HK J Paediatr (new series) 2024;**29**:74-81

Original Article

Correlation of Prenatal and Perinatal Factors with Meconial Calprotectin in Preterm and Healthy-term Newborns

J **Cekovic**, S **Knezevic**, R **Medovic**, B **Vuletic**, Z **Igrutinovic**, M **Stanojevic**, N **Prodanovic**, T **Prodanovic**, AM **Simovic**

Abstract ***Introduction:*** The fundamental goal of this study was to examine prenatal and perinatal factors that may affect the excretion of faecal calprotectin (FC) in healthy-term newborns and premature infants. ***Methods:*** The total number of surveyed newborns was 136 (91 preterm and 45 born in term). FC levels were analysed from the meconium with conventional denzyme-linked immunosorbent assays method on the automated Alegria® system. ***Results:*** Significantly higher FC levels were observed among the infants born prior to ≤31 gestational weeks, who had a 5-minute Apgar score ≤6 at birth, with leukocyte count >30x10^9/L, the enteral feeding <30 ml/kg/24h, with a maximum total bilirubin in serum >250 μmol/L and certain transient metabolic disturbances. ***Conclusion:*** While interpretation the FC measurements, one should take into account the gestational age, the presence of asphyxia syndrome or inflammation, as well as other accompanying disorders such as enteral feeding intolerance, significant bilirubinaemia, and transient metabolic disturbances.

Key words *Faecal calprotectin; Intestinal distress; Newborn; Perinatal factors*

University Clinical Centre of Kragujevac, Paediatric Clinic; and University of Kragujevac, Faculty of Medical Sciences, Department of Paediatrics, Serbia

J **Cekovic** *MD*
S **Knezevic** *MD, PhD*
R **Medovic** *MD, PhD*
B **Vuletic** *MD, PhD*
Z **Igrutinovic** *MD, PhD*
T **Prodanovic** *MD*
AM **Simovic** *MD, PhD*

University Clinical Centre Kragujevac, Clinic for Gynaecology and Obstetrics, Department of Neonatology, Serbia

M **Stanojevic** *MD*

University of Kragujevac, Faculty of Medical Sciences, Department of Surgery; and University Clinical Centre Kragujevac, Clinic for Orthopaedic and Trauma Surgery, Serbia

N **Prodanovic** *MD, PhD*

Correspondence to: Dr N **Prodanovic**
Email: nikolaprodanovickg@gmail.com

Received February 7, 2022

Introduction

For the past 20 years, calprotectin has been evolved as a new, non-invasive biomarker of the local gastrointestinal (GI) inflammation. In the published studies, calprotectin was found not only in faeces, but in various body fluids as well, such as the following: serum, plasma, urine, saliva and synovial fluid, which led to the variations in its expression, with a certain potential to be used for the disease or condition monitoring even outside the GI tract, in case that they have an inflammatory component.[1,2]

As regards the interpretation of obtained results, a difficulty can arise due to the variability in the values of faecal calprotectin (FC) in the control population of healthy children.[1,2] The abovementioned limitations, which need to be defined and acknowledged before the interpretation of the FC results, include: variability in extraction methodology, performance of test kits, gender, age, nutrition type, use of certain medications, and secondly, the need to establish local reference ranges.[2,3] Currently, FC concentration <50 μg/g faeces is the normal range for FC in healthy adult population. However, an

increasing number of authors indicate that the test appears to have better diagnostic precision for inflammatory bowel disease at a cut-off of 100 μg/g.[2,4]

Abnormal intestinal colonisation, gut microbiota imbalance, abnormal immune responses and tolerance mechanisms can result in enteral feeding intolerance in the postnatal period, and gastrointestinal diseases in the childhood as well.[5-8]

Necrotising enterocolitis (NEC), an intestinal inflammatory disease predominantly seen in preterm infants, is characterised by a high mortality rate and clinically significant long-term consequences. Until recently, clinical presentation and radiological findings have been essential in the diagnosis of NEC, despite the fact that they are not specific and that until the moment they become obvious NEC is already in the most advanced stage. During its early phase, FC, which is secreted into the intestinal lumen because of mucosal damage, and is considered a rapid, non-invasive test to confirm inflammation or intestinal distress, could warn neonatologists of the risk of developing NEC with consecutive serial measurements or it could make it easier to differentiate NEC from enteral feeding intolerance.[9] However, the clinical use of FC in the neonatal population has not been precisely defined yet[10] which mean that the fundamental goal of this study is to examine perinatal factors that may affect the excretion of FC in the initial 5 days after birth.

Materials and Methods

Study Type

Data were prospectively collected over a period of time from January 2019 to September 2020, at the Centre for Neonatology of the Paediatric Clinic and at the Gynaecology and Obstetrics Clinic, University Clinical Centre "Kragujevac", Kragujevac, Serbia. After the data collection, a retrospective observational ("case-control") study was conducted. The study subjects were selected by means of random sampling. Ethics approval for this study was obtained from the Ethics Committee of the Clinical Centre Kragujevac (No. 01/19/1973, May 13, 2019), and Faculty of Medical Sciences, University of Kragujevac, Serbia (No. 01-8092, July 01, 2019).

Research Subjects

The experimental group was comprised of preterm infants, if they met the following criteria: gestational age (GA) <37 weeks; absence of any other diseases, apart from those related to premature birth (hyaline membrane disease, ductus arteriosus, anaemia, etc.); adapted dairy formulas to the nutritional requirements of the preterm infants, with or without partial parenteral nutrition (Vaminolact and Soluvit); postnatal age <5 days of life at the moment of the first stool collection.

The control group consisted of healthy-term newborns, from the heathy controlled pregnancies, and breastfed by their own mothers, at the age of <5 days at the moment of the first stool collection.

Exclusion criteria: death in the first 24 hours of life; gestational age <24 and >42 gestational weeks; postnatal age ≥5 days of life at the moment of the first stool collection; gastrointestinal and other congenital anomalies; positive blood culture; chromosomal aberrations; inherited metabolic diseases; transportation into a higher-rank institution.

Collection and Handling of Stool Samples

For the purpose of conducting the current study, we used the data from epacrises documents and other available medical documentation. Additionally, the abovementioned data were used during routine procedures conducted for the purpose of early screening, diagnosis and treatment of newborns, at the Clinical Centre Kragujevac, University of Kragujevac, Serbia.

Calprotectin levels were analysed from the meconium stool in all the newborns included in the study at less first 5 days of life. One sample of spontaneous stool was collected in the morning (up to 10 hours) for each participant. Stool samples were not stored in the refrigerator. Values of FC measurements were analysed immediately by means of enzyme-linked immunosorbent assays methods on the automated Alegria® system (24 Alegria® test strips with a range of 0-1000 μg/g of faeces).

Complete blood count (CBC) parameters were measured by using capillary blood, by means of the NIHON KOHDEN Celltac Es MEK-7300 or BECKMAN COULTER haematology analysers.

- Examination of immature-to-total neutrophil ratio (I/T ratio) was conducted in peripheral blood smears, coloured by using May-Grünwald-Giemsa stain, and observed under a NIKON ECLIPSE E400 microscope;
- Serum urea, creatinine and C-reactive protein (CRP) levels were measured by immunoturbidimetric assays on an Olympus AU 400 biochemistry analyser;
- Procalcitonin (PCT) was determined by Electrochemiluminescence Immunoassay, conducted using the COBAS e411 analyser.

- pH, BE, HCO3, lactate, glycaemia, sodium (Na+), potassium (K+), ionised calcium (Ca++) and glycaemia were determined using arterialised capillary blood, by means of the GEM Premier 300 system; blood was taken for laboratory processing at the same time as the stool was collected.
- Blood culture (taken at the time of admission to the intensive care unit). In the Laboratory of Microbiology at the Clinical Centre Kragujevac, blood sample analysis is usually conducted by culturing the blood under special nutritional conditions for culture; by means of performing a complex Gram stain technique.
- After admission to the neonatal intensive care unit, all the preterm infants were fed a reconstituted formula for preterm infants, as they were transported from 12 distant maternity hospitals. Administration of enteral feed was delayed for 24-48-72 hours and introduced after stool sampling for faecal calprotectin detection for extremely premature infants, as well as those with severe asphyxia, altered consciousness, severe respiratory distress syndrome (RDS), or gastric residual volume >1-2 ml/kg. In stable preterm infants, feeding was initiated after the 6th hour of life, following the minimal enteral intake principle; 2-3 mL per feeding (<1500 g) or 5 mL per feeding (1500-2000 g) given as a bolus every 3 hours. With the achievement of feeding tolerance, the intake was gradually increased by 3-4 mL per feeding (<1500 g) or 5-10 mL per feeding (1500-2000 g), every day.

Statistical Analyses

Statistical data analysis was performed using the IBM® SPSS® Statistics 22. Continuous variables were presented as mean and standard deviation, while categorical variables were presented as frequencies and percentages. The Kolmogorov-Smirnov test was used to examine distribution of continuous variables. Taking into consideration the normal distribution, we used the parametric method (Independent Samples Test) in order to determine whether there was a statistically significant difference between the two groups of data being tested. The Chi-Square Test was used to compare categorical variables. The Pearson Correlation revealed associations among examined continuous variables, whereas the Spearman Correlation measured the strength of associations among examined categorical variables. In all statistical tests, a p-value less than 0.05 ($p<0.05$) was regarded as statistically significant. After the statistical data analysis, the results were displayed in tables.

Results

The total number of observed children was 136 (91 preterm and 45 born in term), with an equal distribution of male and female newborns. The average age in the term newborn group at the time of faeces sampling was 2.125±0.934 days, and in the preterm group, it was 2.664±0.963 days. There was no statistically significant difference between the observed groups in terms of gender and age; in other words, the observed groups were homogeneous.

The distribution of subjects according to body weight at birth is shown in Table 1. No were newborns with birth weight greater than 4500 g, not even below 980 g. When comparing the mean values of FC levels, no statistically significant differences were observed among the studied groups of newborns with different birth weight (BW).

Ninety-one or 66.9% of the surveyed children were born preterm, 35/136 (25.7%) of whom were born prior to 32 weeks of gestation, whereas 10/136 (7.3%) of the children were extremely immature. In the surveyed sample, there were no infants born after 42 gestational weeks (GW). When comparing the mean values of FC levels, significantly higher FC levels were observed among the infants born prior to ≤31 GW. Significant difference was observed among the surveyed newborns who had a 5-minute Apgar score ≤6 at birth, compared with infants whose 5-minute Apgar score was ≥7 (Table 1).

It was observed that the nutrition type (breastfeeding, nasogastric/orogastric feeding tube, specially adapted preterm formula or breastfeeding) had no effect on FC levels. However, enteral feeding volume was shown to be more effective (Table 1). FC in levels were reported to be the highest in infants fed on milk with less than 30 ml/kg bw/day, in the first 5 days of life (Table 1). The subjects (14/136 or 10.3%) who had significantly higher calprotectin levels (141.9±92.8 μg/g faeces) in the first 3 days of life, after the fifth day had increased gastric residue, abdominal distension, or frequent vomiting and required the use of antibiotics.

The number of children born in a vaginal delivery was approximately equal to the number of children born by Caesarean delivery (C-section), whereas there were no subjects delivered by forceps or vacuum extraction. FC levels were shown not to correlate with the ending of

delivery. In addition, it was established that the presence of meconium in the amniotic fluid (16.2% of the subjects) and preterm premature rupture of the membranes (12.5% of the subjects) did not correlate with the FC levels. Moreover, diseases during pregnancies (arterial hypertension, anaemia, hypothyroidism) along with prescription drug use in the prenatal period (antibiotics, Utrogestan, Methyldopa, Dexason, iron (Fe) preparations) did not have a statistically significant impact on the FC levels after birth.

Meconium samples taken from 89% of the surveyed children were submitted to the laboratory for testing, whereas only 5.1% of the subjects had liquid stool with mucus in it. The majority of tests performed on faeces stool samples were done on days 2 (37.5%) and 3 (28.7%) of life. However, not even the visual appearance of stool nor the time of stool sample collection correlated with the FC level.

29.4% of the surveyed children showed signs of RDS, whereas the application of conventional mechanical ventilatory support was required in 19.1% of subjects. As regards the FC levels, there was no statistically significant difference observed among the groups of children with RDS and those receiving mechanical ventilatory support, compared with the group of children without any respiratory symptoms.

Regardless of the small sample size, there was no statistically significant difference observed in the FC level among the children with ductus arteriosus persistent (10.3%) or without it (80.8%). Necrotising enterocolitis symptoms were not found in children included in the examined sample.

Comparing reference CBC values of the surveyed children, we established that the only statistically significant correlation was found between total leukocyte count and FC levels. Significantly higher calprotectin levels were observed in the group of subjects with total leukocyte count greater than $20x10^9/L$ (Table 2). Haemoglobin levels, or more precisely, anaemia (regardless of the need for transfusion), had no impact on FC levels (FC level was 108.4±91.3 in the group of children with anaemia, compared with 114.4±84.5 reported in the group with normal haemoglobin values; p=0.120). Similarly, the same was valid for children with thrombocytopenia (104.4±83.3), compared with the group with normal platelet count (94.4±72.5; p=0.235).

In addition, there was no correlation found between FC levels and systemic inflammatory response syndrome. 21.1% had CRP levels greater than 10, 24.8% had elevated procalcitonin levels (PCT levels were correlated according to days after birth), whereas 15.3% of the subjects had positive blood culture. There was no statistically

Table 1 Faecal calprotectin means values in different groups according to birth weight, gestational weeks and, 5-minute Apgar score in the surveyed newborns

BW (in grams)	<1500	1500-2500	2500-3500	3500-4500	>4500
N (%)	16/136 (11.8)	52/136 (38.2)	44/136 (32.3)	24/136 (17.6)	0
FC (mean±SD)	108.4±76.4	122.7±83.5	113.8±74.2	111.3±89.1	/
p value	**p=0.91**				
Gestational age (weeks)	<28	28-31	32-34	35-36	37-41
N (%)	10/136 (7.3)	25/136 (18.4)	26/136 (19.1)	30/136 (22.0)	45/136 (33.1)
FC (mean±SD)	119.7±69.2	123.7±88.5	63.6±57.2	77.3±76.4	85.1±69.3
p value	**p<0.05**				
Apgar score	≤6	7	8	9	10
N (%)	12.4	11.1	31.2	32.3	22.4
FC (mean±SD)	136.3±79.8	101.2±85.3	97.6±78.4	99.2±85.6	98.1±89.2
p value	**p<0.05**				
Enteral feeding volume (ml/kg/24h)	<30	30-60	60-90	90-120	>120
N (%)	10.3	32.8	27.5	29.4	0
FC (mean ±SD)	141.9±92.8	102.3±85.4	104.4±79.1	91.3±80.3	/
p value	**p<0.05**				

N-number of respondents;

FC-faecal calprotectin in μg/g;

BW-body weight

significant correlation observed between the laboratory reference values for liver function parameters (AST levels (24.1%) and ALT levels (13.8%) were greater than 50 IU/I), kidneys (creatinine - 3.7% of the patients in whom ABI was measured) and FC levels, regardless of the small number of subjects.

The highest FC levels were reported in newborns with severe acidosis at birth (Table 2), but this result had to be accepted with reserve, considering the fact that the FC level greater than 1000 was present in 2 out of 10 surveyed children with severe acidosis. Furthermore, significantly elevated FC levels were observed in the group of newborns with maximum serum total bilirubin value greater than 250 µmol/L (Table 2).

Partial pressure of oxygen and carbon dioxide wasn't shown to be correlated with FC levels. Additionally, electrolyte disorders (of sodium, potassium and bicarbonate) did not have an impact on FC levels (Table 3).

On the other hand, significantly elevated FC levels were observed in the group of newborns with transient hypocalcaemia, compared with the group of newborns with normal levels of ionised calcium (Table 4). Regarding serum blood glucose levels, there were significantly elevated FC levels observed in the group of premature infants with hyperglycaemia, whereas significantly elevated FC levels were reported in the group of mature infants with hypoglycaemia (Table 4).

Table 2 Faecal calprotectin mean values in different groups according to various laboratory reference values in the surveyed newborns

The total leukocyte count (x10^9/L)	<10	10-20	20-30	>30
N (%)	36/136 (26.5)	71/136 (52.2)	15/136 (11.0)	14/136 (10.2)
FC (mean±SD)	109.7±83.2	102.7±75.2	139.3±94.2	147.2±101.8
p value	**p<0.05**			
pH value	<7.10	7.10-7.30	7.30-7.50	>7.50
N (%)	10/136 (7.3)	25/136 (18.3)	93/136 (68.3)	8/136 (5.8)
FC (mean±SD)	178.2±108.3	101.2±87.5	93.6±77.2	97.3±76.4
p value	**p<0.05**			
t-bilirubin (mean±SD)	>300	250-300	150-250	<150
N (%)	11/136 (8.0)	14/136 (10.2)	61/136 (44.8)	50/136 (36.7)
FC (mean±SD)	141.6±92.1	135.1±86.2	93.7±81.4	94.2±81.6
p value	**p<0.05**			

FC-faecal calprotectin in µg/g;

t-bilirubin (maximum serum total bilirubin value in µmol/L)

Table 3 Faecal calprotectin in different groups according to electrolyte levels in the surveyed newborns

Sodium levels (mmol/L)	<120	120-135	135-145	>145
N (%)	7/136 (5.1)	9/136 (6.6)	120/136 (88.2)	0
FC (mean±SD)	105.2±91.8	104.2±88.2	109.4±93.6	/
p value	**p=1.83**			
Potassium levels (mmol/L)	<3.0	3.0-3.6	3.6-5.1	>5.1
N (%)	10/136 (7.3)	21/136 (15.4)	78/136 (57.3)	27/136 (19.8)
FC (mean±SD)	95.3±78.6	101.3±82.5	98.1±83.5	96.9±88.5
p value	**p=0.78**			
Bicarbonate levels (mmol/L)	<15	15-24	24-32	>32
N (%)	20/136 (14.7)	25/136 (18.3)	86/136 (63.2)	5/136 (3.6)
FC (mean±SD)	100.7±90.2	104.1±86.2	103.2±85.3	98.9±89.2
p value	**p=1.04**			

FC-faecal calprotectin in µg/g

Discussion

It is well-known that local inflammation in the GI tract, bacterial or fungal infections, impairment of mucous membranes, intestinal bleeding (caused by stressogenic factors or non-steroidal anti-inflammatory drugs cause an increase in calprotectin concentrations in stool.[1,2,10-12] Its reference values are not precisely defined in the neonatal population, nor is it defined whether there are some pre-, peri-, or postnatal risk factors that can lead to variations in its concentration. Most of the authors,[1,4,11-13] do not observe any significant differences in FC levels compared with gender, body weight, gestational age of subjects, and the mode of delivery (vaginal delivery, "Caesarean section"), which is consistent with our study results.

During our study, there was no significant correlation between the day of faecal sampling and calprotectin levels in the first 5 days, in both observed groups. When repeating calprotectin sampling in the third week of life, preterm infants had significantly lower calprotectin levels, while term newborns could not undergo repeated sampling due to short hospitalisation duration. Yoon et al[13] noted a clear negative linear correlation between calprotectin levels and number of days after birth (p=0.03) (newborns with a gestational age ≥26 weeks but <30 weeks). In addition, Zoppelli et al[14] observed a decrease in FC levels during the first week of life; however, afterwards there was a statistically significant increase in those born between 26 and 32 weeks GA, compared to those born at less than 26 weeks GA who continued to decline. Likewise, it was in our current study that we observed significantly higher calprotectin concentrations in children ≤31 6/7 GA, but one had to take into account a wide range of FC values and a small sample of subjects.

CRP and procalcitonin are examples of other markers that alert clinicians of ongoing inflammatory processes in the body. Gray et al analysed serum and sputum samples during cystic fibrosis exacerbation and noticed that serum calprotectin predicted median time to exacerbation significantly better than CRP.[15] Similarly, in patients with rheumatic disease, calprotectin concentrations, but not CRP, were significantly lower in those with no swollen joints compared to those with one or more swollen joints.[10] Terrin et al[16] while evaluating serum calprotectin levels as a diagnostic marker for sepsis in infants, observed significantly higher serum concentrations (p<0.001) in 62 newborns with confirmed sepsis (3.1±1.0 mg/L) than in either 29 non-infected subjects (1.1±0.3 mg/L) or 110 healthy controls (0.91±0.58 mg/L). Calprotectin showed greater sensitivity (89%) and specificity (96%) than common laboratory inflammation markers, such as white blood cell count (WBC) and CRP. Accordingly, a relationship between FC and WBC was confirmed in our paper, followed by our observation of the relationship between FC and other haematology parameters: haemoglobin, haematocrit and platelet count, markers of acute inflammation (CRP and randomised controlled trials), kidney or hepatic function, which was consistent with the results obtained by Yoon et al.[13] In the published literature, a relationship between an increased FC level and WBC was most commonly explained by frequent migration of white blood platelets due to the intestinal inflammation, that is, there was a significant direct correlation found between faecal calprotectin

Table 4 Faecal calprotectin mean values in different groups according to glycaemia and ionised calcium levels in the surveyed newborns

Glycaemia (mmol/L) premature infants	<2.0	2-3.5	3.5-7	>7
N (%)	9/91 (9.8)	19/91 (20.8)	49/91 (53.8)	14/91 (15.3)
FC (value±SD)	105.6±93.2	101.2±85.1	103.4±92.4	154.3±111.9
p value	**p<0.05**			
Glycaemia (mmol/L) mature infants	<2.8	2.8-3.5	3.5-7	>7
N (%)	4/45 (8.8)	8/45 (17.7)	30/45 (66.6)	3/45 (6.6)
FC (mean±SD)	163.2±118.6	100.1±86.5	98.6±87.2	97.5±82.4
p value	**p<0.05**			
Ionised calcium levels (mmol/L)	<0.80	0.80-1.00	1.00-1.30	>1.30
N (%)	17/136 (12.5)	22/136 (16.1)	97/136 (71.3)	0
FC (mean±SD)	140.7±90.6	137.8±96.1	78.2±75.3	/
p value	**p<0.05**			

FC-faecal calprotectin in μg/g

concentration and the severity of small intestine inflammation.[12,13]

Accordingly, Campeotto et al showed calprotectin could be used an acute marker of intestinal distress.[17] They defined "sick" infants as those being evaluated for sepsis, requiring antibiotics or vasopressors, withholding enteral feeds, or requiring increased ventilator support. "Sick" infants showed higher FC concentrations (380.4±246.3 µg/g) versus "not sick" (122.8±98.9 µg/g, ($p<0.001$)). Similarly to other authors, in our research, an increase in faecal calprotectin was reported in premature infants with enteral feeding intolerance:[2,13,17,18] enteral feeding <30 ml/kg/24h; perinatal asphyxia: Apgar score ≤6, metabolic acidosis (pH≤7.1), or pronounced hyperechogenicity on transfontanellar neurosonography and other signs of organ hypoxia, transient hypo or hyperglycaemia, transient hypocalcaemia (Ca++ <1 mmol/L) or high total bilirubin (maximum serum total bilirubin value greater than 250 µmol/L).

Generally, all studies conducted on newborns[13,14,18] whether born full-term or preterm, have shown high intra- and inter-individual variations in faecal calprotectin values, reflecting variations in calcium-binding protein. Its correlation with observed variables is often confusing and contradictory, depending on the authors. These variations in FC values can be partly attributed to the sampling method, especially if the stool sample remains in the diaper for an extended period. However, Olafsdottir and colleagues[19] have shown that this sampling method, where diapers absorb some water, increases calprotectin concentration by up to 30%, indicating lower variation than measured in different studies. Other hypotheses can also be proposed. The increase in calprotectin levels in the faeces of newborns reflects an increase in granulocytes in the intestinal lumen due to high intestinal permeability, and inter-individual variations may also be related to other environmental factors (diet, colonisation of the intestine by bacteria or fungi, reaction to antigens) or conditions. Gestational diabetes (GDM) was recorded in 7/45 (15.55%) term newborns' mothers, which may explain the association between hypoglycaemia and high FC values. Novel studies have shown that GDM may be associated with disordered gut microbiota in both (mothers and newborns).[20] In the preterm group, hypo/hyperglycaemia was mostly a consequence of infection or perinatal stress.

Bilirubin is known to be a toxic metabolite that should be eliminated from the body through urination or defecation. Unconjugated bilirubin was observed to activate and damage microglia cells, releasing high levels of TNF-α, IL-1β, and IL-6 in a concentration-dependent manner, and in vitro increases the permeability of intestinal epithelium.[21] The impact of biologically active substances on calprotectin levels in faeces can be complex and may vary depending on the specific context or underlying condition. Therefore, further research is needed to better understand the precise mechanisms and interactions between bilirubin and FC. Yoon et al found significantly higher values of total bilirubin and FC in very low birth weight and premature newborns with necrotising enterocolitis.[13] However, it is also possible that there is a random correlation, as indirect hyperbilirubinemia is very common in newborns (especially those born preterm). This is somewhat supported by the fact that found no significant connection between phototherapy and faecal calprotectin levels.[22]

In adults, calprotectin is increased in individuals with insulin resistance and diabetes, indicating that certain metabolic disorders may be associated with an increase in FC if there is systemic inflammation.[23] Other studies link non-intestinal inflammatory conditions to an increase in FC in children and adults, so large multicentre studies should be conducted to monitor newborns with high FC values and metabolic imbalance in the long term.

Conclusion

High levels of calprotectin, in the first 5 days of life can generally indicate active inflammation in the body, which may not be specific to a certain condition but also be a sign of intestinal distress in newborns. While interpreting the FC-values, one should consider the gestational age of a newborn, the presence of perinatal asphyxia or intestinal infection/inflammation, as well as other accompanying problems of immaturity, such as enteral feeding intolerance, significant bilirubinaemia, and transient metabolic disturbances. On the other hand, variability in the methodology of its extraction and different performances of kits for its detection may somewhat interfere with its diagnostic accuracy.

Conflicts of Interest

None of the authors declares any conflict of interest.

Acknowledgments

This work was supported by the Faculty of Medical Sciences (JP 09/22), University of Kragujevac, Serbia.

References

1. Herrera OR, Christensen ML, Helms RA. Calprotectin: Clinical applications in pediatrics. J Pediatr Pharmacol Ther 2016; 21:308-21.
2. Koninckx CR, Donat E, Benninga MA, et al. The use of fecal calprotectin testing in paediatric disorders: A position paper of the European Society for Paediatric Gastroenterology and Nutrition Gastroenterology Committee. J Pediatr Gastroenterol Nutr 2021;72:617-40.
3. Beşer ÖF, Sancak S, Erkan T, Kutlu T, Çokuğraş H, Çokuğraş F. Can fecal calprotectin level be used as a markers of inflammation in the diagnosis and follow-up of cow's milk protein allergy? Allergy, Asthma Immunol Res 2014;6:33-8.
4. Konikoff MR, Denson LA. Role of fecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease. Inflamm Bowel Dis 2006;12:524-34.
5. Rougé C, Butel MJ, Piloquet H, et al. Fecal calprotectin excretion in preterm infants during the neonatal period. PLoS ONE 2010;5:e11083.
6. Ignacio L, AlFaleh K. Feeding intolerance in preterm infants fed with powdered or liquid formula: A randomized controlled, double-blind pilot study. J Clin Neonatol 2013;2:11-3.
7. Moore TA, Wilson ME. Feeding intolerance. Adv Neonatal Care 2011;11:149-54.
8. Indrio F, Riezzo G, Cavallo L, Mauro AD, Francavilla R. Physiological basis of food intolerance in VLBW. J Matern Fetal Neonatal Med 2011;24:64-6.
9. Agakidou E, Agakidis C, Gika H, Sarafidis K. Emerging biomarkers for prediction and early diagnosis of necrotizing enterocolitis in the era of Metabolomics and Proteomics. Front Pediatr 2020;8:602255.
10. Park JS, Cho JY, Chung C, et al. Dynamic changes of fecal calprotectin and related clinical factors in neonates. Front Pediatr 2020;8:326.
11. Pergialiotis V, Konstantopoulos P, Karampetsou N, et al. Calprotectin levels in necrotizing enterocolitis: A systematic review of the literature. Inflamm Res 2016;65:847-52.
12. Kapel N, Campeotto F, Kalach N, Baldassare M, Butel MJ, Dupont C. Faecal calprotectin in term and preterm neonates. J Pediatr Gastroenterol Nutr 2010;51:542-7.
13. Yoon JM, Park JY, Ko KO, Lim JW, Cheon EJ, Kim HJ. Fecal calprotectin concentration in neonatal necrotizing enterocolitis. Korean J Pediatr 2014;57:351-6.
14. Zoppelli L, Güttel C, Bittrich HJ, Andrée C, Wirth S, Jenke A. Fecal calprotectin concentrations in premature infants have a lower limit and show postnatal and gestational age dependence. Neonatology 2012;102:68-74.
15. Gray RD, Imrie M, Boyd AC, Porteous D, Innes JA, Greening AP. Sputum and serum calprotectin are useful biomarkers during CF exacerbation. J Cyst Fibros 2010;9:193-8.
16. Terrin G, Passariello A, Manguso F, et al. Serum calprotectin: an antimicrobial peptide as a new marker for the diagnosis of sepsis in very low birth weight newborns. Clin Dev Immunol 2011;2011:291085.
17. Campeotto F, Kalach N, Lapillonne A, Butel MJ, Dupont C, Kapel N. Time course of faecal calprotectin in preterm newborns during the first month of life. Acta Paediatr 2007;96:1531-3.
18. Yang Q, Smith PB, Goldberg RN, Cotten CM. Dynamic change of fecal calprotectin in very low birth weight infants during the first month of life. Neonatology 2008;94:267-71.
19. Olafsdottir E, Aksnes L, Fluge G, Berstad A. Faecal calprotectin levels in infants with infantile colic, healthy infants, children with inflammatory bowel disease, children with recurrent abdominal pain and healthy children. Acta Paediatr 2002;91:45-50.
20. Li X, Yu D, Wang Y, et al. The Intestinal Dysbiosis of Mothers with Gestational Diabetes Mellitus (GDM) and Its Impact on the Gut Microbiota of Their Newborns. Can J Infect Dis Med Microbiol 2021;2021:3044534.
21. Raimondi F, Crivaro V, Capasso L, et al. Unconjugated bilirubin modulates the intestinal epithelial barrier function in a human-derived in vitro model. Pediatr Res 2006;60:30-3.
22. Bukulmez A, Dogru O, Kundak AA, et al. The effect of phototherapy on fecal calprotectin levels. Am J Perinatol 2013;30:215-8
23. Ortega FJ, Sabater M, Moreno-Navarrete JM, et al. Serum and urinary concentrations of calprotectin as markers of insulin resistance and type 2 diabetes. Eur J Endocrinol 2012; 167:569-78.