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Urine-based detection of intestinal mucosal cell damage in neonates with suspected necrotizing enterocolitis

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Objectives
To assess the diagnostic value of the urinary intestinal fatty acid-binding protein (iFABP) in neonatal necrotizing enterocolitis (NEC) in the early stage of the disease.

Background
NEC is a severe acute gastrointestinal disease affecting preterm newborns. iFABP has been associated with injury to the intestinal mucosa common to NEC.

Patients and methods
This cross-sectional study included 40 preterm neonates divided into two groups: group I included 20 preterm neonates with suspected NEC according to Modified Bell Staging Criteria for NEC and group II included 20 preterm neonates with non-NEC. All the included participants underwent full history taking, full examination, routine laboratory investigations, and assessment of urinary iFABP.

Results
The mean urinary iFABP level was 17.26 ± 3.69 ng/dl in group I and 8.39 ± 2.49 ng/ml in group II. This difference was significantly higher in the suspected NEC group (P = 0.001). The iFABP level at a cutoff more than 9.25 ng/ml had significant power of discrimination of NEC cases at an early stage (P = 0.001) with a sensitivity of 96.0% and specificity of 71.0%. Linear regression revealed that the sampling time was a significant measure for prediction of iFABP (P = 0.001).

Conclusion
There was an association between elevated iFABP levels in urine and NEC, suggesting that iFABP may be useful as a diagnostic biomarker for earlier identification of NEC.

Keywords:
Bell's criteria, suspected necrotizing enterocolitis, urine intestinal fatty acid-binding protein

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Introduction

Necrotizing enterocolitis (NEC) is predominantly a disease of premature low-birth-weight infants rather than those who are small for gestational age [1]. Approximately 90% of NEC cases develop in infants after feedings are initiated. An association between an increase in the incidence of NEC and advancement of formula feedings at rates greater than 20 kcal/kg/day is suggested [2]. Although the precise pathogenesis remains incompletely understood, clinical progress in recent years portends a shift in focus to prevention and the earlier identification of those infants most at risk or with progressive disease [3].

Cytokines and growth factors play a critical role in mediating the interaction among enterocytes, endothelial cells, fibroblasts, and inflammatory cells, which together are critical to the overall cellular pathophysiology of NEC [4]. Significantly reduced levels of salivary and serum epidermal growth factor have been demonstrated in premature infants in whom NEC developed versus age-matched controls [5]. Overproduction of anti-inflammatory cytokines (IL-4, IL-10, and IL-11) may result in excessive suppression of immune function. A number of different inflammatory mediators have been implicated in the pathogenesis of NEC [6].

The intestinal fatty acid-binding protein (iFABP) is one of the most potential biomarkers of NEC. It is exclusively in epithelial cells in the mucosal layer of the small intestine. When intestinal epithelial cells are damaged, iFABP proteins are released into the bloodstream and then excreted by the kidneys [7]. Thus, iFABP can be detected in either blood or urine as a potential biomarker of intestinal mucosal damage caused by NEC. Gollin *et al.*[8] reported that elevated iFABP (in urine) was a predictive biomarker for NEC one day before clinical manifestations.

The aim of this study was to assess the diagnostic value of the urinary iFABP in the early stage of neonatal NEC.

Patients and methods

This cross-section was conducted on 40 preterm neonates divided into two groups; group I included 20 preterm neonates with suspected NEC according to the modified Bell's staging criteria [9], whereas group II included 20 preterm neonates of matched age and sex. Patients were recruited from the neonatal ICU of Menoufia University Hospital and New Damietta Al-Azhar University Hospital, Egypt, within the period from July 2020 and June 2021. After ethical consideration, approval was obtained from the health facility Ethics Committee. An informed consent was taken from the enrolled patients' guardians. All of the included participants underwent full history taking and full examination. Then, venous blood samples of both suspected NEC and non-NEC cases were collected for measurement of CBC using Sysmex kx21n provided by Sysmex Corp., 1-5-1,Wakinohama-kaigandori, Chuo-ku, Kobe, Hyogo 651-0073, Japan; CRP using Spinreact CRP Latex test kit, Girona, Spinreact CRP Latex test kit, Girona, Spain; serum creatinine using creatinine Jaffe provided by Elitech clinical systems SAS Group, Puteaux, France; and ABG, serum sodium, and potassium using Gem premier 3500 provided by Werfen, Barcelona, Spain. Assessment of urine iFABP was performed using a human (iFABP) enzyme-linked immunosorbent assay kit provided by SunRed Corp., Shanghai, China, according to manufacturer instructions.

Inclusion criteria were patients with preterm neonates (≤34 weeks), aged 0–14 days of both sexes, and received a diagnosis of suspected NEC stage IA according to the modified Bell's staging criteria: temperature instability, apnea, lethargy, increased gastric residual, abdominal distension, and occult blood in stool. Exclusion criteria included full-term neonates; infants initially diagnosed as having possible noninfectious etiology, for example, inborn error of metabolism; patients with malignancies; genetic or chromosomal syndrome known to affect the immune system, for example, Down syndrome; extreme low-birth-weight infants (<1000 g); congenital infection; perinatal asphyxia; congenital abnormalities; intracranial hemorrhage; and maternal drug abuse.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences program (SPSS) for Windows, version 22 (SPSS Inc. Chicago, Illinois, USA). Qualitative data were described using number and percent. Quantitative data were described using median and interquartile range for nonparametric data and mean and SD for parametric data after testing normality using Kolmogorov–Smirnov test. Significance of the obtained results was judged at the 0.05 level. χ^2 test was used for comparison of two or more groups for qualitative data. Student *t* test was used to compare two independent groups for normally distributed data, and Mann–Whitney *U* test was used to compare two independent groups for non-normally distributed data. The Spearman correlation was used to determine the strength and direction of a linear relationship between one normally distributed continuous variables and one non-normally distributed continuous variables. Significant correlation was expressed in a scatterplot diagram. Linear regression analysis was used for prediction of independent variables of continuous parametric outcome. Binary stepwise logistic regression analysis was used for prediction of independent variables of binary outcome. Significant predictors in the univariate analysis were entered into regression model using forward Wald method/ Enter. Adjusted odds ratios and their 95% confidence interval were calculated. In addition, receiver operating characteristic curves was constructed to assess cutoff, sensitivity, specificity, and accuracy of the iFABP levels.

Results

There were no statistically significant difference between the studied groups regarding sociodemographic characterization. The most common clinical presentation of NEC cases were gastric residual, abdominal distension, and emesis, followed by temperature instability, lethargy, and apnea (Table 1). Regarding laboratory investigations, platelet count and serum sodium were significantly lower in the suspected NEC group, whereas potassium and CRP were significantly higher in the suspected NEC group ($P < 0.05$). As for urine iFABP, it was significantly higher in the suspected NEC group ($P = 0.001$) (Table 2).

There was a significant positive correlation between iFABP and each of start of feeding, CRP, and serum K ($r = 0.51$, 0.55, and 0.42, respectively). However, platelet count and serum Na showed a significant inverse correlation (r=–0.49, and –0.56, respectively) (Table 3). On the contrary, there was a strong inverse correlation between iFABP and sampling time of suspected NEC group (r=–0.85, $P = 0.001$) (Fig. 1). At a cutoff more than 9.25 ng/ml, iFABP had significant good power of discrimination for suspected NEC cases at early stages, with a sensitivity of 94%, specificity of 71%, area under the curve of 0.96, and accuracy of 82.5% (Fig. 2). The median of sampling time of urinary iFABP was 3.5 (2–5) days in NICU. It was inversely correlated with iFABP of suspected NEC group, (r=–0.85, $P = 0.001$), and it was predictive of iFABP with a standard error of 0.96. Binary logistic regression revealed that both

Table 1 Comparison of demographic and clinical data of the studied groups

Demographics	Suspected NEC group (*n*=20) [*n* (%)]	Non-NEC group (*n*=20) [*n* (%)]	Test of significance	*P*
Sex				
Male	11 (55)	15 (71.4)	χ^2=0.96	0.33
Female	9 (45)	6 (28.6)		
Age (days)				
Min.-max.	3-14	4-14	*U*=162	0.3
Median (IQR)	9 (7-12.75)	7.5 (5-12)		
Weight (g)				
Min.-max.	1125-2600	1150-2500	*t*=0.83|	0.41
Mean±SD	1748.6±421.03	1647±352.3		
RD stage				
RD II	18 (90)	17 (85)	χ^2=7.03	0.13
RD III	2 (5)	1 (5)		
RD IV	0	2 (10)		
Ventilation				
Off oxygen	19 (95)	17 (85)	χ^2=2.1	0.35
CPAP	1 (5)	1 (5)		
MV	0	2 (10)		
Gastric residual	19 (95)	0	χ^2=36.2	0.001*
Abdominal distension	19 (95)	0	χ^2=36.2	0.001*
Emesis	19 (95)	0	χ^2=36.2	0.001*
Intestinal sounds				
Audible	12 (60)	20 (100)	χ^2=10	0.007*
Weak	7 (35)	0		
None	1 (5)	0		
Occult blood	10 (50)	0	χ^2=13.3	0.001*
Temp. instability	15 (75)	0	χ^2=24	0.001*
Apnea	13 (65)	0	χ^2=19.3	0.001*
Bradycardia	12 (60)	0	χ^2=17.1	0.001*
Lethargy	15 (75)	0	χ^2=24	0.001*
Start of feeding (day in NICU)				
Min.-max.	1-17 (1-15)		*U*=109	0.01*
Median (IQR)	2.5 (2-4) (2 (2-4.75)			
Type of feeding				
Formula	20 (100)	16 (80)	χ^2=4.44	0.04*
Formula and BF	0	4 (20)		

χ^2, χ^2 test; BF, breastfeeding; CPAP, continuous positive airway pressure; IQR, interquartile range; max, maximum; min, minimum; MV, mechanical ventilation; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; RD, respiratory distress; *t*, Student *t* test; *U*, Mann-Whitney *U* test. *Statistically significant (*P*<0.05).

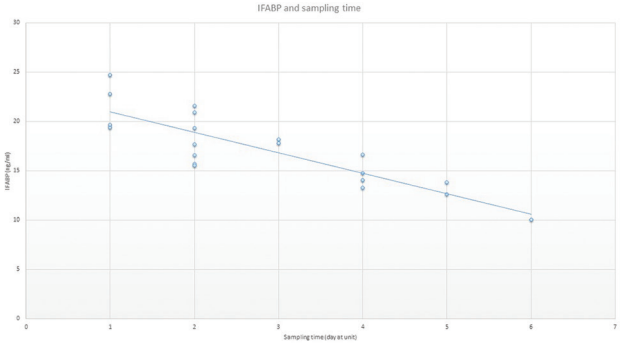
serum potassium and urine iFABP were risk factors for NEC (Table 4).

Discussion

In the present study, the clinical presentation of NEC cases revealed that 95% of them had gastric residual, abdominal distension, and emesis. Overall, 75% had temperature instability and lethargy, 65% had apnea, 60% complained of bradycardia, and 50% had occult blood. Moreover, 60% of them had audible intestinal sounds, 35% had weak intestinal sounds, and 5% had no intestinal sounds.

Early signs of NEC are nonspecific and may be indistinguishable from those of sepsis. Clinical signs include both intestinal and systemic perturbations. Intestinal signs in early NEC can present as feeding intolerance that may manifest as increased prefeeding gastric residuals, emesis, abdominal distension, and bloody stools (hematochezia) [10].

Figure 1



A scatter plot showing the correlation between iFABP of cases and sampling time. iFABP, intestinal fatty acid-binding protein.

Table 2 Laboratory data of the studied groups

Parameters	Suspected NEC group (*n*=20)	Non-NEC group (*n*=20)	Test of significance	*P*
Hb (g/dl)				
Min.-max.	8.9-18.5	9.1-19.9	*t*=0.13	0.27
Mean±SD	14.61±2.5	15.64±3.23		
Platelets (×10^9/l)				
Min.-max.	12-891	11-427	*U*=77§	0.001*
Median (IQR)	193 (101-263.3)	248 (108.3-282)		
WBC (×10^3/mm^3)				
Min.-max.	3.3-30	4.3-24.2	*U*=191.5§	0.82
Median (IQR)	11.9 (7.3-13.6)	10.6 (7.5-12.6)		
CRP (µg/ml)				
Min.-max.	0-48	0-24	*U*=88.5	0.001*
Median (IQR)	4.5 (0-12)	0.5 (0-6)		
Na (mmol/l)				
Min.-max.	125-140	129-140	*t*=−2.9	0.006*
Mean±SD	132.6±3.9	135.8±3.1		
K (mEq/l)				
Min.-max.	3.2-5.7	3.5-4.5	*U*=74	0.001*
Median (IQR)	4.5 (3.8-5.3)	3.5 (3.5-3.9)		
SCr (mg/dl)				
Min.-max.	0.3-0.7	0.3-1.2	*U*=155	0.2
Median (IQR)	0.5 (0.5-0.6)	0.55 (0.43-0.6)		
iFABP (ng/ml)				
Min.-max	14.76-24.7	4.42-12.04	*t*=8.89	0.001*
Mean±SD	17.26±3.69	8.39±2.49		

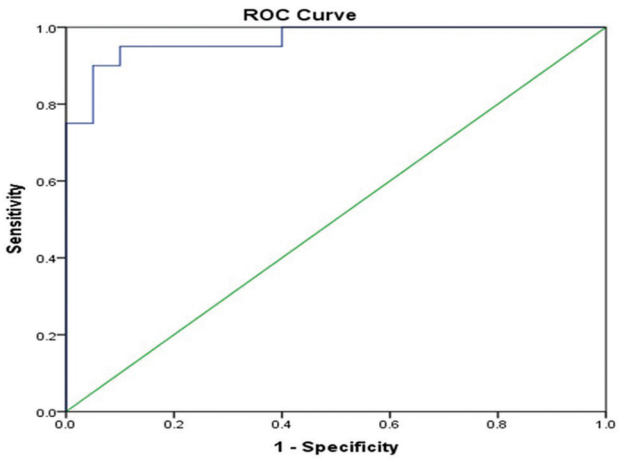
CRP, C-reactive protein; Hb, hemoglobin; iFABP, intestinal fatty acid binding protein; IQR, interquartile range; max, maximum; min, minimum; NEC, necrotizing enterocolitis; SCr, serum creatinine; *t*, Student *t* test; *U*, Mann-Whitney *U* test; WBC, White blood cells. *Statistically significant (*P*<0.05).

Table 3 Correlation between intestinal fatty acid-binding protein and each of demographic and laboratory parameters

Parameters	iFABP (ng/ml)	
	r	*P*
Age (days)	0.29*	0.07
GA (week)	0.26*	0.1
Weight (g	0.12	0.46
Start of feeding (day at unit)	0.51*	0.001*
Hb (g/dl)	0.19	0.25
Platelets (×10^9/L)	−0.49*	0.001*
WBC (×10^3/mm^3)	0.19	0.22
CRP (mg/ml)	0.55*	0.001*
Na (mmol/l)	−0.56*	0.001*
K (mEq/l)	0.42*	0.01*
SCr (mg/dl)	0.05	0.78
Sampling time	−0.85**	0.001*

CRP, C-reactive protein; Hb, hemoglobin; iFABP, intestinal fatty acid binding protein; *r*, Spearman correlation coefficient; SCr, serum creatinine; WBC, white blood cells. *Statistically significant (*P*<0.05).

Figure 2



ROC curve of iFABP for prediction of NEC. iFABP, intestinal fatty acid-binding protein; NEC, necrotizing enterocolitis; ROC, receiver operating characteristic.

The rate and substance of infant feeding have been a major focus of NEC research. Overall, 90% of infants who develop NEC have been fed formula. Altering feeding regimen is a powerful measure for clinicians that can reduce the risk for NEC, although the balance between supporting neonatal growth and hedging a baby's risk of developing NEC is challenging [11].

Our study evaluated the start of feeding and type of feeding, and there was a statistically significant difference regarding both of them. The median time of feeding start was second (2nd–4.75th) day in NEC cases versus 2.5nd (2nd–4th) day in non-NEC cases. All suspected NEC cases versus 80% of non-NEC cases started formula feeding, whereas 20% of non-NEC cases started formula plus breast-feeding. However, Yoon *et al.*[12] evaluated feeding type that revealed that all cases versus 94% consumed formula feeding in the NEC group (*n* = 6) and non-NEC group (*n* = 108), respectively.

Table 4 Binary logistic regression for prediction of necrotizing enterocolitis

Risk factors	*B*	*P*	OR (95% CI)
Start of feeding	−0.45	0.25	0.64 (0.13-31.36)
Platelets (×10^9/l)	−0.002	0.51	0.99 (0.3-1.01)
CRP (mg/ml)	−0.26	0.07	0.77 (0.58-1.01)
Na (mmol/l)	0.29	0.1	1.34 (0.94-1.9)
K (mEq/l)	−3.19	0.02*	0.04 (0.03-0.64)
iFABP	−1.32	0.02*	3.75 (1.19-11.78)

CI, confidence interval; CRP, C-reactive protein; iFABP, intestinal fatty acid binding protein; OR, odds ratio. *Statistically significant (P<0.05).

In this study, the CBC showed only a statistically significant difference between the two groups regarding platelet count. The median platelet count was significantly lower in the suspected NEC group. Thrombocytopenia (platelet count <150 × 10^9/l) is a frequently encountered hematological abnormality in infants with NEC. Patients with confirmed NEC typically show decreased platelet counts within 24 h of disease onset. This thrombocytopenia may worsen until 72 h, and the depth of the nadir correlates with the severity and extent of bowel injury [13]. Moreover, Siahaan *et al.*[14] evaluated the clinical significance of low platelet count in neonates diagnosed with NEC where the nadir platelet count (lowest level during the course of disease) was lower in patients with stage III disease than in patients with stage II disease. The greater the extent of the disease, the lower the platelet count. The nadir platelet count was lower in infants who died than in survivors. None of the patients with platelet count greater than 100 × 10^9/l had died [14]. In addition, Yoon *et al.*[12] reported that there was no significant difference between the two groups regarding hemoglobin, in the suspected NEC group and non-NEC group.

In the current study, both CRP and potassium were statistically significantly higher in the suspected NEC group. However, serum sodium was statistically significantly lower in the suspected NEC group. A study done by Young *et al.*[15] evaluated the association between serum CRP and NEC, where CRP levels were found to be abnormal in both stage II and stage III NEC. In infants with NEC, persistently elevated CRP after initiation of appropriate medical management suggested development of complications of NEC, which often required surgical intervention.

Indications for surgical consultation include abdominal wall cellulitis, fixed dilated bowel, tender abdominal mass, or clinical deterioration not responsive to medical management (metabolic acidosis, thrombocytopenia, increasing respiratory support, hypovolemia, oliguria, leucopenia, leukocytosis, hyperkalemia, and increased third-space losses) [11]. Metabolic derangement, a measure of acuity, was defined by assigning one point per indicator (thrombocytopenia, metabolic acidosis, neutropenia, left shift of segmented neutrophils, hyponatremia, bacteremia, and hypotension) and calculating a total. When two or more indicators of metabolic derangement were present, the odds of mortality dramatically increased [16].

In the current study, urine iFABP was significantly higher in the suspected NEC group Moreover, Gregory *et al.*[17] found that the median iFABP was higher among cases and statistically significant in Bell Stages I and III when they were stratified by Bell stage.

A correlation was performed between urine iFABP and each of demographic and laboratory parameters. We found a significant positive correlation between urine iFABP and each of start of feeding, CRP, and serum potassium, On the contrary, each of platelet count, serum sodium, and sampling time exhibited a significant negative correlation. Sampling time was significant for prediction of urine iFABP.

This study revealed that urine iFABP is a statistically significant predictor of NEC with every 1 ng/ml increase in urine iFABP increases the risk of NEC by 3.75 [odds ratio (OR): 3.75, 95% confidence interval (CI): 1.19–11.78]. On the contrary, Gregory *et al.*[17] reported the OR for a 10-fold change in iFABP was 4.14 (95% CI: 2.20, 7.81) and 6.84 (95% CI: 2.87, 16.31) for 7-day and 3-day analysis, respectively.

In the current study, the best cutoff point of iFABP in differentiating cases with NEC was 9.25 ng/ml with 94% sensitivity, 71% specificity, PPV of 65.4%, NPV of 100%, and total accuracy of 82.5%. In addition, Schurink *et al.*[18] reported that the cutoff values of iFABP for diagnosing NEC were 9 ng/ml. iFABP levels were highest in the first 8 h after symptom onset and gradually decreased over time. Cutoff values for complicated disease were 19 ng/ml.

Conclusion

Our reported findings provide further evidence of the association between elevated iFABP and NEC previously observed in other studies, suggesting that iFABP may be useful as a diagnostic marker for earlier identification of suspected NEC cases.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Rose AT, Patel RM. A critical analysis of risk factors for necrotizing enterocolitis. Semin Fetal Neonatal Med 2018; **23**:374–379.

2 Meister AL, Doheny KK, Travagli RA. Necrotizing enterocolitis: It's not all in the gut. Exp Biol Med (Maywood) 2020; **245**:85–95.

3 Samuels N, van de Graaf RA, de Jonge RC, Reiss IK, Vermeulen MJ. Risk factors for necrotizing enterocolitis in neonates: a systematic review of prognostic studies. BMC Pediatr 2017; **17**:105.

4 Alganabi M, Lee C, Bindi E, Li B, Pierro A. Recent advances in understanding necrotizing enterocolitis. F1000Res 2019; 8:F1000 Faculty Rev–F1000 Faculty 107.

5 Patel RM, Underwood MA. Probiotics and necrotizing enterocolitis. Semin Pediatr Surg 2018; **27**:39–46.

6 MohanKumar K, Namachivayam K, Ho TT, Torres BA, Ohls RK, Maheshwari A. Cytokines and growth factors in the developing intestine and during necrotizing enterocolitis. Semin Perinatol 2017; **41**:52–60.

7 Garg B, Sharma D, Bansal A. Biomarkers of necrotizing enterocolitis: a review of literature. J Matern Fetal Neonatal Med 2018; **31**:3051–3064.

8 Gollin G, Stadie D, Mayhew J, Slater L, Asmerom Y, Boskovic D, *et al*. Early detection of impending necrotizing enterocolitis with urinary intestinal fatty acid-binding protein. Neonatology 2014; **106**:195–200.

9 Bell MJ, Ternberg JL, Feigin, RD, Keating JP, Marshall R, *et al*. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg 1978; **187**:1–7.

10 Sharma R, Hudak ML. A clinical perspective of necrotizing enterocolitis: past, present, and future. Clin Perinatol 2013; **40**:27–51.

11 Gephart SM, McGrath JM, Effken JA, Halpern MD. Necrotizing enterocolitis risk: state of the science. Adv Neonatal Care 2012; **12**:77–89.

12 Yoon JM, Park JY, Ko KO, Lim JW, Cheon EJ, Kim HJ. Fecal calprotectin concentration in neonatal necrotizing enterocolitis. Korean J Pediatr 2014; **57**:351–356.

13 Maheshwari A. Role of platelets in neonatal necrotizing enterocolitis. Pediatr Res 2021; **89**:1087–1093.

14 Siahaan ES, Adriansyah W, Sasmita AP, Fauzi AR, Dwihantoro A, Gunadi ■. Outcomes and prognostic factors for survival of neonates with necrotizing enterocolitis. Front Pediatr 2021; **9**:744504.

15 Young C, Sharma R, Handfield M, Mai V, Neu J. Biomarkers for infants at risk for necrotizing enterocolitis: clues to prevention?. Pediatr Res 2009; **65(Part 2)**:91R–97R.

16 GałĄzka P, Chrzanowska M, StyczyŃski J. Clinical spectrum and outcomes of neonatal necrotizing enterocolitis. *In Vivo* 2021; **35**:585–591.

17 Gregory KE, Winston AB, Yamamoto HS, Eaton S. Urinary intestinal fatty acid binding protein predicts necrotizing enterocolitis. J Pediatr 2014; **164**:1486–1488.

18 Schurink M, Kooi EM, Hulzebos CV, Kox RG, Groen H, Heineman E, *et al*. Intestinal fatty acid-binding protein as a diagnostic marker for complicated and uncomplicated necrotizing enterocolitis: a prospective cohort study. PLoS ONE 2015; **10**:e0121336.