

ORIGINAL ARTICLE

Elevated fecal calprotectin as a biomarker of sepsis in preterm neonates: A cross-sectional study

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Abstract. *Background and aim:* Fecal Calprotectin (FC) is used to determine the presence of infection in the intestine. Some studies have found high concentrations in sepsis infants with necrotizing enterocolitis but have not compared those with sepsis and without sepsis. This study evaluated the difference between FC levels in sepsis and without sepsis. *Methods:* A cross-sectional study was conducted. Infants aged 0 to 14 days with gestational age 28-36 weeks consist of the patient who was diagnosed with sepsis and without sepsis based on the European Medicines Agency (EMA) score. Clinical and biochemical data were collected from medical records. Fecal samples were analyzed for FC levels using the ELISA method. *Results:* We enrolled 88 neonates consisting of 40 neonates with sepsis and 48 neonates without sepsis. FC was higher in the sepsis infants' group than in healthy infants ($P < 0.05$). Multivariate analysis showed no variables significantly determining the FC level in sepsis infants. *Conclusions:* Preterm neonates with sepsis have higher FC levels than preterm neonates without sepsis. (www.actabiomedica.it)

Key words: newborn, neonatal sepsis, fecal calprotectin, preterm infant, biomarker

Introduction

Sepsis is a significant cause of neonatal mortality, particularly among extremely preterm infants, with infections contributing to a substantial number of deaths and leading to prolonged hospital stays and increased risk of morbidities such as retinopathy of prematurity, intraventricular hemorrhage, and bronchopulmonary dysplasia (1). This increased risk is attributed to several factors, including immature immune systems, prolonged hospital stays, and invasive procedures. The incidence and outcomes of sepsis in preterm infants vary, with early-onset sepsis (EOS) and late-onset sepsis presenting distinct challenges and pathogen profiles (1-3). EOS is a significant cause of morbidity and mortality in very preterm infants, defined as an infection occurring within 72 hours after birth due to bacterial pathogens transmitted from mother to infant (2).

EOS occurs in approximately 6% of extremely preterm infants, with *Escherichia coli* and Coagulase-negative Staphylococci being the most common pathogens (1). Fecal calprotectin (FC) is an innate immune system, antimicrobial, calcium, and zinc-binding heterocomplex protein (4). FC levels are elevated in preterm infants with EOS compared to those without the condition, suggesting a link between FC and sepsis-related inflammation (5). Many studies have reported the benefits of FC among several markers associated with acute intestinal mucosal infections. Previous studies have shown that serum calprotectin can differentiate between sepsis and non-sepsis patients in the Intensive Care Unit (6-8). A study in neonates found that the FC concentration was higher in sepsis neonates with necrotizing enterocolitis (NEC) than those without NEC (9,10). However, no one has reported the difference in FC concentration between sepsis and

non-sepsis premature infants. This study aims to compare FC concentrations in preterm infants with sepsis and those without sepsis. If significant differences are observed, FC levels could be used as a potential marker for neonatal sepsis.

Methods

This cross-sectional study was conducted at the Neonatal Intensive Care Unit of Dr. Wahidin Sudirohusodo Hospital, Hasanuddin University Hospital, Khadijah Hospital, and Cahaya Medika Hospital, Makassar, Indonesia, between March 2024 and June 2024. The study participants were selected using consecutive sampling. Participants were taken between 0-14 days of age, and in premature babies with sepsis, complications such as NEC are detected at 14 days of age. The sample size is calculated using the Lemeshow formula for a cross-sectional study (11,12). As a result, each group, sepsis, and non-sepsis, consisted of 40 participants. Inclusion criteria were infants aged 0 to 14 days with gestational age 28–36 weeks consisting of the patient who was diagnosed with sepsis and without sepsis. Subjects were excluded if they weighed < 1000 grams, received antibiotics > 72 hours, and had congenital malformation of the intestinal tract (i.e. congenital infarcted segments, intestinal atresia, malrotation, gastroschisis, or omphalocele). Informed consent was obtained from their parents/caregivers. Data recording was carried out in the form of data on sex, gestational age, birth weight, feeding, and mode of delivery. The patient's parents were thoroughly informed about the study and must provide written informed consent before their children participated. The Faculty of Medicine of Hasanuddin University ethics committee approved the protocol under the code number UH24020071.

Gestational age

Gestational age is the time the fetus spends in the womb, counted from the first day of the last menstrual period until the baby is born. The gestational age in neonates can be determined using the New

Ballard Score. It can be classified as follows: full-term (> 37 weeks), late preterm (34–36 weeks 6 days), moderately preterm (32–33 weeks 6 days), very preterm (28–32 weeks 6 days), and extremely preterm (< 28 weeks) (13,14).

Birth weight

Body mass was measured using a scale or baby scale. In neonates, birth weight is divided into normal (>2500 grams), low birth weight/LBW (1500 grams–2499 grams), very low birth weight/VLBW (1000 grams–1499 grams), and extremely low birth weight/ELBW (<1000 grams) (15). This study combined VLBW and ELBW babies into one group of VLBW.

Sepsis

Sepsis was diagnosed based on the European Medicines Agency (EMA) score (16,17). The interpretation of the EMA score indicates sepsis if there are at least two clinical categories and at least two laboratory tests. First, we determine the EMA score to assess whether the baby has sepsis. If the EMA score is sufficient to diagnose sepsis, we proceed by collecting the baby's stool for FC examination.

Calprotectin measurement

Blood samples were taken for culture, routine blood, c-reactive protein, procalcitonin, and Immature Total Ratio in suspected sepsis patients. Stool samples of 1–5 grams were taken, weighed using a digital scale or about one teaspoon, and placed in a clean container. The samples were immediately sent to the Hasanuddin University Medical Research Center Laboratory for FC examination, and the results were compared between the two groups. The test for FC protocol was used using the Dynex DS2 ELISA Processing systems and DSX Automated ELISA (Chantilly, VA, USA) from CalproLab Calprotectin ELISA with catalog no. CALP0170 (Lysaker, Norway). The results of normal FC levels are 1 g/gram based on the cut-off point in neonates (18,19).

Statistical analysis

Univariate analysis was used for data description in the form of frequency description, mean value, standard deviation, and range. An unpaired T-test was used to compare two different variants with normal distribution. In this case, calprotectin levels were compared in infants with and without sepsis. The Mann-Whitney test compares numerical variables whose data are not normally distributed and have different variants between two unpaired groups. The normality test used the Shapiro-Wilk test. The Lavene test was used for the variance equality test. A p-value of <0.05 indicates a significant result.

Results

Characteristics of participants

The total subjects obtained were 88 neonates consisting of 40 neonates with sepsis and 48 neonates

without sepsis (Table 1). Most of the subjects were female neonates born at late preterm gestation, normal birth weight, cesarean section delivery, and breastfed in both study groups. Both groups had homogeneous characteristics ($p>0.05$).

Table 2 showed that birth weight, delivery method, and feeding type were significantly associated with FC levels in neonates with sepsis (p-values were 0.011, 0.008, and 0.025, respectively). Higher levels were observed in LBW infants compared to VLBW infants, vaginally delivered infants compared to cesarean births, and breastfed infants compared to formula-fed infants. This indicates that certain clinical factors, like birth weight, delivery method, and feeding type, may influence inflammation levels in septic neonates.

Table 3 showed that feeding type was the only variable significantly associated with FC levels in neonates without sepsis. Formula-fed infants had higher levels compared to breastfed infants. Overall, FC levels in non-sepsis conditions were lower compared to sepsis conditions, indicating reduced inflammation. This

Table 1. Patient Characteristics

Variable		Sepsis (n=40) n (%)	Non-sepsis (n=48) n (%)	p-value
Sex				
	Male	19 (47.5)	20 (41.7)	0.583 ^a
	Female	21 (52.5)	28 (58.3)	
Gestational age				
	Late preterm	24 (60.0)	47 (97.9)	N.A ^b
	Moderate preterm	10 (25.0)	0 (0.0)	
	Very preterm	6 (15.0)	1 (2.1)	
Birth weight				
	Normal	1 (2.5)	1 (2.1)	N.A ^b
	LBW	30 (75.0)	47 (97.9)	
	VLBW	9 (22.5)	0 (0.0)	
Delivery				
	Vaginal delivery	6 (15.0)	4 (8.3)	0.305 ^b
	Cesarean birth	34 (85.0)	44 (91.7)	
Feeding				
	Breast Milk	24 (60.0)	31 (64.6)	0.658 ^a
	Formula Milk	16 (40.0)	17 (35.4)	

Abbreviations: N.A, not applicable; LBW, low birth weight; VLBW, very low birth weight; ^aChi-square test; ^bFisher exact test.

Table 2. Fecal calprotectin levels in sepsis conditions based on patient characteristics

Variable		Fecal Calprotectin	p-value
		Sepsis (n=40)	
		Median (min-max)	
Sex			
	Male (n=19)	79.69 (5.57 – 260.76)	0.635 ^a
	Female (n=21)	95.08 (0.81 – 432.08)	
Gestational age			
	Late preterm (n=24)	121.46 (12.80 – 432.08)	0.134 ^b
	Moderate preterm (n=10)	77.67 (5.57 – 195.11)	
	Very preterm (n=6)	13.13 (0.81 – 294.87)	
Birth weight			
	Normal (n=1)	21.89	0.011 ^{b*}
	LBW (n=30)	121.46 (8.15 – 432.08)	
	VLBW (n=9)	11.70 (0.81 – 195.11)	
Delivery			
	Vaginal delivery (n=6)	207.87 (121.46 – 260.76)	0.008 ^{a*}
	Cesarean birth (n=34)	79.12 (0.81– 432.08)	
Feeding			
	Breast Milk (n=24)	138.15 (5.57 – 432.08)	0.025 ^{b*}
	Formula Milk (n=16)	67.00 (0.81 – 226.06)	

Abbreviations: N.A: not applicable; VLBW, very low birth weight; LBW, low birth weight; ^aMann-Whitney test; ^bIndependent T-test; *p<0.05 indicates statistical significance.

suggests that feeding type (breast milk vs. formula) plays a role in influencing FC levels even in non-septic neonates.

None of the variables (birth weight, delivery method, feeding type) showed statistically significant effects on FC levels ($p>0.05$) (Table 4). The delivery method ($p=0.082$) is the closest to being significant, suggesting a potential influence of vaginal delivery on increasing FC levels. The delivery method has the largest Standardized Coefficient (Beta = 0.201), indicating it may have the strongest relative impact on FC levels, even though it is not statistically significant.

FC levels are significantly higher in neonates with sepsis compared to non-sepsis cases ($p=0.001$). This highlights its potential utility in distinguishing septic neonates from non-septic ones (Table 5).

Discussion

In this study, most neonates were female in the sepsis and non-sepsis groups. Previous studies stated that neonatal sex is another factor that influences the risk of neonatal sepsis. Male increases the chance of sepsis in neonates (20). Males are more susceptible to infection because only one X chromosome exists. By diversifying the leukocyte response, the X chromosome is responsible for the dimorphic nature of the inflammatory response during endotoxemia (21). In addition, the female sex is protective, whereas the male sex is harmful due to reduced cellular immune response and cardiovascular function. Androgens, classified as male sex hormones, have been demonstrated to inhibit cell-mediated immune responses. Conversely, female sex hormones display protective properties that may facilitate the inherent advantage observed

Table 3. Fecal calprotectin levels in non-sepsis conditions based on patient characteristics

Variable		Fecal Calprotectin	p-value
		Non-sepsis (n=47)	
		Median (min-max)	
Sex			
	Male (n=20)	23.00 (1.65 – 267.52)	0.933 ^a
	Female (n=28)	34.44 (0.41 – 306.37)	
Gestational age			
	Late preterm (n=47)	26.78 (0.41 – 443.16)	0.159 ^a
	Moderate preterm (n=0)	–	
	Very preterm (n=1)	113.19	
Birth weight			
	Normal (n=1)	60.18	0.367 ^a
	LBW (n=47)	26.78 (0.41 – 443.16)	
	VLBW (n=0)	–	
Delivery method			
	Vaginal delivery (n=4)	7.35 (3.34 – 60.18)	0.361 ^a
	Cesarean birth (n=44)	32.03 (0.41 – 306.37)	
Feeding type			
	Breast Milk (n=31)	25.56 (1.48 -306.67)	0.025 ^{b*}
	Formula Milk (n=17)	39.92 (0.41 – 443.16)	

Abbreviations: N.A: not applicable; LBW, low birth weight; VLBW, very low birth weight; ^aMann-Whitney test; ^bIndependent T-test; *p<0.05 indicates statistical significance.

Table 4. Multiple Linear Regression Analysis Results

Model	Variable	Unstandardized Coefficients		Standardized Coefficients	t	p-value
		B	Std. Error	Beta		
1	(Constant)	22.843	280.042		0.082	0.935
	Birth weight	-0.060	0.058	-0.232	-1.026	0.308
	Delivery method	60.273	34.243	0.201	1.760	0.082
	Feeding type	-4.106	22.031	-0.021	-0.186	0.853

Note: *p<0.05 indicates statistical significance.

Table 5. Comparison of fecal calprotectin in sepsis and non-sepsis neonates

Variable	Sepsis (n=40)	Non-sepsis (n=48)	p-value
	Median (min-Max)	Median (min-max)	
Fecal calprotectin	94.56 (0.81-432.08)	28.59 (0.41-443.16)	0.001*

Notes: Mann Whitney test; *p<0.05 indicates statistical significance.

in women under septic circumstances (22,23). However, studies suggest that sex distribution does not show significant differences between sepsis and non-sepsis (24). In this study, most of the neonates were born at late preterm gestational age, both in the sepsis and non-sepsis groups. The previous study stated that neonates with preterm labor are 3.36 times more likely to experience neonatal sepsis compared to a term neonate. This is because preterm neonates have an immature immune system (low neutrophil storage) and organs that fight infection (25). However, a meta-analysis study stated that gestational age had an insignificant effect on the frequency of neonatal sepsis (26). No correlation was found between gestational age and FC levels. It is possible that FC levels in infants before 34 weeks gestation remained constantly high and did not vary significantly throughout our measurements. Our findings add specificity to previous studies on FC levels and gestational age. In this study, most neonates had low birth weight in sepsis and non-sepsis groups. The previous research stated that neonates with birth weights less than 2.5 kg had a 1.42 times greater risk of neonatal sepsis infection than neonates with normal birth weights. This is because low-weight neonates are mostly born less than a month, have an immature immune system, are unable to suckle, quickly lose heat, have low glucose stores, and are more at risk of hypoglycemia, thus increasing the possibility of infection in neonates (25). Infants under one month of age, characterized by an underdeveloped intestinal mucosa and heightened intestinal permeability, may experience an augmented transepithelial migration of neutrophils, resulting in the secretion of calprotectin into the intestinal lumen. Despite increased study concerning calprotectin in recent years, there persists a deficiency of information regarding FC concentrations in term infants. Therefore, it is important to understand the dynamic pattern of FC and the factors involved in regulating FC, such as increased intestinal permeability and active immune response, factors such as maternal health, birth conditions, and early neonate care. These factors may vary between LBW and LBW infants, potentially contributing to differences in FC levels in LBW infants compared to LBW infants. Gut flora and

immune response may vary significantly between these two groups, potentially leading to higher FC levels in LBW infants (27). In this study, most neonates were born by cesarean section in the sepsis and non-sepsis groups. The delivery route can affect the diversity and nature of microbial flora in neonates. A previous study stated that the most common delivery route in neonatal sepsis patients was cesarean section (66.7%), but did not show a significant relationship between neonatal sepsis and birth route in term infants (9). Results are in line with other studies that vaginal delivery is associated with a lower incidence of neonatal sepsis; however, cesarean section is associated with a greater risk of sepsis (26). Early postnatal microbial colonization through vaginal or vaginal delivery may reduce susceptibility to infection (25). However, other studies indicate that vaginal birth correlates with an elevated likelihood of neonatal sepsis. This is because newborns delivered by cesarean section are not exposed to fecal and vaginal bacteria (27–29). Meanwhile, forceps-assisted vaginal delivery may increase the likelihood of neonatal morbidity and skull injury. The use of forceps during labor increases contact between external instruments, the fetus, and the maternal reproductive tract. Forceps-assisted delivery increases the chance of neonatal sepsis (20). This study showed a significant difference between infants who received breast milk and formula milk on FC levels. Breast milk has microbiota such as *Lactobacilli* and *Bifidobacteria*. The gut microbiota plays a crucial role in promoting the normal maturation of gut-associated lymphoid tissue, the synthesis and secretion of immunoglobulin A, and the regulation of a balanced T helper cell response. In addition, breast milk contributes to the immune system's development by way of the cytokines, hormones, and various immunomodulatory and growth factors present, including granulocyte colony-stimulating factor, epidermal growth factor, insulin-like growth factor, leptin, and ghrelin. Elevated concentrations of FC during the early stages of life may serve as an indicator of the physiological maturation of the immune system (30). This study showed that neonates with sepsis had greater FC than neonates without sepsis. The elevated concentration of calprotectin observed in cases of sepsis can be elucidated by the secretion of this protein

from monocytes and neutrophils into the extracellular space, or its liberation into pus following cellular necrosis or disruption. Calprotectin serves a pivotal function in the modulation of immune responses and is actively involved in inflammatory mechanisms as well as antimicrobial activities. Consequently, calprotectin is integral to the alterations in immune system functionality that are induced by sepsis (31). Calprotectin assumes a crucial role within the human innate immune response to bacterial proliferation and may further influence the inflammatory cascade subsequent to its release. The levels of extracellular calprotectin are elevated during episodes of infection and inflammation, thus serving as an important biomarker indicative of neutrophil activation (32). Calprotectin release is also involved in the inflammatory process by increasing CD11b expression in human monocytes and participating in transendothelial migration mechanisms (6). Calprotectin release from neutrophils is caused by inflammatory cell infiltration and LPS. LPS affects PMN function and stimulates inflammatory cytokines, including IL1 β , TNF, and IL-6 from PMN and monocytes (33). This study provides new information on FC concentration between preterm with and without sepsis. We employed noninvasive tools and procedures in this study. However, our study has limitations: there is still a lack of analysis of factors that affect FC levels in neonates, such as infections suffered by mothers and drugs consumed by mothers (corticosteroids). Therefore, we recommend further study on using FC as a marker of sepsis in neonates.

Conclusion

The study concluded that preterm neonates diagnosed with sepsis exhibited significantly higher FC levels compared to those without sepsis. This finding suggests that FC could serve as a potential biomarker for identifying sepsis in preterm infants. The findings suggest that measuring FC levels could be beneficial in clinical settings for the early detection of sepsis in preterm neonates, potentially leading to timely interventions and improved outcomes.

Acknowledgements: The authors would like to express sincere thanks to the participants in this study. The authors acknowledge all the staff department of Pediatrics, Hasanuddin University, Makassar, Indonesia.

Funding: This research received no external funding.

Ethic Committee: All research designs were reviewed and approved by the Health Research Ethics Committee of Dr Wahidin Sudirohusodo Hospital – Faculty of Medicine, Hasanuddin University (Protocol no. UH24020071).

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Authors' Contribution: LIL: Concept, Design, Resources, Materials, Data Collection and Processing, Analysis, Literature Search, Writing Manuscript; ADB, EA, AJA, HA: Concept, Design, Supervision, Analysis, Literature Search; JS: Concept, Design, Analysis, Critical Review. All authors read and approved the final version of the manuscript.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Received: 20 December 2024

Accepted: 29 January 2025

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