ORIGINAL ARTICLE

Citrulline levels in preterm infants: A clinical comparison between suspected necrotizing enterocolitis and healthy controls

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ABSTRACT

Background: Necrotizing enterocolitis (NEC) is a severe acute ischemic condition of the intestine in neonates, with higher incidence and mortality rates among preterm and low birth weight infants. Reliable biomarkers that reflect intestinal functional integrity are crucial for guiding management and monitoring recovery, yet none are routinely available. Plasma citrulline, synthesized by small intestinal enterocytes, reflects enterocyte mass and absorptive function and may serve as a non-invasive indicator of gut maturity and mucosal injury.
Methods: A case-control study included 38 preterm infants admitted to NICU and Neo-Intermediate units. Citrulline levels were measured on days 1 and 7 and compared between NEC and healthy groups. Correlations with clinical variables were also analyzed.
Results: The NEC group was predominantly male (76.5%; p=0.037) and had significantly higher antibiotic use and CRP levels than the healthy group (p=0.000 and p=0.001). Citrulline levels on day 1 and day 7 did not differ significantly between groups. However, the change in citrulline levels from day 1 to day 7 was significantly different (p=0.001). No significant associations were found with gestational age, birth weight, or NEC severity. A weak but significant correlation was observed between citrulline level on day 1 and birth length (r=0.324, p=0.047).
Conclusion: Differences in citrulline change between NEC and healthy infants suggest potential as a predictive biomarker. Although only weakly correlated with birth length, citrulline may provide insight into NEC risk in shorter preterm infants. Further studies are needed to validate these findings. (www.actabiomedica.it)

Key words: necrotizing enterocolitis, NEC, citrulline, neonates, preterm infants

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Introduction

Necrotizing Enterocolitis (NEC) is the most common gastrointestinal emergency in neonates and remains a major clinical challenge (1). It is characterized by acute intestinal ischemic necrosis. The estimated incidence of NEC is approximately 1–3 per 1,000 live births, and in the United States, it ranges from 1% to 7.7% among NICU admissions (2). Although national data on NEC in Indonesia are limited, a 2009 report from the national referral hospital, Cipto Mangunkusumo General Hospital, documented 31 NEC cases among 737 preterm births (4.2%) (3). The incidence is notably higher in premature infants and those with very low birth weight, particularly under 1,500 grams (4). The average mortality rate of NEC is approximately 35%, with the highest risk observed in infants with lower gestational age, lower birth weight, and underlying comorbidities. The pathogenesis involves intestinal inflammation that may progress to systemic infection, multi-organ failure, and death (5). Diagnosing NEC in its early stages remains challenging due to the lack of a definitive pathognomonic test. Current diagnosis still relies primarily on clinical and radiologic findings such as abdominal distension, bloody stools, and pneumatosis intestinalis, features that often appear after the disease has progressed. Laboratory markers including C-reactive protein and leukocyte indices are nonspecific and vary with postnatal age and concurrent illnesses. Consequently, there remains an unmet need for reliable biomarkers that can reflect intestinal integrity and functional status during the early disease course and throughout recovery (6,7). Given these diagnostic limitations, there is increasing recognition of the need for prognostic, markers that reflect the evolving physiological state of the intestine and can help anticipate disease course and recovery potential rather than merely establish diagnosis. Such biomarkers would enable earlier risk stratification, guide clinical monitoring, and inform therapeutic decisions in vulnerable preterm populations (8). Most serologic biomarkers indicate systemic inflammation and lack specificity, as they may also be elevated in other neonatal conditions (9). One promising biomarker under investigation is plasma citrulline (citrulline-P), a non-essential amino acid and intermediate in the urea cycle synthesized from glutamine and glutamate in the liver and small intestine. Because citrulline produced in the liver is metabolized locally, circulating citrulline primarily reflects small intestinal production, making it a potential marker of intestinal integrity and enterocyte mass (10). Serum citrulline levels have been reported to be lower in preterm neonates with meconium ileus, likely due to impaired intestinal development and function (11). Several studies have also shown that preterm neonates who develop NEC exhibit significantly lower citrulline concentrations compared to those who do not (12–14). Given that prematurity, NEC's most significant risk factor, is closely associated with immature intestinal function, citrulline may serve as a useful biomarker of enterocyte health. This study aims to evaluate the potential of citrulline as a biomarker for identifying preterm neonates at risk of NEC and its severity, while also examining its correlation with factors associated with organ immaturity, such as gestational age, birth weight, and birth length.

Methods

This case–control study included preterm infants (gestational age <37 weeks) admitted to the NICU and Neo-Intermediate units at RSUD Dr. Soetomo between September 2023 and September 2024. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Airlangga–Dr. Soetomo General Hospital. Informed consent was obtained from parents following a detailed explanation of the study. Subjects were categorized into two groups: infants diagnosed with necrotizing enterocolitis (NEC) based on clinical findings and Bell's criteria (15), and those without any signs or symptoms of NEC. Exclusion criteria included congenital abdominal anomalies (e.g., omphalocele, gastroschisis, atresia), intestinal dysfunction, liver or renal disease, inborn errors of metabolism, and a history of exchange transfusion. Controls were defined as preterm infants admitted to the same NICU or Neo-Intermediate units for non-gastrointestinal, non-infectious conditions who showed no clinical or radiologic signs of

NEC throughout admission. Exclusion criteria for controls mirrored those of the NEC group and additionally included any history of feeding intolerance, sepsis, or antibiotic exposure before enrollment. This study was conducted as a pilot exploratory study using a convenience sample of preterm infants who met eligibility criteria during the study period. No formal power calculation was performed. Estimated effect sizes and 95 % confidence intervals from this pilot work will be used to inform future confirmatory studies. Blood samples were obtained on days 1 and 7 following NEC diagnosis (for the NEC group) and at the corresponding chronological ages for matched controls (for example, if Case 1 was diagnosed with NEC at age 2 days, the corresponding control's day 1 was also age 2 days). Plasma citrulline and C-reactive protein (CRP) were measured using standardized enzymatic assays in the hospital laboratory. Demographic and clinical data including, gestational age category, birth weight, birth length, APGAR score, and antibiotic regimen, were recorded for all participants. For infants with NEC, disease severity was classified using the Modified Bell's staging criteria, which guided clinical management and was used for subgroup descriptive analysis. Feeding route and total parenteral nutrition (TPN) exposure at baseline were documented because these factors influence intestinal function and citrulline kinetics in preterm infants (10). After data collection, patient demographics were analyzed descriptively. Data normality was assessed using the Shapiro–Wilk test. Normally distributed data were expressed as mean ± standard deviation (SD), while non-normally distributed data were presented as median (min–max). Initially, group comparisons were performed using independent-sample t-test or Mann–Whitney U test for continuous variables and Chi-square test for categorical variables. These bivariable analyses were used for descriptive purposes and to identify potential covariates for multivariable modeling. The primary endpoint was defined as the day-7 plasma citrulline concentration, adjusted for baseline (day-1) citrulline and prespecified covariates. The primary analysis used analysis of covariance (ANCOVA), with day-7 citrulline as the dependent variable and group (NEC vs. control) as the main fixed factor. Baseline (day-1) citrulline and relevant clinical covariates including sex, gestational-age category, birth weight, day-1 CRP, antibiotic regimen, and feeding/TPN status, were entered as covariates to control for confounding. Results were reported as adjusted mean differences with 95 % confidence intervals, and statistical significance was set at $p < 0.05$. Covariates were prespecified based on biological plausibility and prior evidence of influence on plasma citrulline. Sex, gestational-age category, and birth weight reflect developmental maturity of enterocytes; CRP represents systemic inflammatory burden; antibiotic regimen captures microbiota-related effects on intestinal metabolism; and feeding route together with TPN exposure reflects enteral nutrient supply, a key determinant of citrulline synthesis in preterm infants (10). Adjusting for these covariates aimed to isolate the independent association between NEC status and citrulline concentration.

Results

The characteristics of the research subjects are presented in Table 1. The comparison between preterm infants with and without NEC showed statistically significant differences in sex, degree of prematurity, antibiotic regimens, and CRP levels on day 1, while no significant differences were found in mode of delivery, birth weight, birth length, or APGAR score. Baseline clinical characteristics summarized further demonstrated significant differences in admission indications, including respiratory distress, jaundice, and apnea of prematurity ($p < 0.05$), with a trend toward higher sepsis frequency in the NEC group. These findings highlight background heterogeneity in clinical exposure profiles and underline the need for covariate adjustment in subsequent multivariable analyses.

Comparison of citrulline levels between healthy preterm infants and those with NEC

Table 2 presents citrulline levels on days 1 and 7 in the NEC and healthy groups. On day 1, the median level was lower in the NEC group compared to the healthy group, though not statistically significant. Similarly, on day 7, the NEC group had

Table 1. Subjects' characteristics.

Characteristics	NEC n=17(%)	Controls n=21(%)	p value
Sex			**0,037a***
Female	4(23,5)	12(57,1)	
Male	13(76,5)	9(42,9)	
Degree of Prematurity			**0,042a***
Extremely Preterm (<28 weeks)	0(0,0)	0(0,0)	
Very Preterm (28-<32 weeks)	2(11,8)	4(19,1)	
Moderate Preterm (32-<34 weeks)	8(47,0)	2(9,5)	
Late Preterm (34-37 weeks)	7(41,2)	15(71,4)	
Mode of delivery			0,823[a]
SC	15(88,2)	19(90,5)	
Pervaginam	2(11,8)	2(9,5)	
Birth Weight			0,239[a]
Extremely Low Birth Weight (<1000 gram)	2(11,8)	0(0,0)	
Very Low Birth Weight (1000-1499 gram)	1(5,9)	4(19,0)	
Low Birth Weight (1500-2499 gram)	9(52,9)	8(38,1)	
Normal (≥ 2500 gram)	5(29,4)	9(42,9)	
Birth Length (median (min-max); cm)	47 (33-50)	47 (32-52)	0,615[d]
Antibiotics			**0,000[a]***
First Regimen	1(5,9)	11(52,4)	
Second Regimen	3(17,6)	3(14,3)	
Third Regimen	13(76,5)	0(0,0)	
Not Given	0(0,0)	7(33,3)	
CRP Level on Day 1 (mean + SD; mg/dL)	6,4±8,1	1,0±2,8	**0,001[c]***
APGAR Score on first minute (mean + SD)	4,8±1,2	5,5±1,9	0,214[b]
Admission indication			
Respiratory distress	15(88.2)	9(42.9)	**0.004[a]***
Sepsis	6(35.3)	2(9.5)	0.053[a]
Jaundice	0(0)	7(33.3)	**0.011[b]***
Apneu of Prematurity (AOP)	0(0)	4(19.0)	**0.013[b]***
Congenital Heart Disease	3(17.6)	7(33.3)	0.275[a]
Feeding route at baseline			0.057[a]
Enteral	0(0)	4(19)	
Parenteral	0(0)	0(0)	
Mixed	17(100)	17(81)	

[a]Chi square test; [b]Fisher's Exact test; [c]Independent Sample T-test; [d]Mann–Whitney test
*Significant ($p < 0.05$).

a lower median level than the healthy group, with no significant difference. However, the mean change (Δ) in citrulline levels from day 1 to 7 was significantly lower in the NEC group compared to the healthy group (p=0.001).

Comparative analysis of citrulline levels in preterm infants with NEC by degree of prematurity

In the NEC group, gestational age was categorized as very preterm, moderate preterm, and late

Table 2. Comparison of citrulline levels between healthy preterm infants and those with NEC.

	NEC	Controls	p value
Citrulline Level Day 1 (nmol/mL) Median (Min-Max)	3,27 (1,19 - 11,89)	3,62 (1,31 - 5,88)	0.472[a]
Citrulline Level Day 7(nmol/mL) Median (Min-Max)	4,52 (1,04 – 12,48)	5,74 (1,94 – 11,00)	0.378[a]
Δ Citrulline Level (nmol/mL) Mean±SD	1,13±1,19	2,01±1,10	**0.001**[b]*

[a]Mann-Whitney test; [b]Independent Sample T-test; *significant ($p < 0.05$).

Table 3. Association between body length and citrulline levels.

	Citrulline Level (nmol/mL) Median (Min-Max)	p value	Correlation Coefficient
Day 1	3,42 (1,19-11,89)	**0,047**[a]*	0,324[a]
Day 7	5,05 (1,05-12,48)	0,759[a]	

[a]Spearman test; *significant ($p < 0.05$).

preterm. Median citrulline levels varied across groups on day 1 and increased by day 7 in all categories. However, no significant differences were found between prematurity categories on either day 1 (p=0.914) or day 7 (p=0.591).

Comparative analysis of citrulline levels in preterm infants with NEC by birth weight

Among the 17 preterm infants with NEC, birth weight categories included extremely low birth weight (ELBW), very low birth weight (VLBW), low birth weight (LBW), and normal birth weight. Median citrulline levels varied across groups on day 1 and increased by day 7 in all categories. Analysis showed no significant differences in citrulline concentrations across birth weight categories on either day 1 (p=0.335) or day 7 (p=0.846).

Comparative analysis of citrulline levels in preterm infants with NEC by birth length

The average body length (BL) of all samples was ranging from 32 to 52 cm. Although the healthy group had a slightly greater average BL, statistical analysis showed no significant difference between the groups. Table 3 demonstrates the association between body length and citrulline levels, with a significant correlation observed on day 1. Analysis of citrulline levels in relation to body length revealed a significant association on day 1 ($p = 0.047$), with a low to moderate correlation strength ($r = 0.324$). However, no significant association was observed between body length and citrulline levels on day 7.

Comparative analysis of citrulline levels and the severity of NEC in preterm infants

This study compared citrulline levels in preterm infants with NEC based on the severity of NEC as assessed by Bell's criteria (15). Mean citrulline levels varied across stages on day 1, with no significant differences observed (p=0.843). Although levels increased in all stages by day 7, the differences remained statistically insignificant (p=0.713).

Multivariable analysis of factors associated with day-7 plasma citrulline concentration

A multivariable linear regression analysis was conducted to identify factors associated with day-7 plasma citrulline concentration. Independent variables included NEC status, baseline (day-1) citrulline level, sex, gestational-age category, birth weight, birth length, CRP day-1, antibiotic regimen, and feeding/TPN status. Only baseline citrulline level on day 1 remained significantly associated with day-7 citrulline concentration after adjustment for all covariates, suggesting that initial intestinal function strongly predicts subsequent citrulline levels regardless of

Table 4. Multivariable linear regression of factors associated with *Day-7 Plasma citrulline concentration.*

Variables	B (95% CI)	p value
NEC	−0.330 (−4.02 to 3.36)	0.856
Citrulline Level Day 1	**0.924 (0.408 to 1.439)**	**<0.001***
Birth Weight	−0.001 (−0.003 to 0.002)	0.623
Birth Length	0.020 (−0.313 to 0.353)	0.903
Gender	−0.410 (−2.824 to 2.004)	0.730
Prematurity Category	−0.796 (−2.598 to 1.006)	0.374
Antibiotic regimen	−0.668 (−2.517 to 1.181)	0.466
CRP Level on Day 1	0.001 (−0.002 to 0.002)	0.933

*significant ($p < 0.05$).

disease status or clinical exposures. The adjusted regression coefficients, 95 % confidence intervals, and p-values are presented in Table 4.

Discussion

In this study, most infants with suspected NEC were male, while females predominated in the healthy group (p=0.037). Although some studies have reported a higher incidence of NEC in males , others suggest that both sexes have an equal risk of developing NEC (16,17). CRP levels were significantly higher in the NEC group (p=0.01), indicating a stronger inflammatory response. This finding is consistent with previous studies linking elevated CRP levels to NEC severity (18–20). Citrulline levels on both day 1 and day 7 did not differ significantly between the NEC and healthy groups. This may be explained by the fact that both groups consisted of preterm infants, who typically have lower baseline citrulline levels (21). Additionally, antibiotic use was significantly more frequent in the NEC group (p=0.008), with all infants receiving antibiotics, including a higher proportion treated with second- and third-line regimens (p=0.000). This difference may be influenced by routine prophylactic antibiotic use in preterm infants, particularly those with suspected NEC, and by the fact that many were referral patients who had already received antibiotics prior to admission. This may have introduced bias by disrupting the intestinal microbiota, impairing mucosal integrity, and promoting NEC through an increase in pathogenic bacteria and a reduction in protective species (22,23). These findings are consistent with previous studies identifying antibiotic exposure as a risk factor for NEC and suggest that antibiotic use may directly influence citrulline levels (24,25). This potential confounding effect may help explain the absence of a significant difference in citrulline concentrations between the groups, despite clinical suspicion of NEC. Interestingly, differences in admission indications between groups may have contributed to variations in baseline citrulline levels. Infants admitted for respiratory distress, apnea of prematurity, or sepsis had a higher prevalence in the NEC group, conditions that are themselves associated with hypoxia, systemic inflammation, and delayed enteral feeding, all of which can influence enterocyte metabolism and citrulline synthesis (8-9). In contrast, control infants were more often admitted for relatively benign conditions such as jaundice or congenital heart disease, which may have allowed earlier enteral nutrition and better intestinal perfusion. These background differences further highlight the multifactorial influences on citrulline kinetics beyond NEC itself. This study found that citrulline levels increased on day 7 compared to day 1 in both groups, reflecting intestinal maturation and enhanced absorptive capacity in preterm infants. As part of the nitric oxide pathway, citrulline also contributes to antioxidant and inflammatory responses. Its increase indicates improved enteral readiness, while low levels may result from enzyme deficiencies (CPS1, OTC) commonly seen in prematurity or from severe inflammation (10,21). Since severe inflammation can suppress citrulline production, this aligns with our finding that, in the NEC group, characterized by significant intestinal inflammation, the increase in citrulline levels was significantly lower than in the control group ($p = 0.001$), suggesting impaired enterocyte function. Jawale et al. (26) similarly reported significantly lower citrulline levels in neonates with NEC ($p = 0.009$). As a marker of enterocyte mass, citrulline has also been

shown to decrease in conditions involving mucosal injury, such as celiac disease, chemotherapy-induced mucositis, and immunodeficiency (27–29). Feenstra et al. (30) also observed a decline in citrulline within 48 hours of NEC onset, further supporting our findings. In this study, citrulline concentrations did not differ significantly among very preterm, moderate preterm, and late preterm infants within the NEC group. This contrasts with the findings of Robinson et al. (21), who reported approximately 50% lower citrulline levels in preterm piglets compared to near-term and full-term controls at the initiation of parenteral nutrition ($p < 0.05$). The discrepancy between these findings may be attributed to differences in sample size, subgroup variability, and study design. Moreover, while the previous study compared preterm and full-term subjects, our study included only preterm infants, who generally exhibit lower citrulline levels overall. There were no significant differences in citrulline levels on day 1 or day 7 across NEC severity stages based on the Modified Bell's Criteria in this study. This may be due to the multifactorial nature of NEC and variability in treatment, including antibiotic use. In contrast, Jawale et al. (26) reported significantly lower citrulline levels in Stage II–III NEC compared to Stage I ($p = 0.02$), likely reflecting differences in study design, population, and sampling time. Consistent with our findings, Robinson et al. (21) observed stable citrulline levels throughout NEC progression, suggesting that citrulline may not reliably reflect disease severity. NEC progression is primarily driven by intestinal barrier dysfunction resulting from epithelial injury and impaired repair mechanisms, leading to heterogeneous clinical presentations. Although Bell's staging is widely used to guide prognosis, it may not be fully represented by a single biomarker such as citrulline (31), which may explain the non-significant differences observed across stages in this study. Citrulline concentrations did not differ significantly among NEC infants across different birth weight groups, including ELBW, VLBW, LBW, and normal birth weight. Previous studies have shown that prematurity influences citrulline levels but did not specifically examine birth weight, as it is closely associated with gestational age (32–34). These studies suggest that lower citrulline levels in lower birth weight infants are primarily driven by prematurity. In contrast, Celik et al. reported lower citrulline levels in a preterm NEC cohort with a lower average birth weight compared to Ioannou et al., attributing the difference to birth weight, although they did not explore its direct relationship with citrulline levels (13,14). Unlike birth weight, citrulline levels in this study differed significantly by birth length ($p = 0.047$). Although this association reached statistical significance, it should be interpreted with caution because the analysis was exploratory and not adjusted for multiple comparisons. The limited sample size and the number of subgroup tests increase the likelihood of Type I error. Rather than a confirmatory result, this finding should be viewed as hypothesis-generating and interpreted in conjunction with its effect size and confidence interval (29). Plasma L-citrulline concentration correlates with the length of the remaining small intestine or the severity of intestinal lesions, and therefore shows potential as a translational safety biomarker in toxicology modeling approaches (35). Since small intestinal length increases proportionally with body length during fetal and neonatal development (36), and body length strongly correlates with intestinal length in children (37), longer body length may be associated with greater citrulline production. This exploratory association suggests that birth length could serve as an indirect indicator of intestinal mass and deserves further investigation in larger, prospectively powered studies. To address these potential confounders, a multivariable linear regression analysis was performed including NEC status, baseline citrulline, sex, gestational age, birth weight and length, CRP, antibiotic regimen, feeding route, and TPN exposure. Only baseline citrulline level remained significantly associated with day-7 citrulline concentration ($p = <0.001$), indicating that early enterocyte functional capacity strongly predicts subsequent intestinal performance regardless of disease or clinical exposures. This finding supports the role of day-1 citrulline as a robust baseline biomarker of gut maturity, while the lack of association with NEC status after adjustment suggests that postnatal inflammation and treatment factors exert a more transient effect on citrulline homeostasis (10,21,26).

Study's limitation

This study has several limitations that may affect the interpretation and generalizability of the findings. The relatively small sample size and single-center design may limit statistical power, particularly for subgroup analyses by gestational age, birth weight, and NEC severity. The use of only two time points for citrulline measurement (day 1 and day 7) may not fully capture the dynamic changes in intestinal function during NEC progression or recovery. Additionally, antibiotic exposure, which differed significantly between groups—may have acted as a confounding factor by altering intestinal microbiota and influencing citrulline levels. The lack of histopathological confirmation or direct assessment of intestinal damage also limits the ability to establish a causal link between citrulline concentrations and enterocyte function. While birth length showed a statistically significant correlation with citrulline levels, the strength of this correlation was weak to moderate, which limits its clinical relevance. Furthermore, birth length is not routinely recorded or emphasized in neonatal research, which may affect the applicability of this finding across settings.

Conclusion

This study found a significant difference in citrulline concentration changes from day 1 to day 7 between NEC and healthy infants, indicating impaired enterocyte function in NEC. Although citrulline levels were not associated with birth weight, gestational age, or NEC severity, the temporal change may reflect underlying intestinal injury. A significant but weak to moderate correlation with birth length supports its role as a surrogate marker of intestinal mass and highlights birth length as a potential variable for future research. These findings support citrulline's promise as a non-invasive biomarker for early detection and monitoring of intestinal dysfunction in preterm infants, warranting validation in larger, multi-center studies.

Ethic Approval: Informed consent was obtained from the patient's parent following a detailed explanation of the study's purpose by the researchers. The study received ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Hospital (Ethical Clearance Number: 0773/KEPK/IX/2023).

Conflict of Interest: The authors declare that they have no commercial associations such as consultancies, stock ownership, equity interest, patent/licensing arrangement, that might pose a conflict of interest in connection with the submitted article.

Authors contribution: BSSA contributed to the concept and study design, literature review, data collection, statistical analysis, and drafting of the manuscript. AFA. contributed to the concept and study design, provided critical revisions, interpreted the data, and supervised the study. RE contributed to the concept and study design and provided supervision. AD contributed to data analysis, interpretation of the results, and critically revised the manuscript for intellectual content. KRS assisted with the literature review and manuscript preparation. SMS provided expertise in clinical interpretation and supported the final review of the manuscript. All authors have read and approved the final version of the manuscript.

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References

1. Neu J, Walker WA. Necrotizing Enterocolitis. N Engl J Med. 2011;364(3):255–64. doi: 10.1056/NEJMra1005408.
2. Niño DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. Nat Rev Gastroenterol Hepatol. 2016;13(10):590–600. doi: 10.1038/nrgastro.2016.119.

3. Sidauruk RJM, Amir I, Kadim M, Said M. Faktor Risiko yang Memengaruhi Kolonisasi Mikroflora Saluran Cerna Neonatus Kurang Bulan dengan Enterokolitis Nekrotikans. Sari Pediatri. 2016;15(6):353. Indonesian. doi: 10.14238/sp15.6.2014.353-60.
4. Alganabi M, Lee C, Bindi E, Li B, Pierro A. Recent advances in understanding necrotizing enterocolitis. F1000Res. 2019;8:107. doi: 10.12688/f1000research.17228.1.
5. Tanner SM, Berryhill TF, Ellenburg JL, et al. Pathogenesis of Necrotizing Enterocolitis. Am J Pathol. 2015;185(1):4–16. doi: 10.1016/j.ajpath.2014.08.028.
6. Patel RM, Ferguson J, McElroy SJ, Khashu M, Caplan MS. Defining necrotizing enterocolitis: current difficulties and future opportunities. Pediatr Res. 2020;88(Suppl 1):10–5. doi: 10.1038/s41390-020-1074-4.
7. D'Angelo G, Impellizzeri P, Marseglia L, et al. Current status of laboratory and imaging diagnosis of neonatal necrotizing enterocolitis. Ital J Pediatr. 2018;44(1):84. doi: 10.1186/s13052-018-0528-3.
8. Meng W, Wang Q, Xu Q, Gao H, Zhou Y, Shao W. Biomarkers in the Severity of Necrotizing Enterocolitis in Preterm Infants: A Pilot Study. Int J Gen Med. 2024;17:1017–23. doi: 10.2147/IJGM.S446378.
9. Gordon PV, Swanson JR, Clark R, Spitzer A. The complete blood cell count in a refined cohort of preterm NEC: the importance of gestational age and day of diagnosis when using the CBC to estimate mortality. J Perinatol. 2016;36(2):121–5. doi: 10.1038/jp.2015.162.
10. Maric S, Restin T, Muff J, et al. Citrulline, Biomarker of Enterocyte Functional Mass and Dietary Supplement. Metabolism, Transport, and Current Evidence for Clinical Use. Nutrients. 2021;13(8):2794. doi: 10.3390/nu13082794.
11. Woo HK, Kim EK, Jung YH, et al. Reduced early dried blood spot citrulline levels in preterm infants with meconium obstruction of prematurity. Early Hum Dev. 2015;91(12):777–81. doi: 10.1016/j.earlhumdev.2015.09.004.
12. Becker RM, Wu G, Galanko JA, et al. Reduced serum amino acid concentrations in infants with necrotizing enterocolitis. J Pediatr. 2000;137(6):785–93. doi: 10.1067/mpd.2000.109145.
13. Ioannou HP, Diamanti E, Piretzi K, Drossou-Agakidou V, Augoustides-Savvopoulou P. Plasma citrulline levels in preterm neonates with necrotizing enterocolitis. Early Hum Dev. 2012;88(7):563–6. doi: 10.1016/j.earlhumdev.2011.11.008.
14. Celik IH, Demirel G, Canpolat FE, Dilmen U. Reduced Plasma Citrulline Levels in Low Birth Weight Infants With Necrotizing Enterocolitis. J Clin Lab Anal. 2013;27(4):328–32. doi: 10.1002/jcla.21607.
15. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal Necrotizing Enterocolitis: Therapeutic Decisions Based upon Clinical Staging. Ann Surg. 1978;187(1):1–7. doi: 10.1097/00000658-197801000-00001.
16. Kordasz M, Racine M, Szavay P, et al. Risk factors for mortality in preterm infants with necrotizing enterocolitis: a retrospective multicenter analysis. Eur J Pediatr. 2022;181(3):933–9. doi: 10.1007/s00431-021-04266-x.
17. Lamireau N, Greiner E, Hascoët JM. Risk factors associated with necrotizing enterocolitis in preterm infants: A case–control study. Arch Pediatr. 2023;30(7):477–82. doi: 10.1016/j.arcped.2023.07.003.
18. Young C, Sharma R, Handfield M, Mai V, Neu J. Biomarkers for Infants at Risk for Necrotizing Enterocolitis: Clues to Prevention? Pediatr Res. 2009;65(5 Pt 2):91R-97R. doi: 10.1203/PDR.0b013e31819dba7d.
19. Mohd Amin AT, Zaki RA, Friedmacher F, Sharif SP. C-reactive protein/albumin ratio is a prognostic indicator for predicting surgical intervention and mortality in neonates with necrotizing enterocolitis. Pediatr Surg Int. 2021;37(7):881–6. doi: 10.1007/s00383-021-04879-1.
20. Lin X, Zeng HP, Fang YF, Lin YY, Yang CY. Predictive Indicators for Necrotizing Enterocolitis With the Presence of Portal Venous Gas and Outcomes of Surgical Interventions. Front Pediatr. 2021;9:683510. doi: 10.3389/fped.2021.683510.
21. Robinson JL, Smith VA, Stoll B, et al. Prematurity reduces citrulline-arginine-nitric oxide production and precedes the onset of necrotizing enterocolitis in piglets. Am J Physiol Gastrointest Liver Physiol. 2018;315(4):G638–49. doi: 10.1152/ajpgi.00198.2018.
22. Arboleya S, Sánchez B, Milani C, et al. Intestinal Microbiota Development in Preterm Neonates and Effect of Perinatal Antibiotics. J Pediatr. 2015;166(3):538–44. doi: 10.1016/j.jpeds.2014.09.041.
23. Pace E, Yanowitz TD, Waltz P, Morowitz MJ. Antibiotic therapy and necrotizing enterocolitis. Semin Pediatr Surg. 2023;32(3):151308. doi: 10.1016/j.sempedsurg.2023.151308.
24. Ouaknine Krief J, Helly De Tauriers P, Dumenil C, et al. Role of antibiotic use, plasma citrulline and blood microbiome in advanced non-small cell lung cancer patients treated with nivolumab. J Immunother Cancer. 2019;7(1):176. doi: 10.1186/s40425-019-0658-1.
25. Zhu K, Gao H, Yuan L, Wang L, Deng F. Prolonged antibiotic therapy increased necrotizing enterocolitis in very low birth weight infants without culture-proven sepsis. Front Pediatr. 2022;10:949830. doi: 10.3389/fped.2022.949830.
26. Jawale N, Prideaux M, Prasad M, Miller M, Rastogi S, for Maimonides Neonatal Group. Plasma Citrulline as a Biomarker for Early Diagnosis of Necrotizing Enterocolitis in Preterm Infants. Am J Perinatol. 2021;38(13):1435–41. doi: 10.1055/s-0040-1713406.
27. Barzał JA, Szczylik C, Rzepecki P, Jaworska M, Anuszewska E. Plasma citrulline level as a biomarker for cancer therapy-induced small bowel mucosal damage. Acta Biochim Pol. 2014;61(4):615–31. PMID 25473654.
28. Crenn P, Vahedi K, Lavergne-Slove A, Cynober L, Matuchansky C, Messing B. Plasma citrulline: a marker of enterocyte mass in villous atrophy-associated small bowel disease. Gastroenterology. 2003;124(5):1210–9. doi: 10.1016/S0016-5085(03)00170-7.
29. Crenn P, De Truchis P, Neveux N, Galpérine T, Cynober L, Melchior JC. Plasma citrulline is a biomarker of

enterocyte mass and an indicator of parenteral nutrition in HIV-infected patients. Am J Clin Nutr. 2009;90(3):587–94. doi: 10.3945/ajcn.2009.27448.
30. Feenstra FA, Kuik SJ, Derikx JPM, et al. Plasma citrulline during the first 48 h after onset of necrotizing enterocolitis in preterm infants. J Pediatr Surg. 2021;56(3):476–82. doi: 10.1016/j.jpedsurg.2020.11.020.
31. Mackay S, Frazer LC, Bailey GK, et al. Identification of serum biomarkers for necrotizing enterocolitis using aptamer-based proteomics. Front Pediatr. 2023;11:1184940. doi: 10.3389/fped.2023.1184940.
32. Chapman JC, Liu Y, Zhu L, Rhoads JM. Arginine and citrulline protect intestinal cell monolayer tight junctions from hypoxia-induced injury in piglets. Pediatr Res. 2012;72(6):576–82. doi: 10.1038/pr.2012.137.
33. Badurdeen S, Mulongo M, Berkley JA. Arginine depletion increases susceptibility to serious infections in preterm newborns. Pediatr Res. 2015;77(2):290–7. doi: 10.1038/pr.2014.177.
34. Contreras MT, Gallardo MJ, Betancourt LR, et al. Correlation between plasma levels of arginine and citrulline in preterm and full-term neonates: Therapeutical implications. J Clin Lab Anal. 2017;31(6):e22134. doi: 10.1002/jcla.22134.
35. Yoneyama T, Abdul-Hadi K, Brown A, Guan E, Wagoner M, Zhu AZX. A Citrulline-Based Translational Population System Toxicology Model for Gastrointestinal-Related Adverse Events Associated With Anticancer Treatments. CPT Pharmacometrics Syst Pharmacol. 2019;8(12):951–61. doi: 10.1002/psp4.12475.
36. Bardwell C, El Demellawy D, Oltean I, et al. Establishing normal ranges for fetal and neonatal small and large intestinal lengths: results from a prospective postmortem study. World J Pediatr Surg. 2022;5(3):e000397. doi: 10.1136/wjps-2021-000397.
37. Struijs MC, Diamond IR, De Silva N, Wales PW. Establishing norms for intestinal length in children. J Pediatr Surg. 2009;44(5):933–8. doi: 10.1016/j.jpedsurg.2009.01.031.