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# PECULIARITIES OF THE COURSE AND DIAGNOSTICS OF NECROTIZING ENTROCOLITIS IN PREMATURE BORN CHILDREN

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## Abstract

Necrotizing enterocolitis (NEC) is an acute and potentially fatal disease characterized by inflammation and necrosis in the gastrointestinal tract (GIT). Due to the obscure multifactorial etiology, early diagnosis and effective treatment of NEC is limited. Consequently, effective strategies in the prevention of NEC are critically needed.

**The aim of the study:** to establish clinical and diagnostic features of necrotizing enterocolitis of premature born children.

**Materials and methods:** we examined 57 premature born children with necrotizing enterocolitis in Vinnytsya and Zhytomyr Regional Children's Clinical Hospital. The children were divided into three groups depending on the severity of the NEC: the first group consisted of 20 preterm infants infants with NEC I stage, the second group consisted of 30 preterm infants with NEC II stage, the third group consisted of 7 preterm infants with NEC of the 3rd stage. The control group consisted of 10 preterm infants without NEC.

The content of the protein that binds fatty acids (intestinal fatty acid binding proteins - I-FABP) in the blood serum was determined using the Human I-FABP (Hycult Biotech, Netherlands) assay, according to the manufacturer's instructions.

The statistical processing of the obtained results was carried out using the software package Statistica 6.1. In order to determine the prognostic value of I-FABP in blood serum, we used the Receiver Operating Characteristic (ROC).

**Results:** the analysis of the results of breast feeding allowed to establish that children from III and II groups started enteral nutrition later - by 3.6 ± 0.7 and 2.9 ± 0.4 days of life, than children of group II - by 1.4 ± 0.1 days of life and children from the control group - by 1.2 ± 0.1 days of life ($p < 0.01$). In the clinical picture of necrotizing enterocolitis, among the nonspecific symptoms, the symptoms of a "white spot", and bradycardia ($p < 0.05$) were significantly more likely to occur, among abdominal manifestations - abdominal distension, weak peristalsis, contouring of the intestinal loops, moderate edema of the anterior abdominal wall ($p < 0.05$). In all preterm infants with necrotizing enterocolitis, the content of the protein binding fatty acids (I-FABP) in serum has been elevated ($p < 0.05$). The serum I-FABP value of ≥ 727.50 pg / ml allows to identify necrotizing enterocolitis in premature babies with a sensitivity of 73.6% and a specificity of 72.2%, obtained by ROC analysis, the area under the ROC curve (AUC) is 0.883 [95% CI 0.806-0.961], indicating a high diagnostic value of the model.

**Keywords: necrotizing enterocolitis; intestinal fatty-acid-binding protein; premature babies.**

## Introduction

Necrotizing enterocolitis (NEC) is an acute and potentially fatal disease characterized by inflammation and necrosis in the gastrointestinal tract (GIT). Incidence of NEC is between 7% and 12% in preterm very low birth weight (PVLBW) infants with an estimated mortality of 15%–30% [1]. The pathogenesis of NEC is still incompletely understood, but it is thought that several factors are involved interactively, such as premature birth, low birth weight, ischemia/reperfusion (I/R) injury, abnormal gut bacterial colonization, and inappropriate enteral feeding [2].

Diagnosis of NEC was made by clinical and radiological signs according to modified Bell's staging criteria at present. However, relying on clinical manifestations tends to heighten omission diagnostic rate because both systemic and abdominal signs are nonspecific in NICU patients. These signs include feeding intolerance, abdominal distention, bloody stool, dyspepsia, and ascites [3]. Similarly, the radiological signs often lack sufficient discriminative power due to time delay [4].

Feeding strategies and nutritional interventions play important roles in the modulation of these functions and thus the prevention of NEC [5]. Human milk feeding has been shown to prevent NEC [6], whereas infant formula (IF) feeding is associated with a higher risk of NEC, with a typical risk ratio of 2.77 (95% confidence interval (CI) 1.40 to 5.46) [7].

Fatty acid-binding proteins are small (14-15 kD) proteins that transport fatty acids in enterocytes. Human intestinal fatty acid-binding protein (I-FABP) is found in mature enterocytes of the small intestine. This protein is rapidly released in blood in response to intestinal mucosal injury [8].

Several studies have suggested the level of I-FABP could be used as a promising biomarker for early diagnosis and the prediction of severe NEC, even possibly for timing of surgery [9, 10].

Due to the obscure multifactorial etiology, early diagnosis and effective treatment of NEC is limited. Consequently, effective strategies in the prevention of NEC are critically needed. Accurate and timely diagnosis will limit morbidity, improve patients' living quality, and reduce costs. Therefore, we urgently need a new diagnostic method that is valid and promising for early diagnosis.

**The aim of the study**

To establish clinical and diagnostic features of necrotizing enterocolitis of premature born children.

**Materials and methods**

During the period 2016-2017, we examined 57 premature born children with necrotizing enterocolitis in Vinnytsya and Zhytomyr Regional Children's Clinical Hospital. The NEC diagnosis and severity determination were established according to modified Bell's NEC criteria for M.C.Walsh and R.M. Kliegman (1986). The children were divided into groups depending on the severity of the NEC.

The first group consisted of 20 preterm infants infants with NEC I stage (gestational age 29.9 ± 0.7 weeks, body weight at birth 1277.8 ± 92.1 g).

The second group consisted of 30 preterm infants with NEC II stage (gestational age 28.7 ± 0.6 weeks, body weight at birth 1180.6 ± 95.1 g).

The third group consisted of 7 preterm infants with NEC of the 3rd stage (gestational age 27.3 ± 0.6 weeks, body weight at birth 908.6 ± 79.6 g).

The control group consisted of 10 preterm infants without NEC (gestational age of 30.3 ± 0.6 weeks, body weight at birth 1299.0 ± 72.2 g).

Selection criteria for the study were: premature babies with NEC, gestational age up to 32 weeks, weight at birth < 2000 g without congenital developmental defects of digestive tract.

The research complex included a clinical examination, general laboratory and biochemical blood tests.

The content of the protein that binds fatty acids (intestinal fatty acid binding proteins - I-FABP) in the blood serum was determined using the Human I-FABP (Hycult Biotech, Netherlands) assay, according to the manufacturer's instructions.

The statistical processing of the obtained results was carried out using the software package Statistica 6.1. Indicators that characterize the normal data distribution are presented as mean ± average deviation (SD). Non-parametric data is presented as Median Me (MEDIAN) and the boundaries of the interquartile segment [Q1; Q3] (QUARTILE). The estimation of the probability of differences between independent statistical groups with nonparametric distribution was carried out using the Kolmogorov-Smirnov criterion. For the relative values (percent) the exact method of Fisher was used. The difference in parameters was considered statistically significant at $p < 0.05$. In order to determine the prognostic value of I-FABP in blood serum, we used the Receiver Operating Characteristic (ROC). The results were presented as the area under the ROC curve (AUC) based on the values of the sensitivity and specificity of the test, indicating a 95% confidence interval.

**Results and discussion**

The children of the III group had a significantly lower body weight (908.6 ± 73.7 g) and gestational age (27.3 ± 0.5 weeks) at birth than the children of the control group (body weight - 1299.0 ± 63.5 g and gestational age - 30.3 ± 0.5 g) ($p < 0.05$). Children of I and II groups had lower body weight and gestational age than control group children without a significant difference. It was found that the heavier the NEC stage, the smaller the body weight ($p < 0.05$) and the term of gestation.

All children after birth received enteral nutrition through orogastric probe. The analysis of the results of breast feeding allowed to establish that children from III and II groups started enteral nutrition later - by 3.6 ± 0.7 and 2.9 ± 0.4 days of life, than children of group II - by 1.4 ± 0.1 days of life and children from the control group - by 1.2 ± 0.1 days of life ($p < 0.01$).

The study of the nature of enteral nutrition in the early neonatal period allowed the following patterns to be established: breastfeeding was 4.2 times less than that of the children of group III than from the control group - 1 (14.3%) and 6 (60.0%) children respectively (p

<0.05). Children from the 2nd group were 2.2 times less likely to breastfeed than 8 children (26.7%) than the control group children. All other children started the first enteral nutrition from dry milk mixes with protein content of 2.0-2.3 g / 100 ml: - 6 (30.0%) of the children in first group, 17 (56.7%) of the children of the second group, 3 (42.9%) children from the 3rd group and 3 (30.0%) from the control group; from liquid milk mixes containing protein 2.9 - 3.1 g / 100 ml - 1 (5.0%), 5 (16.7%), 3 (42.9%) and 1 (10.0%) children respectively.

The first clinical symptoms of NEC in children began on average 7.5 ± 1.2 days of life in children from group I, 6.7 ± 1.7 days of life in children from group II and 7.9 ± 3.4 days of life in children from group III. General nonspecific symptoms of NEC are indicated in Table 1.

**Table 1**

**General nonspecific symptoms of NEC premature babies (abs.,%)**

| Symptoms | I group, n=20 | II group, n=30 | III group, n=7 |
|---|---|---|---|
| Not steady body temperature | 10 (50,0) | 21 (70,0) | 5 (71,43) |
| Apnoe | 7 (35,0) | 9 (30,0) | 3 (42,86) |
| Positive symptom of "white spot" | 9 (45,0) | 27 (90,0)* | 6 (85,71)* |
| Bradycardia | 7 (25,0) | 18 (60,0)* | 5 (71,43)* |

Note: * - a significant difference in the indices of children of II and III groups with indicators of children of group I, p <0.05.

Among the nonspecific symptoms that were detected in the examination of children of II and III groups, there was a significant increase in the positive symptom of "white spot" in 27 (90.0%) children and 7 (100.0%) children and bradycardia in 18 (60.0 %) and 5 (71.43%) children respectively (p <0.05).

Abdominal manifestations of NEC in premature babies are shown in Table 2.

The first abdominal manifestations of NEC began in all groups of abdominal distension and weak peristalsis (in 100.0% in both groups), as well as stasis on the stomach probe, which was in 12 children (60.0%) in group I and 22 children (73.3%) in group II, in 7 (100.0%) children of III group (p>0.05). In children of the 2nd and 3rd groups, the following symptoms were significantly more common in the absence of peristalsis in 5 children (16.7%) in group II and 6 (85.7%) in group III, contouring loops of the intestine in 12 children (40.0%) II group and in 5 children (71.4%) of group III and moderate edema of the anterior

abdominal wall that was present in 3 children (10.0%) in group II and in 3 children (42.9%) in group III (p> 0.05).

**Table 2**

**Abdominal manifestations of NEC prematurely born children (abs., %)**

| Symptoms | I group, n=20 | II group, n=30 | III group, n=7 |
|---|---|---|---|
| abdominal distension | 6 (30,0) | 14 (46,7) | 7 (100,0)** |
| regurgitation | 13 (65,0) | 17 (56,7) | 4 (57,1) |
| bloody feces | 2 (10,0) | 8 (26,7) | 4 (57,1)* |
| weak peristalsis | 20 (100,0) | 30 (100,0) | 7 (100,0) |
| absent peristalsis | 0 | 5 (16,7)* | 6 (85,7)** |
| Stasis on the gastric probe | 12 (60,0) | 22 (73,3) | 7 (100,0)** |
| Contouring loops of the intestine | 2 (10,0) | 12 (40,0)* | 5 (71,4)** |
| Swelling of the abdominal wall | 0 | 3 (10,0)* | 3 (42,9)* |

Notes: * - a significant difference in the indices of children of groups II and III with relatively to I group, p <0.05;

** - a significant difference in the indices of children of the III group relatively to groups I and II, p <0.05;

The results of laboratory examinations of children with NEC involved in the study are presented in Table 3.

**Table 3**

**Results of laboratory examinations of children with NEC involved to the study (M±m)**

| Indicators | I group, n=20 | II group, n=30 | III group, n=7 | Control proup, n=10 |
|---|---|---|---|---|
| Hemoglobin(G/l) | 120±8,9 | 99,8±6,4* | 86,4±8,0*# | 135,4±7,2 |
| RBC(G/l) | 3,7±0,3 | 3,5±0,5 | 2,7±0,3*# | 4,0±0,3 |
| WBC (G/l) | 16,3±1,6* | 20,2±6,4* | 22,8±2,9* | 8,6±1,8 |
| PTL (G/l) | 256,3±18 | 160,2±13,9*# | 136,2±32,2*# | 287,4±12,5 |
| C-protein > 6 mg/l, (abs., %) | 7 (35,0) | 10 (33,3) | 6 (85,7)*# | 1 (10,0) |

Notes: * - a significant difference between the respective indicators for the control group, p <0.05;

# - a significant difference between the corresponding indices of children of II and III groups concerning to group I, p <0.05.

In children of II and III groups, the hemoglobin level was significantly lower than in the control group children, whose mean values were (99.8 ± 6.4, 86.4 ± 8.0 and 135.4 ± 7.2 G / l ) respectively ( p <0.05). There is also a significant difference in the level of hemoglobin in children of the III group in relation to children of group I (p <0.05). The number of RBC in the general blood test in children of the III group was significantly lower than in the control group and also in the children of group I (p <0.05). The level of WBC in children of groups I, II and III was significantly higher than the level of WBC in the control group and was 16.3 ± 1.6, 20.2 ± 6.4, 22.8 ± 2.9 and 8, 6 ± 1.8 G / l (p <0.05). Between the groups this indicator did not differ. Thrombocytopenia was more severe in children of groups II and III in relation to children of both control and I groups, and this indicator was 160.2 ± 13.9, 136.2 ± 32.2, 287.4 ± 12.5 and 256.3 ± 18 G / L, respectively (p <0.05). In the study of C- protein, it was found that the increase of its level was in 6 (85.7%) children of the III group, which was significantly more frequent in comparison with both the control group children and the children of groups I and II (p <0.05).

All children with NEC involved in the study had an increase in the serum I-FABP content. Thus, in the I group, the mean I-FABP content was 824.50 ± 77.45 pg / ml, in group II – 1292.07 ± 125.59 pg / ml, in group II – 2067.43 ± 282.19 pg / ml, which significantly exceeded the value of I-FABP in control group children – 381.80 ± 68.25 pg / ml (p <0.05). Also, there was a significant difference in the I-FABP content of preterm infants with NEC, depending on the severity of the disease, the higher the severity of the disease, the higher the I-FABP content. Thus, the I-FABP content of children in group III significantly exceeded the I-FABP content in children of groups II and I, while in children of group II, the I-FABP content was significantly above the content of this indicator in children from group I (p <0 , 05).

The diagnostic value of I-FABP in blood serum of premature babies with necrotizing enterocolitis is determined by ROC analysis (Figure 1).

The results of the ROC analysis showed that the serum I-FABP value of ≥ 727.50 pg / ml allows to identify necrotizing enterocolitis in premature babies with a sensitivity of 73.6% and a specificity of 72.2%, obtained by ROC analysis, the area under the ROC curve (AUC) is 0.883 [95% CI 0.806-0.961], indicating a high diagnostic value of the model. Our data coincides with the data of Aydemir C, Dilli D, Oguz SS, et al. (2011), in which it was shown that I-FABP of the blood allows to identify infants in the early stages of the NEC and icorrelates with the severity of the disease.

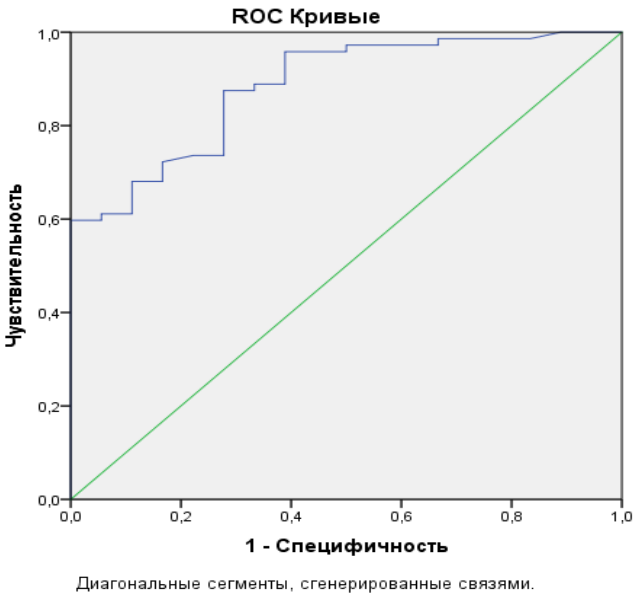


Fig. 1. ROC curve of I-FABP content in serum in preterm infants for prediction the development of necrotizing enterocolitis.

**Conclusions**

1. The heavier course of the NEC is associated with the later enteral nutrition - by 3.6 ± 0.7 days ($p < 0.01$) and the absence of breast milk in 6 (85.7%) children ($p < 0.05$).

2. In the clinical picture of the NEC of III degree, among the common manifestations, the symptoms such as a positive symptom of "white spot" and bradycardia ($p < 0.05$) were significantly more common among abdominal manifestations, such as the absence of peristalsis, contouring of the intestinal loops, moderate edema of the anterior abdominal wall, and also a stasis in the gastric probe and blood in feces ($p < 0.05$).

3. In all preterm infants with necrotizing enterocolitis, the content of the protein binding fatty acids (I-FABP) in serum has been elevated. In the I group, the mean I-FABP content was 824.50 ± 77.45 pg / ml, in group II – 1292.07 ± 125.59 pg / ml, in group II – 2067.43 ± 282.19 pg / ml, which significantly exceeded the value of I-FABP in control group children – 381.80 ± 68.25 pg / ml ($p < 0.05$).

4. The serum I-FABP value of ≥ 727.50 pg / ml allows to identify necrotizing enterocolitis in premature babies with a sensitivity of 73.6% and a specificity of 72.2%, obtained by ROC analysis, the area under the ROC curve (AUC) is 0.883 [95% CI 0.806-0.961], indicating a high diagnostic value of the model.

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