Indian Journal of Experimental Biology
Vol. 61, July 2023, pp. 493-498
DOI: 10.56042/ijeb.v61i07.2467

Diagnostic and prognostic values of abdominal ultrasound combined with urinary intestinal fatty acid binding protein for neonatal necrotizing enterocolitis

Xiaoqiong Tu & Dezhao Jia*

Department of Neonate, Suizhou Hospital, Hubei University of Medicine, Suizhou 441300, Hubei Province, China

Received 19 April 2023; *revised 06 June 2023*

Necrotizing enterocolitis (NEC) triggers extensive or localized necrosis of the small intestine and colon in neonates. Here, we studied the diagnostic and prognostic values of abdominal ultrasound combined with urinary intestinal fatty acid binding protein (I-FABP) for NEC. Ninety-eight NEC neonates were divided into a suspected NEC group (n=57) and a confirmed NEC group (n=41). They were further divided into an internal medicine treatment group (n=54) and an operation group (n=44) according to disease progression. The influencing factors were explored by multivariate analysis. The diagnostic values of abdominal ultrasound and I-FABP alone or in combination were analyzed by receiver operating characteristic (ROC) curves. Further, 1-year survival rates were analyzed by Kaplan-Meier survival curves. Premature birth, meconium-stained amniotic fluid, neonatal septicemia, maternal gestational diabetes mellitus, non-exclusive breastfeeding, non-prophylactic application of probiotics and I-FABP >6.25 ng/mL were risk factors for NEC (P <0.05). The confirmed NEC group had higher proportions of neonates with intestinal wall thickening, portal venous gas, disappearance of intestinal peristalsis, peritoneal effusion and decreased intestinal wall blood perfusion than the suspected NEC group (P <0.05). The area under the ROC curve of combined detection for diagnosing NEC was the largest (P <0.001). The operation group had higher proportions of neonates with intestinal wall thickening, dilatation, disappearance of intestinal peristalsis and peritoneal effusion and increased urinary I-FABP level than the internal medicine treatment group (P <0.05). Abdominal ultrasound combined with urinary I-FABP can improve the diagnostic efficiency of neonatal NEC, with high predictive value for prognosis.

Keywords: Neonates, Receiver operating characteristic (ROC) curves

Necrotizing enterocolitis (NEC), which triggers extensive or localized necrosis of the small intestine and colon in neonates, has an incidence rate of 2-10% and a mortality rate of up to 70% in severe cases[1]. Currently, the etiology of NEC is not entirely understood, which may be related to factors such as infection, hypoxia and improper feeding[2]. If NEC is not diagnosed and treated in time, it may lead to adverse events, such as intestinal perforation, infectious peritonitis and even shock[3]. However, until now, there has been no specific examination method for early diagnosis of NEC. Currently used abdominal X-ray plain film as the main imaging method is reported to be insensitive for early diagnosis of NEC[4]. Majority of NEC neonates can be treated conservatively, but severe cases need surgical intervention. Although free intraperitoneal air and intestinal perforation are currently recognized as the surgical indications of NEC, the coincidence rate of abdominal plain film diagnosis is low, and the rate of missed diagnosis is high, hence plain abdominal film cannot be employed as the criteria for selecting the treatment plan for NEC neonates[5].

In recent years, abdominal ultrasound has played a crucial role in the diagnosis of NEC, with the advantages of real-time dynamic display, no radiation and repeatable operation. Hwang *et al.*[6] observed abdominal ultrasound to have high diagnostic value for neonatal NEC. Intestinal fatty acid binding protein (I-FABP) is a low molecular weight protein that transports fatty acids. Ahmed *et al.*[7] reported that I-FABP, a vital biological marker of neonatal NEC, had a high diagnostic value. However, the diagnostic value of abdominal ultrasound combined with I-FABP for neonatal NEC has seldom been referred. In this context, here, we investigated the diagnostic and prognostic value of abdominal ultrasound combined with urinary I-FABP for neonatal NEC.

Methodology

General data

Ninety-eight NEC neonates treated in our hospital from March 2018 to March 2020 were enrolled as an

*Correspondence:
E-Mail: zeq268112@163.com

observation group, including 57 boys and 41 girls with a mean age of (13.45±6.96) days old and a mean gestational age of (34.86±5.02) weeks. In addition, 65 healthy neonates born in the same period were enrolled as a control group, including 36 boys and 29 girls with a mean age of (12.85±6.42) days old and a mean gestational age of (35.41±5.18) weeks. According to the Bell-NEC classification criteria[8], the NEC neonates were divided into a suspected NEC group (Bell-NEC stage I, n=57) and a confirmed NEC group (Bell-NEC stage II, n=41) based on clinical manifestations and abdominal X-ray examination results. According to disease progression, they were divided into an internal medicine treatment group (n=54) and an operation group (n=44). This study has been approved by the ethical committee of our hospital (approval No. SHHUM201803004), and written informed consent has been obtained from the guardians of all neonates.

Inclusion and exclusion criteria

Inclusion criteria: (i) Neonates meeting the diagnostic criteria for neonatal NEC as in the Patho-physiology of Necrotizing Enterocolitis[9]; (ii/iii) those in Bell-NEC stage I or II; (iv) those with onset within 30 days after birth; and (v) those with complete clinical data.

Exclusion criteria: (i) Neonates with complex congenital heart disease; (ii) those with intestinal malformation symptoms such as intestinal atresia, Hirschsprung's disease and intestinal malrotation; and (iii) those with disappeared abdominal distension within 12 h.

Collection of general data

The general data of neonates were collected through electronic medical records, including date of birth, sex, gestational age, birth weight, mode of delivery (cesarean section or natural delivery), 1-min Apgar score, premature birth, neonatal complications (neonatal asphyxia, neonatal respiratory distress syndrome, neonatal pneumonia, meconium-stained amniotic fluid, neonatal septicemia, neonatal hypoglycemia, septic shock and hyperbilirubinemia), maternal diseases during pregnancy (gestational hypertension, gestational diabetes mellitus and intrahepatic cholestasis of pregnancy), premature rupture of membranes, infection, feeding method (breastfeeding, artificial feeding and mixed feeding), breastfeeding initiation time and prophylactic application of probiotics.

Observation indices

Urine was collected, and urinary I-FABP level was determined by ELISA kit (Shanghai Enzyme-linked Biotechnology Co., Ltd., China). Colour Doppler ultrasonography (GE Healthcare, USA) was used to examine the abdomen as follows: (i) The thickness of the diseased intestinal wall was measured, 1.1-2.6 mm suggested normal thickness, and ≥3.0 mm indicated thickening; (ii) Pneumatosis cystoides intestinalis was detected by scanning while pressurizing the probe, whether there were punctate or granular gas echoes between the intestinal walls was observed, and the location of the gas (lumen or wall) was identified; (iii) Portal vein gas was manifested as gas-like strong echoes moving with blood flow in the portal vein, or punctate, beaded or patchy gas-like echoes along the branches of the portal vein from the hepatic porta; (iv) Intestinal dilatation was detected, and the small intestine ≥2.0 cm suggested dilatation; (v) Free intraperitoneal air was manifested as punctate, linear or mass-like hyperechoic areas outside the intestinal canal; (vi) Intestinal peristalsis was detected, and contraction >10 times/min, ≤10 times/min, and no contraction indicated normal, slowed, and no intestinal peristalsis, respectively; (vii) Peritoneal effusion was detected by observing the nature and quantity of abdominal fluid; and (viii) Blood perfusion of the intestinal wall was observed, and ≤9 points/line in each square meter of the sampling frame indicated decreased blood perfusion.

Follow-up

All subjects were followed up for one year (until March 2021), and the follow-up data were collected through the outpatient clinic or telephone call. The survival of neonates was recorded, and the survival rate was calculated.

Statistical analysis

SPSS 19.0 software was utilized for statistical analysis, and GraphPad Prism 5.0 software was used for plotting. The count data were expressed as percentages, and the chi-square test was performed for intergroup comparison. The measurement data were expressed by mean ± standard deviation, and the independent *t*-test was conducted for intergroup comparison. Multivariate logistic regression analysis was employed to explore the factors affecting neonatal NEC. The diagnostic values of abdominal ultrasound, urinary I-FABP and their combination for neonatal NEC were evaluated by receiver operating characteristic (ROC) curves, and the 1-year survival

Table 1 — General data of observation and control groups

Group	Observn. (n=98)	Control (n=65)	t/χ^2	P
Age (d)	13.45±6.96	12.85±6.42	0.556	0.579
Boy/girl (n)	57/41	36/29	0.123	0.726
Gestational age (week)	34.86±5.02	35.41±5.18	0.676	0.500
Birth weight (kg)	1.92±0.73	1.85±0.68	0.616	0.539
Mode of delivery			1.107	0.293
Cesarean section	25 (25.51)	12 (18.46)	-	-
Natural delivery	73 (74.49)	53 (81.54)	-	-
Apgar score (point)	8.01±1.53	7.56±1.48	1.863	0.064
Premature birth	64 (65.31)	26 (40.00)	10.121	0.001
Neonatal complications				
Neonatal asphyxia	10 (10.20)	1 (1.54)	4.663	0.031
Neonatal respiratory distress syndrome	9 (9.18)	1 (1.54)	3.967	0.046
Neonatal pneumonia	7 (7.14)	0 (0.00)	4.851	0.028
Meconium-stained amniotic fluid	26 (26.53)	5 (7.69)	9.005	0.003
Neonatal septicemia	31 (31.63)	6 (9.23)	11.177	0.001
Neonatal hypoglycemia	6 (6.12)	3 (4.62)	0.170	0.680
Septic shock	5 (5.10)	1 (1.54)	1.400	0.237
Hyperbilirubinemia	1 (1.02)	1 (1.54)	0.087	0.769
Maternal pregnancy diseases				
Gestational hypertension	11 (11.22)	5 (7.69)	0.551	0.458
Gestational diabetes mellitus	34 (34.69)	11 (16.92)	6.175	0.013
Intrahepatic cholestasis of pregnancy	5 (5.10)	2 (3.08)	0.390	0.532
Premature rupture of membranes	34 (34.69)	13 (20.00)	4.112	0.043
Infection	24 (24.49)	8 (12.31)	3.676	0.055
Feeding methods			18.571	0.000
Breastfeeding	19 (19.39)	33 (50.77)	17.715	0.000
Bottle-feeding	57 (58.16)	26 (40.00)	5.159	0.023
Mixed feeding	22 (22.45)	6 (9.23)	4.799	0.028
Breastfeeding initiation time (min)	34.82±5.47	30.26±5.25	5.295	0.000
Prophylactic application of probiotics	14 (14.29)	28 (43.08)	16.936	0.000
Urinary I-FABP (ng/mL)	8.73±1.87	3.12±1.45	20.444	0.000

rate was analyzed by plotting Kaplan-Meier survival curve. P <0.05 suggested that difference was statistically significant.

Results

There were significant differences in premature birth, neonatal asphyxia, neonatal respiratory distress syndrome, neonatal pneumonia, meconium-stained amniotic fluid, neonatal septicemia, maternal gestational diabetes mellitus, premature rupture of membranes, feeding method, breastfeeding initiation time, prophylactic application of probiotics and urinary I-FABP level between observation and control groups (P <0.05) (Table 1).

Multivariate logistic regression analysis revealed that premature birth, meconium-stained amniotic fluid, neonatal septicemia, maternal gestational diabetes mellitus, non-exclusive breastfeeding, non-prophylactic application of probiotics and I-FABP >6.25 ng/mL were risk factors for neonatal NEC (Fig. 1).

The confirmed NEC group had significantly higher proportions of neonates with intestinal wall thickening, portal venous gas, disappearance of intestinal peristalsis, peritoneal effusion (poor acoustic transmissibility) and decreased intestinal wall blood perfusion than the suspected NEC group (P <0.05) (Table 2).

Table 2 — Abdominal ultrasound results of suspected and confirmed NEC groups [n (%)]

Group	Suspected NEC (n=57)	Confirmed NEC (n=41)	χ^2	P
Intestinal wall thickening	10 (17.54)	22 (53.66)	14.144	0.000
Pneumatosis cystoides intestinalis	22 (38.60)	17 (41.46)	0.082	0.775
Portal venous gas	2 (3.51)	19 (46.34)	25.985	0.000
Intestinal dilatation	14 (24.56)	17 (41.46)	3.150	0.076
Decreased intestinal dilatation	16 (28.07)	19 (46.34)	3.467	0.063
Free intraperitoneal air	0 (0.00)	2 (4.88)	2.838	0.092
Slowed intestinal peristalsis	28 (49.12)	15 (36.59)	1.522	0.217
Disappearance of intestinal peristalsis	6 (10.53)	13 (31.71)	6.845	0.009
Peritoneal effusion (good acoustic transmissibility)	26 (45.61)	17 (41.46)	0.167	0.683
Peritoneal effusion (poor acoustic transmissibility)	4 (7.02)	24 (58.54)	31.014	0.000
Decreased intestinal wall blood perfusion	0 (0.00)	16 (39.02)	26.584	0.000

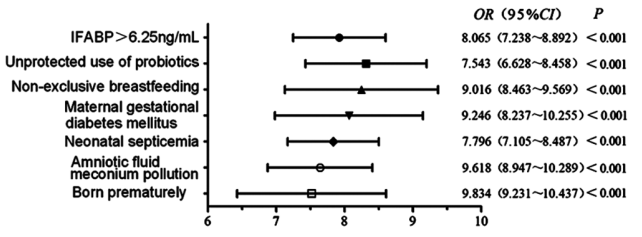

Fig. 1 — Multivariate analysis results of factors affecting occurrence of NEC in newborns.

The area under the ROC curve, sensitivity, specificity, positive predictive value and negative predictive value of combined detection were significantly higher than those of abdominal ultrasound and urinary I-FABP alone (P <0.005), showing higher diagnostic value (Table 3 and Fig. 2).

The operation group had significantly higher proportions of NEC neonates with intestinal wall thickening, intestinal dilatation, disappearance of intestinal peristalsis and peritoneal effusion (poor acoustic transmissibility) and a significantly higher urinary I-FABP level than the internal medicine treatment group (P <0.05) (Table 4).

The NEC neonates with urinary I-FABP >6.25 ng/mL and intestinal wall thickening, intestinal peristalsis, disappearance of intestinal peristalsis or peritoneal effusion (poor acoustic transmissibility) were assigned to group A (n=38), those with urinary I-FABP ≤6.25 ng/mL and no intestinal wall thickening, intestinal dilatation, disappearance of intestinal peristalsis or peritoneal

Table 3 — Diagnostic values of abdominal ultrasound, I-FABP and their combination for NEC

Diagnostic index	AUC	Optimal cut-off	P	Sensitivity (%)	Specificity (%)	+ve predictive value (%)	–ve predictive value (%)
Urinary I-FABP	0.752	6.25	<0.001	71.35	65.42	68.47	82.14
Abdominal ultrasound	0.817	/	<0.001	77.41	72.25	75.52	87.12
Combination of abdominal ultrasound and urinary I-FABP	0.854	/	<0.001	88.28	84.79	81.68	92.43

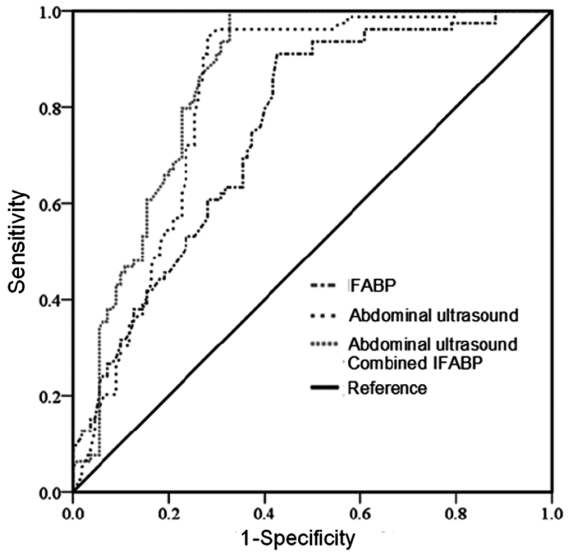

Fig. 2 — ROC curves of diagnostic values of abdominal ultrasound, I-FABP and combination for NEC.

effusion (poor acoustic transmissibility) were assigned to group B (n=19), while the rest neonates were assigned to group C (n=41). During follow-up for 1 year, 20 (52.63%), 17 (89.47%) and 32 (78.05%) neonates survived in groups A, B and C, respectively. Group B had a significantly higher survival rate than those of groups A and C (P <0.05) (Fig. 3).

Discussion

NEC mainly occurs in premature infants and very low birth weight infants, with an incidence rate of about 5-14% and a mortality rate of 25-40%[10]. Premature birth and meconium-stained amniotic fluid are risk factors for neonatal NEC[11]. Besides, neonatal septicemia and non-breastfeeding are risk factors for NEC[12]. Additionally, prophylactic application of probiotics is a protective factor against neonatal NEC[13]. Herein, premature birth, meconium-stained amniotic fluid, neonatal septicemia, maternal gestational diabetes mellitus, non-exclusive breastfeeding and non-prophylactic application of probiotics were all risk factors for neonatal NEC. In clinical practice, reasonable prevention and control measures should be formulated based on the above risk factors.

Table 4 — Abdominal ultrasound results and urinary I-FABP levels of internal medicine (IM) treatment and operation groups

Group	IM treatment (n=54)	Operation (n=44)	t/χ^2	P
Intestinal wall thickening	10 (18.52)	22 (50.00)	10.927	0.001
Pneumatosis cystoides intestinalis	21 (38.89)	18 (40.91)	0.041	0.839
Portal venous gas	8 (14.81)	13 (29.55)	3.125	0.077
Intestinal dilatation	12 (22.22)	19 (43.18)	4.925	0.026
Decreased intestinal dilatation	15 (27.78)	20 (45.45)	3.300	0.069
Free intraperitoneal air	0 (0.00)	2 (4.55)	2.506	0.113
Slowed intestinal peristalsis	22 (40.74)	21 (47.73)	0.481	0.488
Disappearance of intestinal peristalsis	2 (3.70)	17 (38.64)	18.930	0.000
Peritoneal effusion (good acoustic transmissibility)	23 (42.59)	20 (45.45)	0.081	0.776
Peritoneal effusion (poor acoustic transmissibility)	4 (7.41)	24 (54.55)	26.397	0.000
Decreased intestinal wall blood perfusion	6 (11.11)	10 (22.73)	2.395	0.122
Urinary I-FABP (ng/mL)	7.52±1.65	9.26±1.77	5.026	0.000

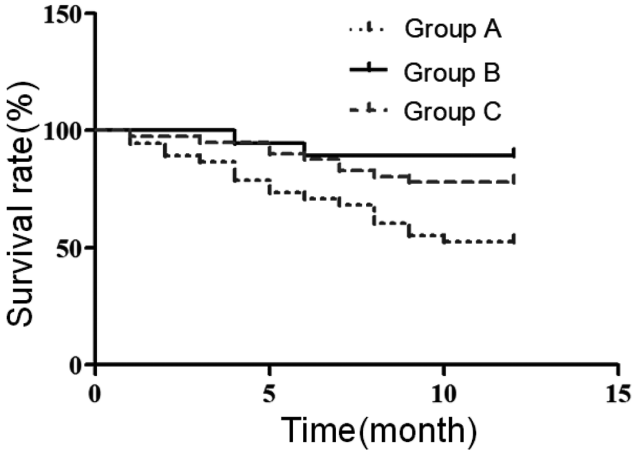

Fig. 3 — Survival curve analysis results of NEC neonates

I-FABP, a low molecular weight protein transporting fatty acids, is mainly expressed in small intestinal and gastric mucosal cells, and has good organ specificity. Elfarargy *et al.*[14] found that the serum I-FABP level within 24 h after the onset of symptoms in the NEC group was significantly higher than that in the healthy control group, so serum I-FABP may be a predictive index for the early diagnosis of neonatal NEC. In this study, the urinary I-FABP level in NEC neonates was significantly higher than that in healthy controls, and I-FABP >6.25 ng/mL was a risk factor for neonatal NEC.

Abdominal ultrasound not only displays intestinal peristalsis, structural echo changes, abdominal cavity condition, intestinal wall thickness and intestinal wall blood perfusion in real time, but also reduces the exposure of neonates to radiation[15]. It has high application value for the diagnosis and prognosis of neonatal NEC[16]. In this study, the confirmed NEC group had significantly higher proportions of neonates with intestinal wall thickening, portal vein gas, disappearance of intestinal peristalsis, peritoneal effusionpoor acoustic transmissibility and decreased intestinal wall blood perfusion than the suspected NEC group. Li & Sheng[17] reported that abdominal ultrasound combined with fecal calprotectin, procalcitonin, interleukin-6 and hypersensitive C-reactive protein improved the clinical diagnosis of neonatal NEC and well predicted the prognosis. In this study, AUC of combined detection for diagnosing neonatal NEC was 0.854 which exceeded those of abdominal ultrasound and urinary I-FABP alone, indicating that the combined detection augmented the diagnostic efficiency.

Free intraperitoneal air in abdominal ultrasound is a definite surgical indication for NEC neonates, and intestinal wall thickening, intestinal dilatation, disappearance of intestinal peristalsis and peritoneal effusion (poor acoustic transmissibility) can be employed to predict the poor clinical outcomes of NEC neonates[18]. Likewise, in this study, the operation group had significantly higher proportions of NEC neonates with intestinal wall thickening, intestinal dilatation, intestinal peristalsis, peritoneal effusion (poor acoustic transmissibility) and a markedly higher urinary I-FABP level than the internal medicine treatment group. Furthermore, the survival rate of group B was 89.47% which was significantly higher than those of groups A and C, indicating that the combined detection improved the prognosis.

Conclusion

Results of the above investigation indicate that abdominal ultrasound combined with urinary I-FABP can improve the diagnostic efficiency of neonatal NEC and has a high predictive value for the prognosis, and, this method has potential clinical application. Regardless, this study is limited. Prematurity is a known cause for NEC, hence the effects of other risk factors on term and preterm NEC babies should be further evaluated.

Conflicts of interest

Authors declare no competing interests.

References

1 Zhang K, Zhang X, Lv A, Fan S & Zhang J, Saccharomyces boulardii modulates necrotizing enterocolitis in neonatal mice by regulating the sirtuin 1/NF-κB pathway and the intestinal microbiota. *Mol Med Rep*, 22 (2020) 671.

2 Yue G, Yang H, Jin M, Deng Y & Ju R, Portal venous gas by ultrasound in advance of impending necrotizing enterocolitis of a very low birth weight infant. *J Clin Ultrasound*, 48 (2020) 178.

3 Xiong T, Maheshwari A, Neu J, Ei-Saie A & Pammi M, An Overview of Systematic Reviews of Randomized-Controlled Trials for Preventing Necrotizing Enterocolitis in Preterm Infants. *Neonatology*, 117 (2020) 46.

4 Tracy SA, Lazow SP, Castro-Aragon IM, Fujii AM, Estroff JA, Parad RB, Staffa SJ, Zurakowski D & Chen C, Is Abdominal Sonography a Useful Adjunct to Abdominal Radiography in Evaluating Neonates with Suspected Necrotizing Enterocolitis? *J Am Coll Surg*, 230 (2020) 903.

5 Qi G, Huang S, Lai D, Li J, Zhao Y, Shen C, Huang J, Liu T, Wei K, Tou J & Shu Q. An improved joint non-negative matrix factorization for identifying surgical treatment timing of neonatal necrotizing enterocolitis. *Biomol Biomed*, 22 (2022) 972.

6 Hwang M, Tierradentro-García LO, Dennis RA & Anupindi SA. The role of ultrasound in necrotizing enterocolitis. *Pediatr Radiol*, 52 (2021) 702.

7 El-Abd Ahmed A, Hassan MH, Abo-Halawa N, Abdel-Razik GM, Moubarak FA & Sakhr HM, Lactate and intestinal fatty acid binding protein as essential biomarkers in neonates with necrotizing enterocolitis: ultrasonographic and surgical considerations. *Pediatr Neonatol*, 61 (2020) 481.

8 Sekhon MK, Grubb PH, Newman M & Yoder BA, Implementation of a probiotic protocol to reduce rates of necrotizing enterocolitis. *J Perinatol*, 3 (2019) 1315.

9 Bazacliu C & Neu J, Pathophysiology of Necrotizing Enterocolitis: An Update. *Curr Pediatr Rev*, 15 (2019) 68.

10 Zvizdic Z, Sefic Pasic I, Dzananovic A, Rustempasic N, Milisic E, Jonuzi A & Vranic S, Bowel Dilatation on Initial Plane Abdominal Radiography May Help to Assess the Severity of Necrotizing Enterocolitis in Preterm Infants. *Children* (Basel), 7 (2020) 9.

11 Kaplina A, Kononova S, Zaikova E, Pervunina T, Petrova N & Sitkin S, Necrotizing enterocolitis: the role of hypoxia, gut microbiome, and microbial metabolites. *Int J Mol Sci*, 24 (2023) 2471.

12 Su Y, Xu RH, Guo LY, Chen XQ, Han WX, Ma JJ, Liang JJ, Hao L & Ren CJ, Risk factors for necrotizing enterocolitis in neonates: A meta-analysis. *Front Pediatr*, 10 (2023) 1079894.

13 Beghetti I, Panizza D, Lenzi J, Gori D, Martini S, Corvaglia L & Aceti A, Probiotics for preventing necrotizing enterocolitis in preterm infants: a network meta-analysis. *Nutrients*, 13 (2021) 192.

14 Elfarargy MS, El Farargy MS, Atef MM, El-Deeb OS, Elsharaby RM & Elhady HAE, Early Biomarkers in Neonatal Necrotizing Enterocolitis: A Pilot Study. *J Popul Ther Clin Pharmacol*, 26 (2019) e1.

15 Guo Y, Si S, Jia Z, Lv X & Wu H, Differentiation of food protein-induced enterocolitis syndrome and necrotizing enterocolitis in neonates by abdominal sonography. *J Pediatr* (Rio J), 97 (2021) 219.

16 Chan B, Gordon S, Yang M, Weekes J, Dance L, Gephart SM & Quinn M, Abdominal ultrasound assists the diagnosis and management of necrotizing enterocolitis. *Adv Neonatal Care*, 21 (2021) 365.

17 Li Z & Sheng L, Significance of dynamic evolution of TNF-α, IL-6 and intestinal fatty acid-binding protein levels in neonatal necrotizing enterocolitis. *Exp Ther Med*, 15 (2018) 1289.

18 Alexander KM, Chan SS, Opfer E, Cuna A, Fraser JD, Sharif S & Khashu M, Implementation of bowel ultrasound practice for the diagnosis and management of necrotising enterocolitis. *Arch Dis Child Fetal Neonatal Ed*, 106 (2021) 96.