium Status and Clinical Outcome

The association between serum calcium status and clinical outcome is presented in Table 4. Mortality was more frequent among neonates with lower serum calcium concentrations (<8.3 mg/dL) than among those with higher concentrations (34.8% vs. 16.2%). Conversely, clinical improvement was more frequent among neonates with serum calcium concentrations >8.3 mg/dL than among those with lower concentrations (83.8% vs. 65.2%).

Table 4. Association between serum calcium levels and clinical outcomes in full-term infants with neonatal sepsis

Serum Calcium Level	Improved, n (%)	Died, n (%)	Total, n (%)	*p* value	OR (95% CI)
<8.3 mg/dL	15 (65.2)	8 (34.8)	23 (100.0)	0.098[a]	0.363 (0.107–1.235)
≥8.3 mg/dL	31 (83.8)	6 (16.2)	37 (100.0)		
Total	46 (76.7)	14 (23.3)	60 (100.0)		

Values are presented as n (%). [a]Fisher's exact test. OR, odds ratio; CI, confidence interval. $p < 0.05$ was considered statistically significant.

3.6 Discussion

This prospective cohort study evaluated the prognostic relevance of serum calcium in full-term infants with neonatal sepsis. The baseline characteristics were generally comparable between infants who improved and those who died, with no significant differences in sex, gestational age, or birth weight. This relative baseline comparability strengthens the interpretation of the association between calcium status and clinical outcome by reducing the potential influence of these demographic and perinatal factors. Similar observations regarding the limited prognostic contribution of sex and birth characteristics after the onset of neonatal sepsis have been reported by Hermawan et al. (2015), although the prognostic effects of these variables may vary according to population characteristics and illness severity.[12]

The principal finding of this study was that serum calcium concentrations were significantly lower among infants who died than among those who improved. Importantly, this difference was observed even though most participants did not meet the conventional biochemical definition of hypocalcemia. This finding suggests that the prognostic information provided by calcium may extend beyond a simple dichotomous classification of hypocalcemia versus normocalcemia. A relatively lower calcium concentration within the normal range may reflect greater systemic physiological disturbance and therefore may provide additional information regarding disease severity. This interpretation is consistent with Liu et al. (2020), who reported that reduced ionized calcium was associated with mortality in neonatal sepsis, supporting the potential role of calcium homeostasis as an indicator of disease severity.[13]

The biological plausibility of this association is supported by the complex interaction between systemic inflammation and calcium regulation during sepsis. Inflammatory mediators may interfere with parathyroid hormone activity, vitamin D metabolism, renal calcium handling, and cellular calcium homeostasis. Kutílek et al. (2019) reported an inverse relationship between calcium concentrations and inflammatory markers in neonates, suggesting that declining calcium may accompany an intensifying inflammatory response.[7] Similarly, Ahmad et al. (2018) demonstrated that electrolyte disturbances were associated

with adverse neonatal outcomes.[14] Taken together, these findings support the concept that alterations in calcium homeostasis may represent a marker of systemic physiological dysregulation rather than an isolated metabolic abnormality.

The relatively lower frequency of hypocalcemia observed in this study compared with that reported by Liu et al. (2020) may be explained by differences in population and laboratory methodology.[13] The present study specifically enrolled full-term infants, whereas previous studies have frequently included premature or low-birth-weight neonates, who have different calcium stores and endocrine regulation. Furthermore, the present study assessed total serum calcium, whereas Liu et al. (2020) evaluated ionized calcium. These measurements are not interchangeable because total calcium is influenced by albumin concentration, whereas ionized calcium more directly reflects the biologically active fraction. Pillai et al. (2022) emphasized that hypoalbuminemia can reduce total calcium without necessarily indicating a comparable reduction in ionized calcium.[15] Thus, differences in gestational maturity and calcium measurement methodology should be considered when comparing the prevalence of hypocalcemia across studies.

Although lower calcium concentrations were more frequent among infants with adverse outcomes, the categorical association between hypocalcemia and clinical outcome was not statistically significant. The limited sample size may have reduced statistical power; however, the significantly lower calcium concentration among infants who died suggests that calcium may provide prognostic information beyond a binary hypocalcemia classification. Thus, serum calcium may be more informative when evaluated as a continuous biomarker or using validated thresholds. Clinically, serum calcium is an accessible and inexpensive marker that may complement established indicators of sepsis severity and support early risk stratification. However, its role should not be interpreted as an independent determinant of outcome, and whether correcting hypocalcemia improves survival cannot be established from this observational study.

A strength of this study was its focus on full-term neonates, providing a relatively homogeneous population, together with the prospective cohort design and standardized diagnostic criteria. Nevertheless, the small sample size, single-center setting, single calcium measurement, and use of total rather than ionized calcium limit statistical power, assessment of calcium trajectories, and generalizability. Residual confounding, particularly from disease severity and treatment-related factors, also cannot be excluded. Overall, lower serum calcium was associated with poorer clinical outcomes, although categorical hypocalcemia was not significantly associated with mortality. Serum calcium may therefore serve as an adjunctive prognostic marker, but larger multicenter studies incorporating serial and ionized calcium measurements are needed to establish its independent prognostic value.

This study has several limitations. First, total serum calcium was measured without simultaneous albumin assessment or correction, which may have limited the accuracy of estimating biologically active calcium status. Second, calcium was measured only once at the time of sepsis diagnosis; therefore, temporal changes in calcium homeostasis during the subsequent clinical course could not be evaluated. Serial measurements may provide greater prognostic value by capturing trends in calcium decline or recovery. Finally, the relatively small sample size and single-center design may have limited statistical power and the generalizability of the findings. Further multicenter studies with larger cohorts, serial calcium measurements, and assessment of ionized calcium are warranted.

4. CONCLUSION

In full-term infants with neonatal sepsis, normocalcemia was more common than hypocalcemia, while lower serum calcium concentrations were associated with poorer clinical outcomes. Although categorical hypocalcemia was not statistically associated with mortality, infants who died had significantly lower serum calcium concentrations than those who improved, indicating that calcium status may provide clinically relevant prognostic information beyond the conventional hypocalcemia threshold. Serum calcium should therefore be considered as an adjunctive marker for early risk stratification and monitoring in neonatal sepsis, while larger multicenter studies with serial and ionized calcium measurements are needed to establish its independent prognostic value.

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AUTHOR CONTRIBUTIONS

IKF: Conceptualization, methodology, investigation, data curation, formal analysis, and writing—original draft.

EA: Supervision, methodology, and critical revision of the manuscript.

ADF: Supervision, investigation, and critical revision of the manuscript.

HA: Supervision, investigation, and critical revision of the manuscript.

AJB: Supervision, methodology, and critical revision of the manuscript.

UF: Supervision, investigation, and critical revision of the manuscript.

All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ETHICS STATEMENT

The study was approved by Biomedical Research Ethics Committee for Human Subjects, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (date: 22.01.2026, number: 125/UN4.6.4.5.31/PP36/2026). Written informed consent was obtained from the parents or legal guardians of all participating infants before enrollment.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request, subject to applicable ethical and institutional restrictions.

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