https://doi.org/10.15360/1813-9779-2023-2-2304

Inter-Alpha Inhibitor Proteins as a Predictor of Necrotizing Enterocolitis in Newborn Infants

Safaa A. ELMeneza*, Neveen M. Arafat, Iman M. El-Bagoury, Amal Gaber

Faculty of Medicine for Girls, AL-Azhar University
383C+QMC Youssef Abbas Str., Nasr city, 11751 Cairo, Egypt

For citation: *Safaa A. ELMeneza, Neveen M. Arafat, Iman M. El-Bagoury, Amal Gaber.* Inter-Alpha Inhibitor Proteins as a Predictor of Necrotizing Enterocolitis in Newborn Infants. *Obshchaya Reanimatologiya = General Reanimatology.* 2023; 19 (2): 33–39. https://doi.org/10.15360/1813-9779-2023-2-2304 [In Russ. and Engl.]

***Correspondence to:** Safaa A. ELMeneza, safaa5@hotmail.com

Summary

Necrotizing enterocolitis is a devastating emergency, multifactorial disease. Inter-alpha inhibitor proteins are serine protease inhibitors involved in many physiological and pathological activities.

Aim: this study was designed in order to assess the value of inter-alpha inhibitor proteins in predicting and improving accuracy of diagnosis of NEC in newborn infants with non- precise abdominal and intestinal manifestations.

Materials and Methods. This study was prospective longitudinal research that included 80 newborn infants presented with non-specific abdominal manifestations. Infants were divided into two groups. Group A; infants who developed necrotizing enterocolitis, they had stage II or III necrotizing enterocolitis according to modified Bell's criteria. Group B; included infants who did not develop necrotizing enterocolitis. Serum inter alpha inhibitor proteins level was measured by ELISA.

Results. In necrotizing enterocolitis group, the median inter-alpha inhibitor protein level was (9.38 mg/L), this was significantly lower than non-necrotizing enterocolitis group (44.40 mg/L), $P<0.01$. Inter-alpha inhibitor protein was reduced in stage IA than stage IIIB. Inter-alpha inhibitor protein values were decreased in preterm and full term infants with sensitivity of 98 % and specificity of 96% at cutoff <19.42 and <19.96 mg/L. The cut off in non-survival cases was >13.29 mg/L with sensitivity of 53.33 % and specificity of 92.31%.

Conclusion. Inter-alpha inhibitor protein levels were reduced in full term and preterm infants with necrotizing enterocolitis, consequently it may improve diagnosis of necrotizing enterocolitis in newborn infants. It has prognostic value and correlate with severity of necrotizing enterocolitis. It might predict non- survival cases.

Keywords: necrotizing enterocolitis; newborn infants; inter-alpha inhibitor proteins; surgical neonatal emergencies; newborn infants; preterm infants

Conflict of interest. The authors declare no conflict of interest. There are no financial or nonfinancial benefits have been received or will be received from any party related directly or indirectly to the subject of this article.

Introduction

Necrotizing enterocolitis (NEC) is a multifactorial overwhelming disease typically occurs without clinical warning [1]. Diagnosis of NEC can be difficult, because symptoms and signs may be nonspecific especially in preterm and sick full term infants who have feeding intolerance and non-specific abdominal disorders. A presumption of NEC will be followed by restraint of enteral feeding for 3 days at least. It is important to detect useful biomarkers to confirm the diagnose and establish the seriousness of NEC. Early diagnosis will promote early intervention, safe care and better outcome of newborn infants with inconclusive diagnosis of NEC and prevent unnecessary discontinue of enteral feeding and use of parenteral nutrition and antibiotics especially in developing countries with limited resources [2].

Inter-alpha inhibitor proteins (IαIp) molecules are part of innate immunity and play a critical role during inflammation. IαIp molecules have unique immunomodulatory effects by reducing TNF-α during systemic inflammation and augmenting anti-inflammatory IL-10 during sepsis in neonatal rats [3–5], but a few researches confirm its role in diagnosis of NEC.

Our research question was can IαIp predict the diagnosis of NEC in newborn infants? Hence, this study was designed in order to assess the value of Inter-alpha inhibitor proteins in predicting and improving the accuracy of diagnosis of NEC in newborn infants with non- precise abdominal and intestinal manifestations.

Materials and Methods

This was prospective longitudinal study carried out in NICU. It involved 80 newborn infants admitted to the NICU.

Ethical approval. The study protocol was approved by the ethics committee of Faculty of Medicine for Girls, AL-Azhar University. Approval number is 202010422 on 6/10/2020.

Informed consent was obtained from the parents after explaining the aim of the study. The aim, steps of the study, were discussed with the parents. Confidentiality of all data was ensured. The researchers

explained to the parents the nature of the study, the possible benefits to understand the nature of the disease. There was no additional risk/pain or invasive procedures as the extra test will be performed with the routine investigations. Also we informed parents that they can withdraw at any time.

The inclusion criteria included newborn infants presented with non-precise abdominal and intestinal manifestations as feeding intolerance, increased gastric aspirates, abdominal distention, and abdominal tenderness.

The exclusion criteria included newborn infants with congenital anomalies and symptoms suggestive errors of metabolism.

According to the results of investigations, the newborn infants were divided into two groups: Group A; infants who developed NEC and group B; infants who did not develop NEC. Modified Bell's staging criteria was used to estimate NEC stages [6].

All studied neonates were subjected to complete prenatal and natal history taking, thorough clinical examination, radiological and laboratory investigations including the serum inter alpha inhibitor proteins at the time of initial presentation.

Plasma IαIp levels were measured quantitatively using a competitive enzyme-linked immunosorbent assay with a monoclonal antibody against human IαIp.

The neonates were evaluated for the increase in abdominal girth, abdominal tenderness or redness, absence of intestinal sound as well as amount and colour of gastric residuals. Sign of respiratory distress or circulatory failure and hematological disorders as DIC were assessed. Also cases were assessed for temperature instability, apnea, bradycardia, lethargy, hypotension, emesis or blood in stool. Results of laboratory investigation as metabolic acidosis, and thrombocytopenia, and radiological findings determine the plane of treatment.

The newborn infants who proved to be NEC were assessed for need of ventilatory support when there was frequent apnea or respiratory failure, or need of vasopressors/inotropes as well as surgical consultation.

Statistical Analysis. Data were collected, coded, revised and entered to the Statistical Package for Social Science (SPSS) version 20. The statistical significance criterion was $P \leqslant 0.05$.

The criterion for assessing the normality of the parameters distribution, descriptive statistics was assessed using the kolmogorov smirnov test and Shapiro wilk test. To measure the diagnostic

Table 1. Comparison between infants who developed NEC and those who did not developed NEC/suspected group.

Parameters	Value in groups		Test	
	Not developed NEC	Developed NEC	χ^2/t*	*p*
Gender, *n* (%)				
Male	30 (57.70)	15 (53.60)	0.126	0.723
Female	22 (42.30)	13 (46.40)		
Gestational age, weeks				
Mean ±SD	33.96±3.85	34.39±3.56	–0.490*	0.625
Maturity, *n* (%)				
Pre term	33 (63.5)	17 (60.7)	0.059	0.809
Term	19 (36.5)	11 (39.3)		
Type of delivery, n (%)				
NVD	15 (28.8)	8 (28.6)	0.001	0.979
Postnatal age, days				
Mean ±SD	1.84±1.45	1.82±1.44	0.064*	0.949
Consanguinity, n (%)				
Negative	41 (78.8)	19 (67.9)	1.172	0.279
Positive	11 (21.2)	9 (32.1)		
Outcome, n (%)				
Non-survival	10 (19.2)	18 (64.8)	16.24	<0.001
Survival	42 (80.8)	10 (35.2)		

Note. * — Independent *t*-test; χ^2 = Chi square test.

Table 2. Comparison between infants who developed NEC and those who did not develop NEC as regard to abdominal examination.

Indicators	Value in groups, *n* (%)		Chi square test	
	Not developed NEC	Developed NEC	χ^2	*P*-value
Abdominal distension	38 (73.1)	23 (82.1)	0.826	0.363
Abdominal tenderness	14 (26.9)			
Occult blood in stool				
Negative	47 (90.4)	0 (0.0)	21.945	<0.001
Positive	5 (9.6)	28 (100.0)		
Gastric aspirate				
Bloody	2 (3.8)	26 (92.86)	27.619	<.001
Bilious	0 (0.0)	2 (7.14)		
Negative	50 (96.2)	0 (0.0)		

ability of the IaIp, the ROC curve was used for mapping of the sensitivity versus for all possible values of the cut-off point between cases and controls.

The cut-off point chosen was the best point for balancing the sensitivity and specificity on the curve. The cut-off point corresponding to these sensitivity and specificity values is the one closest to the (0, 1) point and was taken to be the cut-off point that best differentiates between the NEC cases and non NEC cases.

Results

Description of the study population

The study population included 80 newborn infants. Thirty cases were full term infants and fifty cases were preterm infants. Twenty-eight cases developed NEC of stage II and III according to Bell's classification, while 52 cases did not develop NEC.

There was no significant difference regarding gestational age, gender, mode of delivery, postnatal age, and anthropometric measurements between infants who developed NEC and those who did not develop NEC, $P>0.05$.

In the NEC group, 17 (60.7%) newborn infants were preterm and 11 (39.3%) newborn infants were full-term (Table 1).

Clinical presentation

There was non-significant difference between the NEC group and non NEC group regarding abdominal distension; 82.1% of newborn infants of NEC group had abdominal distension, while 73.1% of the non NEC group had abdominal distension, $P>0.05$. Our study showed that gastric residuals is an indicator for newborn infants who developed NEC. All the patients in NEC group had gastric aspirates; 92.86. % of cases had bloody brownish residual and 7.10% had bilious residual (Table 2).

Laboratory findings

In the NEC group, the median inter-alpha inhibitor protein level was 9.38 mg/L which was significantly lower than the non NEC group (44.40 mg/L), $P<0.01$. Inter-alpha inhibitor protein level was reduced in stage IIIB than IA stage (Table 3, 4).

The total leukocytic count was significantly increased in NEC group, $P<0.001$, there was insignificant difference in hemoglobin level and red blood cells count or platelets.

The Table 2 shows that there was no statistically significant difference between the two groups as regard to abdominal examination with $P>0.05$ while

Table 3. Comparison between infants who developed NEC and those who did not develop NEC as regard to inter-alpha inhibitor protein level, *Median* (*IQR*).

Indicators	Inter-alpha inhibitor protein, mg/L	Mann-Whitney test	
		U	*P*-value
All cases			
No NEC	44.40 (28.8–62.02)	–7.344	<0.001
NEC	9.38 (4.45–14.64)		
Preterm			
No NEC	29.04 (13.04–57.73)	–5.745	<0.001
NEC	7.75 (3.17–13.04)		
Full term			
No NEC	25.96 (15.33–44.38)	–4.497	<0.001
NEC	13.52 (9.10–16.79)		
No NEC			
Preterm	29.04 (13.04–57.73)	–0.353	0.724
Full Term	25.96 (15.33–44.38)		
NEC			
Preterm	7.75 (3.17–13.04)	–1.717	0.086
Full Term	13.52 (9.10–16.79)		
Outcome			
All non-survival	21.22 (13.73–47.92)	1.242	0.214
All survival	34.32 (13.2–54.42)		
Blood culture			
Positive	21.54 (11.21–35.23)	–4.150	<0.001
Negative	54.81 (44.38–67.90)		

Table 4. Inter-alpha inhibitor protein levels in relation to stage of NEC, *Median* (*IQR*).

Staging	*n*	Inter-alpha inhibitor protein,+ mg/L	Kruskall–Wallis test	
			K	*P*-value
IA	35	57.73 (44.38–67.9)	44.045	0.001
IB	17	25.1 (21.56–28.4)		
IIA	6	17.98 (17.35–19.42)		
IIB	10	13.07 (9.65–13.52)		
IIIA	6	6.75 (4.5–7.75)		
IIIB	6	1.03 (0.73–1.92)		

occult blood in stool and gastric aspirate showed highly statistically significant difference between the two groups with $P>0.001$.

Blood culture was positive in 38.5% of NEC group and in 3.6% of non NEC group, $P<0.01$ (Table 5, 6).

The figure shows the cut off point for the inter-alpha inhibitor protein in relation to gestational age as well as for prediction of mortality among the studied groups.

Discussion

Necrotizing enterocolitis is the most serious gastrointestinal disorder to occur in the newborn infant population. Mortality approaches 100% in the most severe cases with perforation, peritonitis, and sepsis. NEC mainly affect premature infants, but it is recognized in full-term infants too [7]. There are new markers that may help diagnosis of NEC, but with variable significance [8, 9]. IαIp is a fairly new marker with high sensitivity and specificity in detection of neonatal sepsis [10], but a few researches confirm its role in diagnosis of NEC. This study was designed in order to assess the value of inter-alpha inhibitor proteins in predicting and improving the accuracy of diagnosis of NEC in newborn infants with non-precise abdominal and intestinal manifestations.

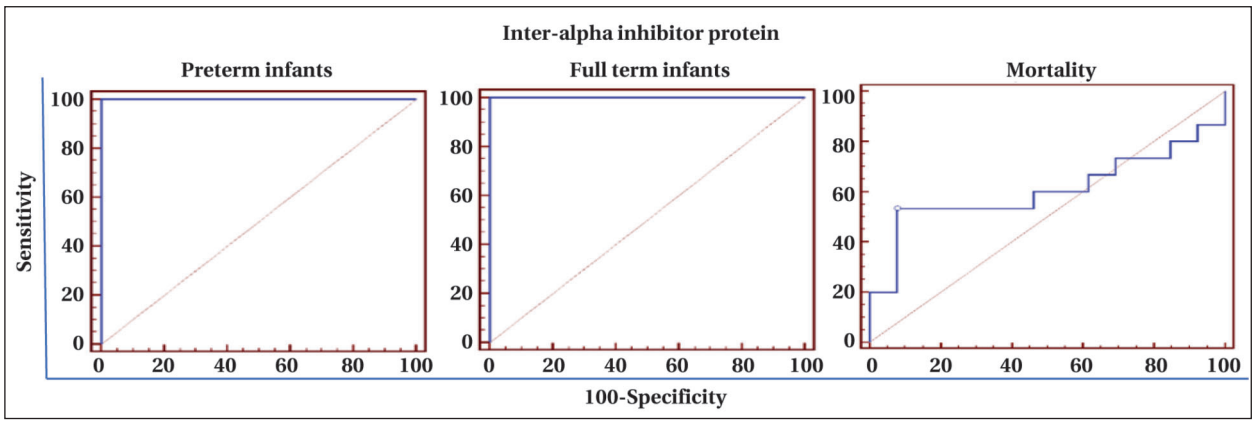
In this study, 60.7% of newborn infants who developed NEC were preterm and 39.3% were full term infants. Premature babies are prone to develop NEC due to multiple risk factors including immature mucosal barrier, gastrointestinal dysmotility and stasis which lead to malabsorption, bacterial growth and microbial dysbiosis that lead to mucosal injury [11].

Table 5. Comparison between infants who developed NEC and those who did not develop NEC as regard to hematological investigation.

Parameter	Value in groups		Independent *t*-test	
	Not developed NEC	Developed NEC	*t*	*P*-value
	Mean ± SD			
RBCs/µL	4.05±1.00	4.08±0.76	–0.158	0.875
PLT/µL	83.23±34.89	81.68±32.97	0.194	0.847
Hb(g/dL)	14.19±3.09	14.15±3.36	0.059	0.953
			Mann–Whitney test	
			Z	*P*-value
	Median (IQR)			
WBCs/µL	19.25 (12.15–27.5)	31.2 (21.8–66.5)	–3.657	<0.001
Lympho (%)	28.45 (14.5–46.1)	25.55 (15–35.8)	–0.042	0.967
Mono (%)	4.3 (1.8–10.6)	10.45 (6.75–13.2)	–1.825	0.068
Eosino (%)	0 (0–0)	0 (0–0)	–0.126	0.900

Table 6. Comparison between infants who developed NEC and those who did not develop NEC as regard to blood culture.

Blood culture	Value in groups, *n* (%)		Chi square test	
	Developed NEC	not developed NEC	χ^2	*P*-value
Negative	32 (61.5)	27 (96.4)	11.444	0.001
Positive	20 (38.5)	1 (3.6)		



Indicators	Cut-off point	AUC	Sensitivity	Specificity	PPV	NPV
Preterm	<19.42 mg/L	0.996	98	96	96.1	98
Full term	<19.96 mg/L	0.993	98	96	96	98
Mortality	>13.29 mg/L	0.70	53.33	92.31	88.9	63.2

Fig. Receiver operating curve of inter-alpha inhibitor protein.

The current study showed that mean levels of inter-alpha inhibitor protein were significantly decreased in the NEC group (9.38 mg/L) than the non NEC group (44.40 mg/L), $P<0.01$. This was in agreement with previous study done by Chaaban et al. who reported that the mean IαIp level in the confirmed NEC group was significantly lower than the control group [12] and with study of Shah et al. who found that IαIp levels were significantly decreased in infants with NEC compared to newborn with spontaneous intestinal perforation and matched controls. The diagnostic accuracy of IαIp for NEC was superior to that of CRP [13].

In this study, further analysis of the IαIp mean values in relation to gestational age, showed that IαIp can predict NEC in preterm and full term infants; the mean values were decreased in preterm infants and full term infants with NEC than in preterm and full term infants who did not develop NEC, $P<0.01$. Furthermore, there was no significant differences in IαIp levels between full term infants with NEC and preterm infant with NEC.The cutoff point for diagnosis of NEC was <19.42 mg/L in preterm infants and <19.96 mg/L in full term infants. The sensitivity was 98% and specificity was 96% in preterm and full term infants. These data showed that IαIp can be used to predict NEC in preterm as well as full term infants with suspected abdominal manifestations, up to our knowledge, this is first study to show the cutoff value in preterm and full term infants.

A probable justification for low values of IαIp among NEC cases may be due to down-regulation of IαIp synthesis, it is considered as a negative acute-phase reactant [14]. Also IαIp is very susceptible to proteolysis by numerous proteinases implicated in inflammation — namely plasmin, thrombin and kallikrein. Plasma IαIp is particularly sensitive to cleavage by neutrophil elastase, and the light chain bikunin released from the IαIp complex exerts its inhibitory activity on serine proteases [15].

Our study showed association between IαIp and severity of NEC as IαIp level was reduced among neonates with stage IIIB than those with stage IA, also there was significant decrease in IαIp among cases who showed ascites and pneumoperitoneum. In severe sepsis there is significant consumption of systemic IαIp and extended secretion of elastase that decompose IαIp [16]. Hepatic IαIp biosynthesis is also down regulated throughout severe inflammation like advanced NEC stages.

Also we found significant decrease of IαIp in cases with positive blood culture than those with negative blood culture, this finding is not matched with the previous study. 12 It is reported that the defending impacts of IαIp may be due to its effect as potent inhibitors of furin which is endogenous cell membrane-associated serine endoprotease, that has role in incomplete initiation of proteolysis of bacterial toxins [17].

Also, IαIp values insignificantly decreased among non-survival cases. The cutoff point for mortality was >13.29 mg/L with sensitivity of 53.33 % and specificity of 92.31%. As far as we know this is first study to look at the differences between IαIp in survival and dead cases with NEC. IαIp values were inversely related with mortality in adult with sepsis. Failure of recovery of IαIp levels over the course of sepsis is associated with an unfavorable outcome [18].

There is shortage of precise clinical and laboratory findings that constrains early diagnosis of NEC and results in over diagnosis with subsequent vigorous treatment. The typical clinical diagnostic features of necrotizing enterocolitis may be delayed, while early diagnostic symptoms and signs as feeding intolerance are not specific and may be related to prematurity or other illness as sepsis. There are no single conclusive laboratory tests to diagnose necrotizing enterocolitis; abnormal leukocytes count or platelets could be related to infection. The early imaging may be normal or shows mild ileus as fixed dilated loops of bowel, that may need to be repeated to confirm the diagnosis, this carry risk of exposure to radiation. Extraluminal air outside the intestine or pneumatosis intestinalis are sign of advanced necrotizing enterocolitis. IaIp can be added to diagnostic tool of NEC due to its high sensitivity, specificity in predicting NEC and its prognostic value.

Biomarker research has remarkable capacity to expand our realizing of the pathogenesis of NEC and consequently progress in early diagnosis and management [19]. Although there is emerging role for proteomic or a metabolomic studies, but it is expensive and needs multidisciplinary studies and efforts by researchers with diverse expertise [20].

Conclusion

From these findings we conclude that inter-alpha inhibitor protein levels were reduced in full term and preterm newborn infants with NEC, consequently use of inter-alpha inhibitor protein as potential marker may improve the diagnosis of NEC in neonates with nonspecific and suspected abdominal disorders. Inter-alpha inhibitor protein had prognostic values and its level is associated with the severity of NEC and might predict mortality.

References

1. *Yee W.H., Soraisham A.S., Shah V.S., Aziz K., Yoon W., Lee S.K.* Incidence and timing of presentation of necrotizing enterocolitis in preterm infants. *Pediatrics* 2012; 129 (2): e298–e304. DOI: 10.1542/peds.2011-2022. PMID: 22271701.
2. *ELMeneza S.* Egyptian neonatal safety standards; 2014. ENSTN/Editor: ENSTN/ISBN: 14851 Available from https: //www.researchgate.net/publication/322831357_Egyptian_Neonatal_Safety_Standards [accessed 15 January 2023].
3. *Lim Y.P., Bendelja K., Opal S.M., Siryaporn E., Hixson D.C., Palardy J.E.* Correlation between mortality and the levels of inter-alpha inhibitors in the plasma of patients with severe sepsis. *J Infect Dis*. 2003; 188 (6): 919–926. DOI: 10.1086/377642. PMID: 12964125.
4. *Kingsmore S.F., Kennedy N, Halliday H.L., Van Velkinburgh J.C., Zhong S., Gabriel V., Grant J., Beavis W.D., Tchernev VT., Perlee L., Lejnine S., Grimwade B., Sorette M. Edgar J.D.M.* Identification of diagnostic biomarkers for infection in premature neonates. *Mol Cell Proteomics* 2008; 7 (10): 1863–1875. DOI: 10.1074/mcp.M800175-MCP200. PMID: 18622029.
5. *Singh K., Zhang L.X., Bendelja K., Heath R., Murphy S., Sharma S., Padbury J. F, Lim, Y.-P.* Inter-alpha inhibitor protein administration improves survival from neonatal sepsis in mice. *Pediatr Res*. 2010; 68 (3): 242–247. DOI: 10.1203/PDR.0b013e3181e9fdf0. PMID: 20520583.
6. *Walsh M.C., Kliegman R.M.* Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin North Am*. 1986; 33 (1): 179–201. DOI: 10.1016/s0031-3955 (16)34975-6. PMID: 3081865.
7. *Ginglen J.G., Butki N.* Necrotizing enterocolitis. [Updated 2022 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan. PMID: 30020729. Bookshelf ID: NBK5133. Available from: Necrotizing Enterocolitis — PubMed (nih.gov).
8. *ELMeneza S., Okasha A., Abd El-Hafez M.A., Hussein N.* Ischemia-modified albumin as a novel marker for diagnosis of necrotizing enterocolitis in newborn infants. *J. Pediatr Neonat Individual. Med*. 2021; 10 (2): e100205. DOI: 10.7363/100205.
9. *Puddu M., Marcialis M.A., De Magistris A., Irmesi R., Coni E., Mascia L., Fanos V.* From the «old NEC» to the «new NECs». *J Pediatr Neonat Individual Med*. 2014; 3 (2): e030245. DOI: 10.7363/030245.
10. *Chaaban H., Singh K., Huang J., Siryaporn E., Lim Y-.P., Padbury J.F. T*he role of inter-alpha inhibitor proteins in the diagnosis of neonatal sepsis. *J. Pediatr*. 2009; 154 (4): 620–622. e621. DOI: 10.1016/j.jpeds.2008.10.008. PMID: 19324226.
11. *ELMeneza S., Amin W.* Study of superior mesenteric blood velocity among normal and high risk newborn infants. *Hepato-Gastroenterology*. 1999; 46: SUPPL.II: 1480. https: //www.researchgate.net/publication/324686238_Study_of_superior_mesenteric_blood_velocity_among_normal_and_high_risk_newborn_infants; [accessed 15 January 2023].
12. *Chaaban H., Shin M., Sirya E., Lim Y.-P., Caplan M., Padbury J.F.* Inter-alpha inhibitor protein level in neonates predicts necrotizing enterocolitis. *J Pediatr*. 2010; 157 (5): 757–761. DOI: 10.1016/j.jpeds.2010.04.075. PMID: 20955849.
13. *Shah B.A., Migliori A., Kurihara I., Sharma S., Lim Y.-P., Padbury J.* Blood level of Inter-alpha inhibitor proteins distinguishes necrotizing enterocolitis from spontaneous intestinal perforation. *J Pediatr*. 2017; 180: 135–140.e1. DOI: 10.1016/j.jpeds.2016.09.016. PMID: 27745748.
14. *Daveau M., Rouet P., Scotte M., Faye L., Hiron M., Lebreton J.P., Salier J.P.* Human inter-a-inhibitor family in inflammation: simultaneous synthesis of positive and negative acute-phase proteins. *Biochem. J*. 1993; 292 (Pt 2): 485–492. DOI: 10.1042/bj2920485. PMID: 7684902.
15. *Hirose J., Ozawa T., Miura T., Isaji M., Nagao Y., Yamashiro K., Nii A., Kato K., Uemura A.* Human neutrophil elastase degrades inter-alpha-trypsin inhibitor to liberate urinary trypsin inhibitor related proteins. *Biol Pharm Bull*. 1998; 21 (7): 651–656. doi: 10.1248/bpb.21.651. PMID: 9703243.
16. *Balduyck M., Albani D., Jourdain M., Mizon C., Tournoys A., Drobecq H., Fourrier F, Mizon J.* Inflammation-induced systemic proteolysis of inter-alpha-inhibitor in plasma from patients with sepsis. *J Lab Clin Med*. 2000; 135 (2): 188–198. DOI: 10.1067/mlc.2000.104462. PMID: 10695665.
17. *Neijens J.H., Abbink J.J., Wachtfogel Y.T., Colman R.W., Eerenberg A.J., Dors D., Kamp A.J., Strack van Schijndel R.J., Thijs L.G., Hack C.E.* Plasma elastase alpha 1-antitrypsin and lactoferrin in sepsis: evidence for neutrophils as mediators in fatal sepsis. *J Lab Clin Med*. 1992; 119 (2): 159–168. PMID: 1740629.
18. *Opal S.M., Lim Y.-P., Siryaporn E., Moldawer L.L, Pribble J.P., Palardy J.E., Souza S.* Longitudinal studies of inter-alpha inhibitor proteins in severely septic patients: a potential clinical marker and mediator of severe sepsis. *Crit Care Med*. 2007; 35 (2): 387–392. DOI: 10.1097/01.CCM.0000253810.08230.83. PMID: 17205024.

19. *Goldstein G.P., Sylvester K.G.* Biomarker discovery and utility in necrotizing enterocolitis. *Clin Perinatol*. 2019; 46 (1): 1–17. DOI: 10.1016/j.clp.2018.10.001. PMID: 30771811.
20. *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 (8): 602255. DOI: 10.3389/fped.2020.602255. PMID: 33425815.

Received 26.12.2022
Accepted 21.02.2023