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Fecal calprotectin, lactoferrin and morbidity associated with immature digestive tract in preterm infants

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Introduction. Excessive intestinal inflammation in preterm infants is one of the key factors in the development of necrotizing enterocolitis (NEC), early- (EOS) and late-onset neonatal sepsis (LOS).

Purpose — to evaluate the connection between fecal calprotectin (FC), enteral use of lactoferrin (LF), and the occurrence of NEC, EOS, and LOS in preterm infants.

Materials and methods. FC was measured in 2@ newborns with gestational age (GA) y32 weeks and birth weight y1500 g. Feces were collected twice: in the first 7 days of life and at the postmenstrual age (PMA) of 3@ weeks. The main group included 15 infants with either EOS, LOS or NEC. The remaining 11 infants formed the comparison group. Eleven infants received LF (4 in the main group and 7 in the comparison group), which was randomly administered in the first 3 days of life.

Results. FC in the first 7 days of life was higher in the main group (p>0.05). At the PMA of 3@ weeks, FC decreased in the main group and increased in the comparison group (p>0.05). FC in the first week of life was higher in infants with EOS compared to newborns without the diseases (p=0.03), followed by a decrease at the PMA of 3@ weeks (p=0.04). There was no significant difference in FC levels depending on the development of LOS or NEC. FC levels increased in all infants who received LF and decreased in babies who did not receive LF (p>0.05).

Conclusions. The occurrence of EOS is associated with a significant increase in FC which subsequently decrease by the PMA of 3@ weeks. FC in the first week of life are not associated with the development of NEC or LOS. Enteral use of LF at a dose of 100 mg/day is associated with an increase in FC levels (p>0.05).

The research was carried out in accordance with the principles of the Helsinki Declaration. The study protocol was approved by the Local Ethics Committee of the participating institution. The informed consent of the patient was obtained for conducting the studies.

No conflict of interests was declared by the authors.

Keywords: fecal calprotectin, lactoferrin, neonatal sepsis, necrotizing enterocolitis, preterm infants.

Фекальний кальпротектин, лакто$ерин і захворюваність, пов'Язана з недорозвиненістю травного каналу, у передчасно народжених немовлЯт

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Вступ. Надмірна запальна відповідь у травному каналі передчасно народжених немовлят є одним із ключових патогенетичних чинників виникнення некротизуючого ентероколіту (НЕК), а також раннього (РНС) і пізнього (ПНС) неонатального сепсису.

Мета — оцінити зв'язок між рівнями фекального кальпротектину (ФК), ентеральним застосуванням лактоферину (ЛФ) і виникненням НЕК, РНС, а також ПНС у передчасно народжених немовлят.

Матеріали та методи. У 2@ дітей із когорти передчасно народжених немовлят із терміном гестації у32 тиж та масою тіла при народженні у1500 г визначали кальпротектин у калі. Матеріал збирали двічі: у перші 7 діб життя і на момент досягнення постменструального віку (ПМВ) 3@ тиж. Основну групу сформували 15 немовлят, які хворіли на РНС, ПНС або НЕК. Решта 11 дітей увійшли до групи порівняння. Одинадцятеро дітей отримували ЛФ (4 дитини з основної групи і 7 дітей у групі порівняння), якій рандомізовано призначали в перші 3 доби життя.

Результати. ФК в перші 7 діб життя був вищим у немовлят з основної групи (p>0,05). На момент досягнення ПМВ 3@ тиж вміст ФК знижувався у немовлят з основної групи і зростав у дітей із групи порівняння (p>0,05). Вміст ФК в перший тиждень життя був значно вищим у дітей з РНС порівняно з немовлятами без захворювань (p=0,03), з подальшим зниженням на момент досягнення ПМВ 3@ тиж (p=0,04). Концентрації ФК суттєво не відрізнялися залежно від розвитку ПНС або НЕК. Рівні ФК зростали у всіх немовлят, які отримували ЛФ, та знижувався у дітей, які не отримували ЛФ (p>0,05).

Висновки. Виникнення РНС асоціюється зі значним підвищенням рівнів ФК з наступним їх зниженням на момент досягнення ПМВ 3@ тиж. Показники ФК на першому тижні життя не пов'язані з розвитком НЕК або ПНС. Ентеральне застосування ЛФ у дозі 100 мг/добу асоціюється з підвищеннями рівнів ФК (p>0,05).

Дослідження виконано відповідно до принципів Гельсінської декларації. Протокол дослідження ухвалено Локальним етичним комітетом зазначеної в роботі установи. На проведення досліджень отримано інформовану згоду батьків дітей.

Автори заявляють про відсутність конфлікту інтересів.

Ключові слова: фекальний кальпротектин, лактоферин, неонатальний сепсис, некротизуючий ентероколіт, передчасно народжені діти.

Introduction

Morbidity associated with an immature digestive tract is one of the most important causes of mortality in very low birth weight (BW) <1500 g infants. Excessive inflammation in combination with insufficient local immunity, increased permeability of the intestinal mucous membrane, reduced motor function and insufficient blood supply to the intestines, as well as enteral nutrition of preterm infants in neonatal intensive care units (NICU) are associated with the occurrence of necrotizing enterocolitis (NEC) [15,23]. These factors can also play

a critical role in the pathogenesis of other diseases, primarily sepsis, due to damage to the mucous membrane of the intestines and the subsequent entry of microorganisms into the bloodstream [17].

Fecal calprotectin (FC) is a protein that makes up to 60% of the soluble protein in human neutrophils, it is also found in monocytes, macrophages and epithelial cells [28]. FC is released during the inflammatory process in the digestive tract and is easily detected in feces. Due to the presence of calcium, the structure of calprotectin can remain stable in feces for up to 7 days [8]. The FC concentration in feces directly correlates with the severity of the inflammation in the intestine [1]. Considering the pathogenesis of NEC and neonatal sepsis, FC can be a potential marker of these diseases, as it can indicate the presence of a subclinical inflammatory process.

Lactoferrin (LF) is a multifunctional iron-binding glycoprotein, which is found in the largest amount in breast milk and plays a key role in innate immunity [26]. In addition to antiviral and bactericidal properties, LF can prevent the occurrence of an excessive inflammatory response [11], stimulate the proliferation and differentiation processes of the epithelium of the small intestine, which affects its weight and length, as well as the production of digestive enzymes [12,13]. Enteral use of LF is one of the potential means of modulating postnatal adaptation of the digestive tract and prevention of diseases associated with its immaturity in preterm infants.

Purpose of this study — to evaluate the associations between FC levels, enteral LF administration, and the occurrence of NEC and sepsis in preterm infants.

Materials and methods of the study

Twenty-six newborns in whom we measured FC levels were involved in the study. The newborns originate from the cohort of preterm infants with gestational age (GA) y32 weeks, BW y1500 g, and age y72 h, who were treated in the Lviv Regional Clinical Hospital. At enrollment, all infants were tolerating at least trophic feeds, they did not have significant congenital malformations or complications that significantly reduced the chances of survival (in particular, stage 3 or 4 intraventricular hemorrhages (IVH) detected in the first 72 hours after birth). The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Local Ethics Committee for all participants. The informed parents' consent was obtained for all patients.

The feces were collected twice — after enrollment, during the first seven days of life, and at the postmenstrual age (PMA) of 36 weeks. If necessary, the feces were stored in a test tube for up to 12 hours at a temperature of +2–8°C until delivery to the laboratory. FC concentration was measured by the enzyme-linked immunosorbent assay (ELISA) using a Sunrise photoelectric analyzer (Tecan).

Among the 26 enrolled infants, 15 who suffered from early- (EOS) and late-onset neonatal sepsis (LOS) or NEC retrospectively formed the main group. The remaining 11 infants without this morbidity were included in the comparison group.

Eleven infants were randomly assigned to bovine LF suspension (4 infants from the main group and 7 children in the comparison group) at a dose of 100 mg/day, starting from the first 3 days of life, until reaching PMA of 36 weeks.

FC levels were compared in the retrospective subgroups of babies with certain diseases (EOS, LOS, or NEC) and infants from the comparison group. Additionally, a separate comparison of FC levels was performed in infants, who received LF (LF subgroup, n=11) and infants, who received standard treatment (ST subgroup, n=15).

NEC was diagnosed according to the Bell's criteria in modification of Kleigman [10]. EOS was diagnosed in first 72 hours of life and LOS was diagnosed after 72 hours of life based on the positive blood culture and/or on agreed criteria and the predictive model of the European Medicines Agency:

- the need to increase the percentage of oxygen in the inhaled gas mixture or to start respiratory support;
- increased frequency of apnea and bradycardia;
- instability of body temperature;
- violation of tolerance to enteral nutrition and/or flatulence;
- oliguria <1 ml/kg/h;
- signs of peripheral hemodynamic disturbances (symptom of «white spot» longer than 3 seconds, marble pattern of the skin);
- arterial hypotension (with a clinical need to prescribe additional fluid or inotropes);
- signs of tactile hyperesthesia, lethargy or muscle hypotonia;
- increase in the level of C-reactive protein >15 mg/l;
- number of leukocytes < 4 / > 20 x 10^9/l or thrombocytopenia <100x10^9/l;

Table 1

Demographic and baseline clinical characteristics of the groups

Indicator	Main group (n=15)	Comparison group (n=11)	p
GA, weeks	28.0 (27.0–31.0)	30.0 (29.0–32.0)	–
GA <28 weeks	7 (4@.@)	0	**0.05**
BW, g	870.0 (7@0.0–970.0)	1300.0 (1150.0–14@0.0)	**0.03**
Males, n (%)	7 (4@.@)	5 (45.5)	0.95
Multiple pregnancies, n (%)	5 (33.3)	1 (9.1)	0.14
Mother's age, years	28.0 (23.0–31.0)	31.0 (24.0–37.0)	0.34
AB treatment during pregnancy, n (%)	2 (13.3)	3 (27.3)	0.37
Antenatal steroids, n (%)	10 (@@.7)	8 (72.7)	0.74
SGA, n (%)	1 (@.7)	1 (9.1)	0.78
APGAR score at 5 minutes <7, n (%)	13 (8@.7)	@ (54.5)	0.34
Need for resuscitation, n (%)	11 (@8.@)	5 (45.5)	0.43
Surfactant administration, n (%)	11 (73.3)	8 (72.7)	0.97
Age at the time of hospitalization, hours	4.0 (2.0–9.0)	@.0 (2.0–25.0)	0.42
MV required, n (%)	11 (73.3)	4 (3@.3)	0.0@
LF administration, n (%)	7 (4@.@)	4 (3@.3)	0.@0

Notes: GA — gestational age; BW — birth weight; AB — antibiotics; SGA — small for gestational age; MV — mechanical ventilation; LF — lactoferrin.

- leukocyte index >0.2;
- violation of glucose tolerance (glucose level < 2.2/> 10 mmol/l);
- metabolic acidosis (base deficit > -10 mmol/l).

In NICU, standard protocols for respiratory support, hemodynamic stabilization, nutrition, prescribing antibiotics and antifungal drugs, correcting anemia and metabolic disorders were used. All infants were continuously monitored for vital functions.

Standard methods of descriptive, comparative statistics and analysis of covariance (ANCOVA) using the χ^2, Mann—Whitney, Wilcoxon, Fisher, and Spearman correlation (rS) coefficients were applied. To assess the possibility of predicting the occurrence of EOS using the concentrations of FC the ROC (навести) curve was used with the determination of the area under the curve and calculation of sensitivity, specificity, as well as positive and negative predictive values of certain measurements. Continuous variables are presented as median (lower and upper quartiles) unless otherwise stated. All results were considered significant if p<0.05.

The research was carried out in accordance with the principles of the Helsinki Declaration. The study protocol was approved by the Local Ethics Committee of the participating institution. The informed consent of the patient was obtained for conducting the studies.

Results of the study

Infants in the main group had significantly lower GA and BW at a birth (Table 1). They were also more likely to be born from multiple pregnancies, needed resuscitation at a birth with mechanical ventilation (MV), and had an Apgar score at 5 minutes of less than 7, although the differences were not statistically significant (Table 1). Thus, the most immature newborns or infants with significant disorders of early postnatal transition were more likely to suffer from sepsis and NEC. Infants in the main group required longer periods of MV and continuous positive airway pressure (CPAP) and, subsequently, suffered from bronchopulmonary dysplasia (BPD) more often. Neurological morbidity was also higher in the main group (Table 2). These babies later achieved a full amount of enteral feeds, required longer minimal enteral nutrition and antibacterial therapy, more blood transfusions, and longer duration of the NICU stay. All infants enrolled in the study were fed with formula or with formula and breastmilk. The proportion of babies who were fed with their mothers' milk at the time of collecting the feces samples did not differ between the groups (Table 2).

FC levels in the first seven days of life in infants in the main group were higher than in the comparison group but the difference was not statistically significant. The median age of infants at the time of collecting the first sample was 3 days in both groups (3.0 (3.0–6.0) days in the main group vs. 3.0 (2.0–5.0) days in the comparison group, p=0.7). At PMA of 36 weeks there was a decrease in FC levels in infants in the main group and an increase in babies in the comparison group, however, this difference between the groups remained

Table 2

Morbidity and therapeutic interventions in the groups

Indicator	Main group (n=15)	Comparison group (n=11)	p
NEC, n (%)	3 (20.0)	0 (0)	0.11
EOS, n (%)	7 (4@.7)	0 (0)	**0.008**
LOS, n (%)	8 (53.3)	0 (0)	**0.003**
BPD, n (%)	4 (2@.7)	0 (0)	0.0@
IVH grade 3–4, n (%)	5 (33.3)	0 (0)	**0.03**
PVL, n (%)	1 (@.7)	0 (0)	0.38
MV duration, hours	1@ (0–88.0)	0 (0–14.0)	**0.05**
CPAP duration, hours	182 (97.0–439.0)	54,0 (45.0–107.0)	**0.006**
Need for repeated tracheal intubations, n (%)	3 (20.0)	2 (18.2)	0.59
Blood transfusions, n (%)	13 (8@.7)	2 (18.2)	**0.01**
Postnatal steroids, n (%)	2 (13.3)	0	0.13
AB duration, days	38.0 (30.0–4@.0)	27.0 (17.0–34.0)	**0.05**
Trophic feeds duration, days	4.0 (3.0–9.0)	3.0 (2.0–4.0)	**0.04**
Age of achievement of full enteral feeds, days	27.5 (20.0–35.5)	10.0 (7.0–15.0)	**0.001**
Feeding with mother`s milk, n (%)	8 (53.3)	@ (54.5)	0.95
NICU stay, days	38.0 (10.0–45.0)	7.0 (@.0–10.0)	**0.0001**
Died, n (%)	3 (20.0)	0	0.12
Transition to another hospital, n (%)	1 (@.7)	0 (0)	0.38
In-hospital stay, days*	83.0 (57.0–104.0)	@0.0 (45.0–74.0)	0.25

Notes: * — only for survived patients; NEC — necrotizing enterocolitis; EOS — early–onset sepsis; LOS — late–onset sepsis; BPD — bronchopulmonary dysplasia; IVH — intraventricular hemorrhage; MV — mechanical ventilation; CPAP — continuous positive airway pressure; AB — antibiotic; NICU — neonatal intensive care unit; BM — breast milk.

Table 3

FC in groups, μg/g

Indicator	Main group (n=15)	Comparison group (n=11)	p	Main group (n=15)	Comparison group (n=11)	p
Age	First 7 days of life			PMA of 3@ weeks		
FC	413.5 (241.9–800.0)	274.2 (195.5–@39.1)	0.25	23@.7 (10.3–722.9)	4@2.3 (247.@–72@.8)	0.45
FC in infants with EOS	800.0 (294.4–800.0) (n=7)		**0.03**	480.3 (134.2–800.0)* (n=7)		0.82
FC in infants with LOS	374,7 (70.4–@80.2) (n=6)		0.88	1@0,4 (100.2–313.1) (n=5)		0.23
FC in infants with NEC	334.4 (228.7–800.0) (n=3)		0.44	–		–

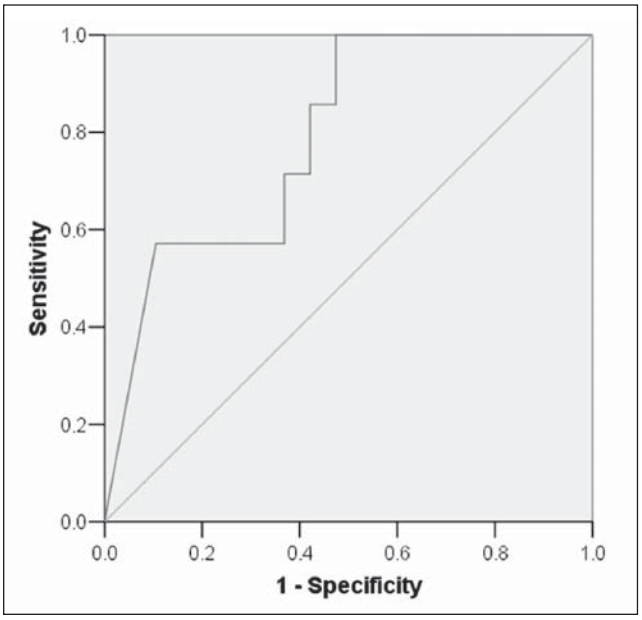
Notes: * — significant changes between 2 measurements (p=0.04); FC — fecal calprotectin; PMA — postmenstrual age; EOS – early–onset sepsis; LOS — late-onset sepsis; NEC — necrotizing enterocolitis.

unreliable, and the changes of concentrations were not statistically significant either (Table 3).

FC and EOS. EOS was diagnosed in seven infants. FC levels in the first week of life were significantly higher in these infants compared to newborns in the comparison group (Table 3). Up to the PMA of 36 weeks, the FC levels in infants with EOS decreased significantly (p=0.0G). Based on the results of the ROC analysis, it was established that the FC value of 373.9 mcg/g and more had a sensitivity of 71% and a specificity of 63% for the detection of EOS in our cohort of preterm infants (area under the curve 0.79; 95% confidence interval 0.81–0.97, p=0.026), (Fig.). The positive predictive value of this FC level was 41.6%, and the negative predictive value of the result of <373.9 mcg/g was 85.7%. According to the results of the analysis of covariance which as independent variables included EOS and GA the latter as a covariate had no significant effect on FC levels in the first week of life (F=0.1G, p=0.72).

FC and LOS. Eight patients developed LOS. In 2 infants the disease was combined with NEC, so the results of FC measurements in them were not taken into account during the relevant comparisons with the comparison group. According to the results of both measurements, the FC levels in infants with LOS did not differ from infants in the comparison group, and changes of the values were not reliable (Table 3).

FC and NEC. NEC was diagnosed in 3 infants. FC levels during the first week of life did not significantly differ between babies with NEC and infants from the comparison group (Table 3).



Notes: FC — fecal calprotectin; EOS — early-onset sepsis.

Fig. ROC-curve describing fecal calprotectin cut-off values for the prediction of EOS in very preterm infants

Two cases of NEC ended lethally, and the third infant was transferred to another hospital for further treatment, which made it impossible to determine and analyze FC levels at the time of PMA of 36 weeks.

FC and GA. No reliable correlations between GA and FC concentrations in the first 7 days of life (rS=-0.17, p=0.39) and at PMA of 36 weeks (rS=0.32, p=0.13) were found. Among the patients enrolled in the study, 7 infants (26.9%) were extremely preterm (GA <28 weeks). FC levels in the first week of life in these infants were higher compared to the infants with GA 28 weeks, but the difference was not statistically significant (507.5 (29G.G–800.0) mcg/g vs. 277.8 (195.5–680.2) mcg/g, p>0.05).

FC and LF. FC levels in the first 7 days of life in infants received LF were lower compared to the rest of the babies, but this difference was not statistically significant (27G.2 (195.5–639.1) mcg/g in the LF subgroup vs. 413.5 (274.2–800.0) mcg/g in infants in ST subgroup; p=0,55). At PMA of 36 weeks, FC levels of infants who received LF increased, while in patients in ST subgroup they decreased, butthedifferencebetweensubgroupswas not significant either (631.1 (232.2–800.0) mcg/g vs. 274.7 (144.8–599.6) mcg/g respectively, p=0.16). The changes of FC levels of infants in both subgroups were not statistically reliable. Based on the results of similar comparisons of FC concentrations depending on the administration of LF separately in the main group and the comparison group, no differences were found.

Discussion

In this study, we have examined the relationship between FC, the occurrence of neonatal sepsis and NEC, and the use of enteral LF in very preterm infants. We have established the only association between the concentrations of FC in the first week of life and the occurrence of EOS: there was a trend toward higher FC levels in infants receiving LF compared to children receiving ST, but the differences were not significant.

The occurrence of severe neonatal morbidity in this study adversely affected the main clinical outcomes of the infants in the main group, who were characterized by higher overall morbidity and mortality, as well as longer hospitalization. This could also have happened because the newborns in the main group were significantly more immature and smaller (Table 2). Severe IVH were detected in these infants after 72 hours of life. One case of PVL occurred in an infant with NEC who died.

The lack of differences in FC levels between the groups can be explained by the fact that at the time of the first measurement (the first 7 days of life), in some infants in the main group LOS or NEC have not yet occurred. During the second measurement at PMA of 36 weeks, the vast majority of them did not have an active inflammatory process, and the differences in the type of nutrition and the proportion of the infants who were fed with native mothers' BM were insignificant. At the same time, a significant increase in FC levels in the first week of life in the infants with EOS may indicate that the immature digestive tract could be the entrance gate of infection and may participate in the formation of a systemic inflammatory response. It is also known that in babies with EOS, not only the concentration of FC but also calprotectin in the blood serum may increase [4,21]. Although the occurrence of EOS in high-income countries is reasonably well-controlled, and due to the use of antenatal antibiotics, the corresponding incidence is minimized, the frequency of clinically suspected EOS in low- or lower-middle-income countries can be as high as 16%, and the incidence of laboratory-confirmed EOS can reach almost 5% [16]. Effective diagnostics of neonatal sepsis can be challenging in resource-limited settings [24]. The sensitivity and negative predictive value of FC <373.9 mcg/g at the level of 71% and 85.7%, respectively, indicate that FC can be an additional diagnostic marker of EOS in the first days of life in infants. At the same time, further research is needed to confirm this conclusion.

Additional factors may have influenced the FC level in our patients. In particular, FC levels may depend not only on the morbidity but also on GA and PMA or chronological age. Josefsson et al. described a decrease in FC levels during the first week of life and a gradual increase between the second and eighth weeks of life [9]. Rouge et al. found a weak negative linear relationship between FC and GA [19]. Yoon et al. described a positive linear association between GA and FC in babies born at GA of <26 weeks and a negative correlation between GA and FC in babies born at GA of 26 to 30 weeks [27]. Although we found that FC levels in infants with GA of <28 weeks were almost twice as high as in infants with GA of 28–32 weeks, this difference was not statistically significant. We did not find any correlations between GA and FC, and covariance analysis did not confirm an independent effect of GA on FC concentrations in our patients. Therefore, considering the inconsistency of the available data, the relationship between GA and FC requires further study.

The possibility of early detection of NEC or an existing subclinical inflammatory process, which may cause the next occurrence of NEC, is one of the most important issues in the context of studying FC in preterm infants. The results of the study by van Zoonen et al. indicate that serial FC measurements did not help to predict NEC, as there were no differences between FC levels in infants who developed NEC and control babies from birth to the first clinical suspicion of NEC [25]. At the same time, some studies indicate increased FC levels in infants with NEC. Thus, to confirm a diagnosis of NEC, Aydemir O et al. proposed a threshold value of FC of 792 mcg/g with a sensitivity of 76% and a specificity of 92 % [3]. However, Zhang et al. demonstrated a specificity of 88.2% and a sensitivity of 82.6% for a much lower concentration of 281 mcg/g [29]. The results of the study by MacQueen et al. which enrolled 250 infants with GA <35 weeks showed that the value of 299 mcg/g had the best diagnostic characteristics with a sensitivity of 71% and a specificity of 88% [1G]. The most important, from a practical point of view, is the possibility of predicting NEC or making its early diagnosis before the appearance of obvious clinical symptoms of the disease. Van Zoonen et al. found no significant differences between FC levels either on days 3–5 and 6–8 of life or at 48 hours before the appearance of clinical symptoms in preterm infants who developed NEC, compared to controls [25]. In contrast, Thibault et al. described that it was possible to detect an increase in FC, as well as a combined increase in FC and lipocalin-2 7–10 days before the appearance of clinical symptoms of the disease due to daily sampling of the material [22]. In our study, baseline FC levels in infants who later developed NEC were not significantly different from those of infants in the comparison group. We did not have the opportunity to routinely measure FC levels before the occurrence of NEC. Among the three infants in our study who developed NEC, only one had had a FC level greater than 792 mcg/g, but the other 5 infants with FC levels in the first week of life above this limit did not develop NEC.

It is known that LOS is also characterized by an increase in serum FC and calprotectin levels [21], although according to Pirr et al., reduced levels of serum calprotectin in the first days of life were associated with a higher risk of sepsis in preterm infants [18]. We have failed to detect any connections between FC levels in the first week of life or at PMA of 36 weeks and the occurrence of LOS. It is possible that this result could be related to an insufficient number of measurements since the concentration of FC might increase shortly before the appearance of clinical signs of the disease similar to NEC, as it was described above.

We observed an increase in FC levels in preterm infants who received LF enterally, but these changes were not statistically significant. Several authors demonstrated that healthy infants fed with BM had had higher FC levels compared to formula-fed infants [2,20]. Groer et al. described a trend toward a gradual increase in FC levels in breastfed infants compared to children who received pasteurized donor milk or were fed with formula and BM [7]. At the same time, Rougé et al. found a positive correlation between FC levels and volume of enteral feeding. This may suggest that food antigens together with commensal bacteria cause a «physiological» subclinical inflammatory process in the immature digestive tract [19]. One of the results of our prospective cohort study on the clinical effectiveness of LF was that infants who received LF were more likely to achieve full enteral nutrition faster [5]. A possible reason for the increase in FC levels could be the effect of immunologically active components of BM. In particular, this could be an effect of LF since the increase in FC levels was not observed in infants who were fed with formulas or donor BM, because the pasteurixation process significantly affects its immunological properties [6]. Also, the available data on the relationship between enteral nutrition and FC may indicate that an increase in FC levels is not always associated with the occurrence of inflammation but may reflect the physiological pro-

cess of postnatal transition and maturation of the digestive tract in infants.

Our study has several limitations. First, insufficient resources did not allow the enrollment of a larger number of infants to measure the levels of FC. Second, we did not have the opportunity to perform multiple interval measurements of FC in the infants involved in the study (e.g. weekly), which could give a more reliable picture of the changes of FC levels depending on the occurrence of LOS and/or NEC, as well as enteral use of LF. Third, we did not have a technical ability to determine FC concentrations exceeding 800 mcg/g which could have caused an underestimation of their significance. This could also affect the determination of the significance of changes of FC levels.

The obtained results indicate the need for further studies on the influence of immune nutrition, in particular enteral use of LF, on the inflammatory status of the digestive tract, and on the values of relevant biomarkers, in particular FC. Our data combined with the results of other authors suggest that significant fluctuations in individual FC levels in very preterm infants and dynamic changes in FC associated with feeding characteristics and postnatal transition of the digestive tract significantly reduce the prognostic value of FC as an early marker of NEC and sepsis, rather reflecting the state of the channel's physiological reaction to the presence of food antigens and commensal bacteria.

Conclusions

FC levels in the first week of life are not associated with the later occurrence of NEC or LOS in very preterm infants. At the same time, newborns who suffered from EOS had significantly higher FC concentrations compared to babies who did not have sepsis and NEC. FC levels at PMA of 36 weeks in infants with LOS did not differ from FC concentrations in infants who did not have this disease. FC value of <373.9 mcg/g allows to exclude the presence of EOS with an average probability of 86%. Enteral administration of LF at a dose of 100 mg/day was associated with an increase in FC levels, but this effect was not statistically significant.

No conßict of interectc 0ac declared by the authorc.

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