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ORIGINAL ARTICLE

Hypoxic-ischemic enterocolitis: a proposal of a new terminology for early NEC or NEC-like disease in preterm infants, a single-center prospective observational study

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Abstract

We aimed to investigate the role of hypoxia-ischemia in the pathophysiology of early NEC/NEC like disease (ENEC) and classic NEC/NEC like disease (CNEC) in preterm infants. In this pilot study, preterm infants who developed the clinical symptoms and signs of NEC/NEC like disease were divided into two groups as early (≤ 7 days, ENEC) or late (> 7 days, CNEC) groups. Beside clinical variables, serum L-lactate, endothelin-1 (ET-1), platelet activating factor (PAF), and intestinal fatty acid binding protein (I-FABP) levels were measured from umbilical/peripheric venous blood in the first hour of life and during the clinical presentation in all groups. A total of 86 preterm infants were enrolled in the study. In the ENEC group, the incidences of fetal umbilical artery Doppler velocimetry abnormalities, IUGR, and delayed passage of first meconium were higher. In addition, mean levels of L-lactate, ET-1, PAF, and I-FABP were higher in the first hour of life.

Conclusion: Our study firstly showed that the dominant pathophysiological factor of ENEC is prenatal hypoxic-ischemic event where intestinal injury and inflammation begin in-utero and become clinically apparent in the first week of life. Therefore, we propose a new term "Hypoxic-Ischemic Enterocolitis (HIEnt)" for the definition of ENEC in preterm infants with prenatal hemodynamic disturbances and IUGR. This new sight can provide individualized preventive and therapeutic strategies for preterm infants.

What is Known:

- *The pathophysiology of early necrotizing enterocolitis (NEC) or NEC-like disease which is seen in the first week of life seems different than classic necrotizing enterocolitis (CNEC) which is always seen after the first week of life.*

What is New:

- *This study suggests that perinatal hypoxic-ischemic process with inflammation is the point of origin of fetal intestinal injury leading to ENEC.*
- *We propose a new term "Hypoxic-Ischemic Enterocolitis (HIEnt)" for the definition and differentiation of this unique clinical entity.*

Keywords Necrotizing enterocolitis · PAF · I-FABP · ET-1 · Lactate

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Abbreviations

AREDF	absent or reversed end-diastolic blood flow
CNEC	classic necrotizing enterocolitis
ENEC	early necrotizing enterocolitis
ET-1	endothelin-1
I-FABP	intestinal fatty acid binding protein
IUGR	intrauterine growth restriction
NEC	necrotizing enterocolitis
NICU	neonatal intensive care unit
PAF	platelet activating factor

Introduction

Necrotizing enterocolitis (NEC) is the most important acquired gastrointestinal disease of the newborn infants and is characterized by intestinal injury, systemic inflammation, and multisystem organ failure [1]. The combination of multiple risk factors including genetic predisposition, intestinal structural and functional immaturity, hypoxia-ischemia, abnormal microbial colonization, timing of initiation, composition, and rate of enteral feedings causes NEC [2, 3]. The pathophysiologic mechanism and the time of onset of NEC seem to be related. In very low gestational age (GA) preterm infants, classic NEC develops almost always after the first week of life probably related to abnormal intestinal microbiota [4–7]. In late preterm or term newborn infants, NEC usually develops in the first week of life due to perinatal hypoxic-ischemic events or diseases such as congenital left heart obstructive lesions, polycythemia, and intrauterine growth restriction (IUGR) [8]. Spontaneous intestinal perforation (SIP) which is mostly seen in preterm growth restricted infants in the first week of life has been also related to an intestinal hypoxic-ischemic insult [9, 10].

Beside these entities, there is another group of preterm infants who develop early NEC or NEC-like disease in the first week of life. These infants almost always have IUGR and fetal hemodynamic disturbances such as absent or reversed end-diastolic blood flows (AREDF) in umbilical artery. The clinical presentation varies from feeding intolerance to typical NEC. In these infants, postnatal superior mesenteric artery Doppler studies have showed the continuation of redistribution which has started prenatally [11, 12].

Gordon et al. [13] reported 14 different subsets of NEC and suggested that "day of diagnosis-centric data" can help in understanding the NEC pathophysiology. Timing of onset was the starting point of our study to distinguish NEC pathophysiology. We aimed to investigate the role of hypoxia-ischemia in the pathophysiology of early NEC/NEC like disease (ENEC) and classic (late) NEC/NEC like disease (CNEC) in preterm infants by the biomarkers which are indicative for hypoxia-ischemia and intestinal cell injury[14–16].

Methods

This single-center prospective observational study (Hacettepe University Research Grant No: 011 D04 101008) was conducted in the neonatal intensive care unit (NICU) of Hacettepe University Ihsan Dogramaci Children's Hospital, Ankara, Turkey between 1 July 2010-1 September 2012. The Institutional Ethics Committee approved the study (No: HEK 10/91) and informed consent forms were received from all parents before inclusion in the study.

Study population

Preterm infants (GA < 37 weeks) who were admitted to NICU for any reason were eligible for study. Infants who developed NEC/NEC like disease in the first 7 days of life (≤ 7 days) constituted "early NEC (ENEC) group," while preterm infants who developed NEC/NEC like disease after the 7th day of life (>7 days) constituted "classic NEC (CNEC)" group. Preterm infants who did not develop any NEC/NEC like disease during the entire NICU stay served as the control group. The study and control population included only *inborn* preterm infants whose parents agreed to participate in the study during the study period. Also, after reaching a reasonable number of cases in the control group, we stopped including new cases to the control group. Infants with congenital or chromosomal abnormalities, inherited metabolic diseases, infants with culture positive early neonatal sepsis in the first week of life, infants with SIP, and infants who were hospitalized shorter than 7 days because of discharge or death were excluded. In addition, symptomatic infants without occult/gross blood in stool and infants who were proven to swallow maternal blood by Apt test were not enrolled in order to exclude infants with feeding intolerance.

Definition of NEC/NEC like disease

All infants who developed at least one gastrointestinal sign or symptom such as gastric residuals, vomiting and bilious drainage from enteral feeding tubes, abdominal distention, prominent intestinal loops, abdominal tenderness and discoloration, abdominal mass with radiological features such as dilated intestinal loops, fixed loop, pneumatosis intestinalis, portal air or free air in the abdomen, gasless abdomen + and/or systemic signs mimicking feeding intolerance or NEC such as; temperature instability, hypotension, apnea, respiratory failure, lethargy, were taken into study groups. Infants who had occult or gross blood in stool in addition to mild gastrointestinal/systemic signs were classified as stage I NEC/NEC like disease. Infants with stage II or III NEC were defined according to the modified Bell's criteria [17]. The diagnosis was confirmed by a senior neonatologist who was blind and not involved in the study.

Nutritional protocol and procedures

According to our NICU enteral nutrition protocol, the first choice was breast milk and the second choice was preterm infant formula in case of complete absence or inadequate amount of maternal breast milk. Minimal enteral nutrition was initiated on the first day of life at a volume of 10–20 ml/kg/day in infants with a birth weight of < 1500 g and 21–40 ml/kg/day in infants with a birth weight of ≥ 1500 g. The enteral nutrition volume was increased by 10–15 ml/kg/day if

possible and finally reaching 150–180 ml/kg/day. All infants received enteral nutrition every 3 h (× 8/day). Infants who could not suck bottles adequately were fed by orogastric feeding tubes. All infants were kept in a supine position with their head and back 30° above the horizontal position until the end of the first hour after each enteral feeding. Total parenteral nutrition was initiated according to nursery protocol in very low birth weight (< 1500 g) infants and in those for whom enteral nutrition was not sufficient to achieve an energy supply of 120–150 kcal/kg/day. For these infants, intravenous protein was initiated at a dose of 1.5 g/kg/day on day 1 and reached 3.5 g/kg/day on day 3, while intravenous lipid was initiated at 1 g/kg/day on day 2 and reached 2–2.5 g/kg/day on day 3. In addition, a glucose infusion was initiated at a rate of 6–8 mg/kg/min and increased to 12–14 mg/kg/min as tolerated.

Data collection

Prenatal data included intrauterine growth (as defined by growth curve of Fenton et al. [18]), maternal and obstetric diseases (chronic hypertension, pregnancy induced hypertension, preeclampsia-eclampsia, chorioamnionitis), fetal umbilical artery Doppler flow velocimetry disturbances (defined as the following: (a) AREDF in the umbilical artery seen on at least 50% of waveforms on at least one occasion during pregnancy or (b) cerebral redistribution (umbilical artery pulsatility index> 95th centile and middle cerebral artery pulsatility index < 5th centile for gestation [19])), and prenatal steroid therapy. Natal data including gender, GA, birth weight, mode of delivery, 5th minute Apgar score, and presence of aggressive resuscitation at birth (positive pressure ventilation through bag and mask or endotracheal tube, chest compression, or drug administration) were recorded. Also, postnatal morbidities such as respiratory distress syndrome, congenital pneumonia, surfactant therapy, patent ductus arteriosus, intraventricular hemorrhage, bronchopulmonary dysplasia, durations of parenteral nutrition, mechanical ventilation, supplemental oxygen, and hospitalization were noted. All infants were fed according to our institutional NICU nutritional protocol. In the study, all systemic, gastrointestinal and radiological signs, day of onset of NEC/NEC like disease, duration (day) and volume (ml/kg/day) of enteral nutrition at the onset of NEC/NEC like disease, and the presence of delayed passage of the first meconium (> 48 h) [20] were recorded.

Serological markers

We studied "plasma L-lactate" and "ET-1" levels as indicators of tissue hypoxia-ischemia, "PAF" as a good indicator of inflammation, and I-FABP as a marker for intestinal cell injury [21, 22]. In order to clarify the pathophysiologic and chronologic differences between ENEC and CNEC, levels of these biomarkers were measured from umbilical/peripheral venous blood samples both in the first hour of life and then during the clinically symptomatic period. After admission to the NICU, 2 ml of venous blood was collected in tubes containing EDTA for ELISA analysis and heparin for blood gas analysis (pH and L-lactate) from all preterm infants. In infants who developed gastrointestinal +/- systemic signs and symptoms, second venous blood samples (2 ml) were obtained for the same analysis. All EDTA plasma samples were centrifuged at room temperature, 1500 rpm, for 15 min and stored at − 80 °C until ELISA analysis for I-FABP, PAF, and ET-1 levels. In the control group who did not develop any NEC/NEC like disease, the second blood samples were taken randomly during the 5–14 days of life.

The determination of plasma I-FABP concentrations was performed by HK-406 Human I-FABP Elisa Kit (Hycult Biotech Inc., PA, USA). Plasma PAF levels were measured with Human PAF Elisa Kit (Cusabio Biotech Co. Ltd., PRC), and plasma levels of ET-1 were determined using Human Endothelin Kit (1-21) (Biomedica Medizinprodukte GmbH & Co KG, Austria). All the procedures proceeded according to the manufacturers' instructions. Plasma L-lactate levels were measured by an automated blood gas analyzer (Radiometer, Copenhagen, Denmark).

Statistical analysis

The statistical data was analyzed using "Statistical Package for Social Sciences" (SPSS for Windows 15.0, Chicago, USA) software on a personal computer. Power analysis was not performed as there was a limitation for the study period as defined by 2 years due to fellowship program. All data were initially controlled for normality of distribution according to the Kolmogorov Smirnov test. The data were presented as a mean ± standard deviation (SD), median (minimum–maximum), frequency, and percentage. For descriptive statistical analysis, mean ± standard deviation (SD) was used for normally distributed data, median was used for non-parametric data and percentage was used for categorical variables. Continuous variables were compared using Mann–Whitney for nonparametrically distributed data. Categorical variables were analyzed using the $\chi 2$ test or Fisher's exact test. Also, ANOVA, Welch ANOVA, Kruskal Wallis, and Conover's test were used for statistical analysis. P value of < 0.05 was accepted as statistically significant. We performed a multivariate analysis correcting for possible known confounders.

Results

During the study period, a total of 86 infants were enrolled in the study and of these, 24 (27.9 %) were in the ENEC group, 19 (22.1 %) were in the CNEC group, and 43 (50.0%) were in the control group. The demographic and clinical

characteristics of the control, ENEC, and CNEC groups were given in Table 1. There were no statistically significant differences between the mean GA and birth weights of the ENEC and CNEC groups ($p = 0.391$, 0.172, respectively). In spite of similar mean GA, the lower mean birth weight of the ENEC group than the control group was due to the significantly higher incidence of IUGR infants in the ENEC group ($p < 0.001$).

The incidences of AREDF, IUGR, and delayed first meconium passage were significantly higher in the ENEC group than the CNEC and control groups. There were no significant differences in the incidences of major neonatal morbidities and mortality between ENEC and CNEC groups. Also, NEC stages were similar between the study groups ($p = 0.568$) (Table 1). The percentage of infants who received no enteral feeding including minimal enteral nutrition (MEN) before the onset of NEC/NEC like disease were significantly higher in the ENEC group than the CNEC group ($p = 0.027$)(Table 2).

In the first blood samples, mean plasma levels of L-lactate, PAF, I-FABP, and median ET-1 levels were significantly higher in the ENEC group than CNEC and control groups ($p = 0.006$, 0.000, 0.000, 0.007, respectively) (Table 3). For the first blood samples, multivariate analysis was performed including the following parameters: prenatal Doppler disturbances, maternal hypertension, IUGR, prenatal betametasone, gestational age, birth weight, cord pH, and Apgar score. This analysis revealed that plasma L-lactate level was affected by cord pH and IFABP level was affected by prenatal Doppler disturbances.

In the second blood samples, plasma mean PAF and I-FABP levels were significantly higher in the CNEC group (Table 4). For the second blood samples, multivariate analysis was performed including the following parameters: RDS, PDA, IVH, pneumonia, BPD, C-reactive protein (CRP), and procalcitonin. We detected no effect of these parameters on plasma L-lactate, ET-1, and IFABP levels in the second blood samples. However, plasma PAF level was found to be affected by serum CRP level and PDA. In the second blood samples, plasma L-lactate, ET-1, PAF, and I-FABP levels were significantly higher both in stage I and II/III infants of ENEC and CNEC groups than the control group as expected ($p = 0.070$, 0.024, 0.000, 0.000, respectively). Although there was no statistical significance, L-lactate, ET-1, and IFABP levels were higher in infants with stage I NEC/NEC like disease than infants with stage II/III NEC (Table 5).

Discussion

For the first time in the literature, this study suggests that *prenatal* systemic/intestinal hypoxia-ischemia could be a major leading factor to intestinal cell injury and inflammation causing an early NEC or NEC-like disease especially in preterm infants with IUGR. The higher plasma levels of L-lactate, ET-1, PAF, and I-FABP in the first blood samples (which is almost reflecting cord blood levels) and higher plasma L-lactate in the second sample in the ENEC group have supported this pathophysiology.

In our study, the most important characteristics of ENEC group were the significantly higher incidences of AREDF, IUGR, and delayed first meconium passage when compared with CNEC group (79.2% vs 31.6%, 75.0% vs 36.8% and 54.2% vs 21.1% , $p = 0.002$, 0.012, 0.039, respectively). Previously, it was reported that the risk of NEC increases in infants with IUGR and AREDF with an OR of 2.13 (95% CI 1.49–3.03) [23]. In the IUGR fetus, hypoxemia produces circulatory redistribution toward the brain and away from the viscera (especially gastrointestinal system) and placenta. The prolonged redistribution may cause structural, neuromotor, secretory, and mucosal function alterations of the intestinal tissue so that postnatally it is more susceptible to dismotility, abnormal colonization, and bacterial invasion [24]. After delivery, oxygenation improves but circulatory redistribution persists [12].

Another remarkable point of the ENEC group was the significantly higher frequency of infants who have not received any enteral feeding before the onset of NEC/NEC like disease when compared with the CNEC group (25% vs 0%, $p = 0.027$). This was due to withholding of even MEN as these infants were seriously symptomatic from the first hours or days of life. Therefore, it seems that enteral nutrition is not a prerequisite for the development of ENEC, and on the contrary, enteral nutrition could be rather preventive [25].

Serum L-lactate is frequently used in adults with acute mesenteric ischemia where it is elevated significantly [26]. However, increased expression of ET-1 has been shown in the removed bowel segments with NEC [27]. In our study, in the first blood samples, mean L-lactate and ET-1 levels were significantly higher in the ENEC group than the CNEC and control groups. Our results suggest that infants in the ENEC group have already been born with ischemic intestinal injury which has started prenatally as a result of fetal hemodynamic disturbances. However, in the control and CNEC groups, mean serum L-lactate and ET-1 levels were similar both in the first and second blood samples. These results support the hypothesis that perinatal (or postnatal) hypoxia-ischemia is not a primary causative factor in the pathophysiology of CNEC.

The increase in plasma I-FABP and PAF levels in NEC have been well demonstrated [15, 28, 29]. In our study, in the first blood samples, mean PAF and I-FABP levels were significantly higher in the ENEC group than CNEC and control groups. It is clear that not only hypoxia-ischemia but also intestinal inflammation leading to intestinal injury have begun in utero in the ENEC group. In the second blood samples, significantly higher levels of I-FABP and PAF were noted in

Table 1 Prenatal, natal, and postnatal demographic and clinical characteristics of the control, ENEC, and CNEC groups

Demographic and clinical characteristics	Control[a] *n* = 43	ENEC[b] *n* = 24	CNEC[c] *n* = 19	p1	p2
Gender (M/F), *n* (%)	20/23 (46.5/53.5)	17/7 (70.8/29.2)	12/7 (63.2/36.8)	0.055[a-b] 0.227[a-c] 0.594[b-c]	0.129
Gestational age (wk)*	31.9 ± 2.1 (27.0–35.6)	30.9 ± 2.4 (27.4–34.7)	30.3 ± 2.6 (27.0–36.0)	0.089[a-b] 0.011[a-c] 0.391[b-c]	0.030
Birth weight (g)*	1605 ± 308 (980–2100)	1137 ± 424 (620–2150)	1292 ± 270 (870–1800)	0.000[a-b] 0.000[a-c] 0.172[b-c]	0.000
Chorioamnionitis, *n* (%)	5 (11.6)	2 (8.3)	4 (21.1)	1.000[a-b] 0.437[a-c] 0.232[b-c]	0.440
Maternal hypertensive disorders, *n* (%)	8 (18.6)	13 (54.2)	5 (26.3)	0.003[a-b] 0.513[a-c] 0.066[b-c]	0.009
Abnormal fetal umbilical artery Doppler flow velocimetry, *n* (%)	2 (4.7)	19 (79.2)	6 (31.6)	0.000[a-b] 0.008[a-c] 0.002[b-c]	0.000
Intrauterine growth restriction, *n* (%)	5 (11.6)	18 (75.0)	7 (36.8)	0.000[a-b] 0.034[a-c] 0.012[b-c]	0.000
Type of delivery (V/CS), *n* (%)	1/42 (2.3/97.7)	1/23 (4.2/95.8)	2/17 (10.5/89.5)	1.000[a-b] 0.220[a-c] 0.575[b-c]	0.333
Apgar score (5th minute)**	8.0 (6–10)	7.0 (2–10)	7.0 (5–9)	0.004[a-b] 0.047[a-c] 0.360[b-c]	0.013
First blood sample pH* (umbilical/peripheric venous blood samples in the first hour of life)	7.31 ± 0.0 (7.22–7.39)	7.26 ± 0.10 (7.02–7.37)	7.32 ± 0.11 (6.95–7.43)	0.005[a-b] 0.939[a-c] 0.125[b-c]	0.073
Resuscitation in the delivery room, *n* (%)	18 (41.9)	15 (62.5)	8 (42.1)	0.105[a-b] 0.986[a-c] 0.183[b-c]	0.231
Delayed (> 48 h) first meconium passage, *n* (%)	7 (16.3)	13 (54.2)	4 (21.1)	0.001[a-b] 0.172[a-c] 0.039[b-c]	0.005
Stage of NEC/NEC like disease, n (%) Stage I NEC/NEC like disease Stage II NEC Stage III NEC	- - -	12 10 2	7 11 1	0.568[b-c]	0.568
Respiratory distress syndrome, *n* (%)	19 (44.2)	11 (45.8)	7 (36.8)	0.897[a-b] 0.589[a-c] 0.553[b-c]	0.820
Patent ductus arteriosus, *n* (%)	14 (32.6)	10 (41.7)	11 (57.9)	0.456[a-b]	0.172

Table 1 (continued)

Demographic and clinical characteristics	Control[a] $n = 43$	ENEC[b] $n = 24$	CNEC[c] $n = 19$	p1	p2
Intraventricular hemorrhage, *n* (%)	2 (4.7)	2 (8.3)	2 (10.5)	0.061^{a-c} 0.290^{b-c} 0.614^{a-b} 0.580^{a-c} 1.000^{b-c}	0.635
Bronchopulmonary dysplasia, *n* (%)	4 (9.3)	3 (12.5)	5 (26.3)	0.695^{a-b} 0.117^{a-c} 0.432^{b-c}	0.214
Duration of mechanical ventilation (day)**	0 (0–38)	2.5 (0–24)	0.5 (0–21)	0.763^{a-b} 0.891^{a-c} 0.664^{b-c}	0.815
Duration of oxygen support (day) **	3 (0–63)	5.0 (0–52)	4 (0–49)	0.597^{a-b} 0.535^{a-c} 0.892^{b-c}	0.396
Duration of hospitalization (day)* *	12 (8–18)	23 (16.75–34)	28 (19–47)	0.005^{a-b} 0.000^{a-c} 0.974^{b-c}	0.000
Mortality, *n* (%)	2 (4.6)	3 (12.5)	4 (21.1)	0.341^{a-b} 0.066^{a-c} 0.680^{b-c}	0.132

*Mean ± SD (range) **Median (range), *NEC* necrotizing enterocolitis, *ENEC* early NEC/NEC like disease, *CNEC* classic NEC/NEC like disease, *V* vaginal, *CS* cesarean section

p1: ^{a-b}p value representing the comparison of the data of control and ENEC groups

^{a-c}p value representing the comparison of the data of control and CNEC groups

^{b-c}p value representing the comparison of the data of ENEC and CNEC groups

p2: *p* value representing the comparison of the data of all groups

Table 2 Nutritional characteristics of the control, ENEC, and CNEC groups

Nutritional characteristics	Control[a] $n = 43$	ENEC[b] $n = 24$	CNEC[c] $n = 19$	p1	p2
Absence of enteral feeding (including MEN) before the onset of NEC (n, %)	-	6 (25.0)	-	0.027[b-c]	0.027
Day of onset of the clinical picture of NEC**	-	3.0 (2–7)	12.0 (8–43)	0.000[b-c]	0.000
Duration of enteral feeding before NEC (day)**	7.0 (7–8)[†]	2.0 (0–6)	11.0 (4–40)	0.000[a-b] 0.187[a-c] 0.000[b-c]	0.000
Enteral feeding volume at the time of onset of NEC (ml/kg/day)*	94 ± 23 [†] (50-120)	26 ± 24 (0-103)	106 ± 40 (50-216)	0.000[a-b] 0.473[a-c] 0.000[b-c]	0.000
Duration of parenteral nutrition (day)*	7.9 ± 7.3 (0–28)	18.3 ± 12.5 (2–62)	22.2 ± 11.3 (8–48)	0.000[a-b] 0.000[a-c] 0.304[b-c]	0.000

NEC necrotizing enterocolitis, *ENEC* early NEC/NEC like disease, *CNEC* classic NEC/NEC like disease,

MEN minimal enteral nutrition

*Mean ± SD (range)

**Median (range)

[†] At the time of the second blood sample in the control group

p1: [a-b] p value representing the comparison of the data of control and ENEC groups

[a-c] p value representing the comparison of the data of control and CNEC groups

[b-c] p value representing the comparison of the data of ENEC and CNEC groups

p2: p value representing the comparison of the data of all groups

the CNEC group than the ENEC group. This finding suggests that the inflammatory process and the disease severity were more profound in the CNEC group.

The first limitation of our study was being a single center study and having a small sample size, while the second limitation was inclusion of infants with Bell's stage I NEC/NEC like

Table 3 First hour (first sample) plasma levels of the biomarkers in the control, ENEC, and CNEC groups*

Biomarkers	Control[a] $n = 43$	ENEC[b] $n = 24$	CNEC [c] $n = 19$	p1	p2
L-Lactate (mmol/L)***	3.46 ± 0.42 (2.62–4.31)	3.87 ± 0.38 (3.10–4.65)	3.13 ± 0.42 (2.28–3.99)	0.000[a-b] 0.846[a-c] 0.005[b-c]	0.006
Endothelin-1(fmol/ml)**	1.9 (0.9–4.5)	3.5 (0.8–8.1)	1.4 (0.6–5.6)	0.000[a-b] 0.192[a-c] 0.003[b-c]	0.007
PAF (ng/ml)*	6.9 ± 5.5 (0.8–25.2)	16.5 ± 10.4 (0.8–43.8)	10.6 ± 5.9 (0.8–29.6)	0.000[a-b] 0.023[a-c] 0.036[b-c]	0.000
I-FABP (pg/ml)***	112.26 ± 248.81 (− 384.81–609.33)	2066.39 ± 227.62 (1611.65–2521.13)	900.79 ± 251.21 (398.93–1402.65)	0.000[a-b] 0.004[a-c] 0.002[b-c]	0.000

NEC necrotizing enterocolitis, *ENEC* early NEC/NEC like disease, *CNEC* classic NEC/NEC like disease, *PAF* platelet-activating factor, *I-FABP* intestinal fatty acid binding protein

*Mean ± SD (range)

**Median (range)

***Estimated mean ± SE (range)

p1: [a-b] p value representing the comparison of the data of control and ENEC groups

[a-c] p value representing the comparison of the data of control and CNEC groups

[b-c] p value representing the comparison of the data of ENEC and CNEC groups

p2: p value representing the comparison of the data of all groups

Table 4 Plasma levels of the biomarkers during the symptomatic disease (second sample) in the control, ENEC, and CNEC groups

Biomarkers	Control [a] $n = 43$	ENEC [b] $n = 24$	CNEC [c] $n = 19$	p1	p2
L-Lactate (mmol/L)*	3.11 ± 2.06 (0.30–7.20)	6.85 ± 4.13 (2.40–19.10)	3.78 ± 2.10 (1.00–8.10)	0.000^{a-b} 0.324^{a-c} 0.010^{b-c}	0.002
Endothelin-1 (fmol/ml)**	1.1 (0.6–2.3)	0.9 (0.3–4.8)	0.8 (0.3–4.8)	0.730^{a-b} 0.836^{a-c} 0.251^{b-c}	0.063
PAF (ng/ml)***	7.86 ± 2.14 (3.56–12.17)	12.95 ± 2.59 (7.73–18.16)	20.67 ± 2.44 (15.76–25.57)	0.000^{a-b} 0.000^{a-c} 0.022^{b-c}	0.000
I-FABP (pg/ml)*	778 ± 321 (318–1546)	1395 ± 787 (241–3483)	2285 ± 1134 (474–3683)	0.000^{a-b} 0.000^{a-c} 0.004^{b-c}	0.000

NEC necrotizing enterocolitis, *ENEC* early NEC/NEC like disease, *CNEC* classic NEC/NEC like disease,
PAF platelet-activating factor, *I-FABP* intestinal fatty acid binding protein
*Mean ± SD (range)
**Median (range)
***Estimated mean ± SE (range)
p1: ^{a-b}p value representing the comparison of the data of control and ENEC groups
^{a-c}p value representing the comparison of the data of control and CNEC groups
^{b-c}p value representing the comparison of the data of ENEC and CNEC groups
p2: p value representing the comparison of the data of all groups

disease. Recently, there is a consensus on exclusion of Bell's stage 1 NEC and SIP among research networks [30, 31]. In our opinion, this approach inhibits the inclusion of the real patients who are at the initial "subclinical" phase of a developing disease and decreases patient number. Despite the risk of overlapping clinical pictures of feeding intolerance and stage I NEC, we have to evaluate all stages of NEC as naturally the severity of the disease increases stepwise from stage I to III.

Table 5 Comparison of the plasma levels of biomarkers during the symptomatic disease (second sample) in stage I, stage II-III disease, and the control group

Biomarkers	Control[a] $n = 43$	Stage I NEC/NEC like disease[b] $n = 19$	Stage II-III NEC[c] $n = 24$	p1	p2
L-Lactate (mmol/L)*	3.1 ± 2.1 (0.3–7.2)	6.4 ± 4.7 (2.1–19.1)	4.9 ± 2.6 (1.0–12.1)	0.005^{a-b} 0.331^{b-c} 0.143^{a-c}	0.007
Endothelin-1 (fmol/ml)**	1.4 ± 0.4 (0.6–2.3)	1.7 ± 1.5 (0.4–4.8)	1.1 ± 1.1 (0.3–4.8)	1.000^{a-b} 0.225^{b-c} 0.021^{a-c}	0.024
PAF (ng/ml)*	9.6 ± 3.2 (4.3–15.9)	17.8 ± 10.3 (0.8–44.0)	21.3 ± 7.1 (12.6–39.7)	0.000^{a-b} 0.201^{b-c} 0.000^{a-c}	0.000
I-FABP (pg/ml)*	778.4 ± 321.8 (318–1546)	1963.9 ± 1016.2 (241–3585)	1650.5 ± 1067.2 (474–3683)	0.000^{a-b} 0.384^{b-c} 0.000^{a-c}	0.000

NEC necrotizing enterocolitis, *ENEC* early NEC/NEC like disease, *CNEC* classic NEC/NEC like disease,
PAF platelet-activating factor, *I-FABP* intestinal fatty acid binding protein
*Mean ± SD (range)
**Median (range)
p1: ^{a-b}p value representing the comparison of the data of control and Stage I NEC/NEC like disease groups
^{a-c}p value representing the comparison of the data of control and Stage II-III NEC groups
^{b-c}p value representing the comparison of the data of stage I NEC/NEC Like disease and stage II-III NEC groups
p2: p value representing the comparison of the data of all groups

Further in our study, plasma levels of serological biomarkers in infants with stage I NEC/NEC like disease were as high as the levels in infants with stage II/III NEC and were significantly higher than the levels in infants of the control group (Table 5). Therefore, infants with stage I NEC/NEC like disease were in fact seriously affected by intestinal ischemia and inflammation. However, the clinical disease severity seems to be affected by other unknown or individual factors. Therefore, we think that selective approach considering only infants with stage II/III NEC in clinical studies should be re-evaluated.

Conclusion

Today "NEC" is a "roof term" which is believed to include more than one disease with different pathophysiological etiologies and clinical presentations. Therefore, there is an obvious need to redefine and re-classify neonatal gastrointestinal diseases presenting like NEC as Bell's criteria have been developed only for disease severity staging. In our study, prenatal intestinal/mesenteric hypoxia-ischemia seems to be the primary pathophysiological factor leading to ENEC/NEC like disease in the first week of life in preterm infants with AREDF and IUGR. ENEC seems to begin "in utero," and these infants are born with already injured and "programmed" gastrointestinal system and significant systemic inflammation. Therefore, we would like to propose a new terminology *Hypoxic-Ischemic Enterocolitis (HIEnt)* for preterm infants presenting with early NEC/NEC like disease and who have prenatal hemodynamic disturbances and IUGR. This new term has been firstly created and used by our colleague Dr. Ayse Korkmaz a few years ago. We believe that this new terminology would raise awareness on the pathophysiology of ENEC and contribute to providing individualized, preventive, and therapeutic strategies for preterm infants after multicenter prospective new studies.

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Author contributions Dr. Surmeli Onay organized the data collection, carried out the initial analyses, drafted the initial manuscript.

Dr. Korkmaz conceptualized and designed the study, proposed the new terminology, controlled the analyses, drafted the first and final manuscripted as submitted.

Drs Yigit and Yurdakok reviewed the manuscript and approved the final manuscript as submitted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The Institutional Ethics Committee approved the study (No: HEK 10/91).

Informed consent Informed consent forms were received from all parents before inclusion in the study.

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