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Clinical Characteristics of Necrotizing Enterocolitis Diagnosed by Independent Adjudication of Abdominal Radiographs, Laparotomy, or Autopsy in Preterm Infants in the "Connection Trial"

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Abstract

Objective Necrotizing enterocolitis (NEC) classically is diagnosed by radiographic demonstration of pneumatosis intestinalis/portal venous gas (PI/PVG). This study examines clinical characteristics of NEC confirmed by independent evaluation of abdominal radiographs, taken for clinical signs of NEC, or by pathologic findings at laparotomy or autopsy (confirmed NEC [cNEC]).

Study Design The investigated cohort included 1,382 extremely low birth weight (BW) infants (BW range: 500–1,000 g) with median 27 weeks (range: 23–32) gestational age (GA) at birth. They were randomized into the placebo-controlled "Connection Trial" of the new biological drug candidate IBP-9414 with cNEC as one primary endpoint.

Results Total 119 infants (8.6%) had cNEC diagnosed at median 14 days of age by confirming PI/PVG at X-ray adjudication ($n = 111$) and/or by surgery/autopsy ($n = 21$). Sixteen percent of cNEC cases died. Adverse events of NEC were reported in 8.5% of infants and 4.1% had NEC diagnosed by radiology and surgery/autopsy at the participating centers. Regression analyses showed that the risk of cNEC decreased by 11 to 30% for every 100-g increment in BW and single-week increment in GA and

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- ELBW infants
- abdominal radiography
- necrotizing enterocolitis
- clinical associations
- risk factors

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associated cNEC with odds ratios (ORs) > 2.0 for gastrointestinal (GI) perforation and obstruction, hypotension, hypokalemia, hypophosphatemia, and death. Comparing risks of cNEC in infants below and above 750-g BW showed higher ORs (2.7–4.3) for GI perforation, hypotension, hypokalemia, and renal complications in the smaller infants, whereas the bigger infants had higher ORs (1.9–3.2) for serious non-GI events, late-onset sepsis (LOS), and death. Predictors of cNEC (hazard ratio, HR > 1.5) included serious non-GI events (mainly infections), hyponatremia, and hyperglycemia, whereas the HR was 0.52 for intravenous antibiotics. After cNEC diagnosis, there were higher rates of GI perforation and obstruction, hypotension, hypokalemia, and LOS.

Conclusion Independent adjudication of abdominal radiographs increased radiological recognition of NEC and proved to be feasible in a multicenter study setting as well as able to diagnose clinically relevant NEC.

Key Points

- Independent adjudication of abdominal radiographs in ELBW infants increased NEC recognition.
- Risk of NEC decreased by 11 to 30% with every 100-g increment in BW and GA week.
- In infants with BW 750 to 1,000 g, the risk of death from NEC was almost twice that in infants with BW 500 to 749 g
- Infants with NEC received antibiotics during one-third and parenteral nutrition during half of the first 7 postnatal weeks.

Necrotizing enterocolitis (NEC) is a complex, multifactorial, and heterogeneous inflammatory disorder that occurs in approximately 5 to 10% of very low birth weight (VLBW) infants.[1–4] The global incidence seems to increase over time despite multiple improvements in neonatal intensive care, like standardized feeding protocols promoting use of mother's milk rather than infant formulas, antibiotic stewardship initiatives, and improved ventilation techniques.[5–7] NEC is associated with clinically significant morbidities and the mortality is approximately 20% with rates up to approximately 35% in the extremely low birth weight (ELBW) infants.[5,8–10] Despite the significant adverse events (AEs) of NEC, no treatment nor prophylaxis for NEC have been evaluated in health authority-agreed drug development programs. The disorder consequently lacks any formally approved, specific medical therapy.

The diagnosis of NEC in VLBW infants most commonly occurs within 6–30 days of age with a majority presenting before 34 weeks postmenstrual age (PMA).[11] In cases without a fulminant debut leading to early laparotomy or autopsy, NEC evaluation characteristically is initiated by the recognition of clinical signs of an intraabdominal complication, such as abdominal distension and discoloration, and radiological findings such as intestinal wall pneumatosis intestinalis (PI) and/or portal venous gas (PVG). Plain-film abdominal radiography is a classic modality for NEC recognition.[12–14] The image interpretation requires expertise and has been associated with both inter- and intraobserver variability.[15,16] In the design of a health authority-approved program on the efficacy, safety, and tolerability of IBP-9414 as a prophylactic therapy for NEC, encouragement was made to use independent adjudication of abdominal radiographs for the diagnosis of NEC. The aim of the current study is to display the clinical characteristics of NEC confirmed by such independent adjudication and/or by verification at laparotomy/autopsy.

Materials and Methods

The "Connection Trial"

The "Connection Trial" is a phase 3, placebo-controlled study on the safety and efficacy of IBP-9414 (ClinicalTrials.gov Id: NCT03978000), which is conducted in 10 countries with approval of Institutional Review Boards and Ethical Committees as appropriate for the 95 participating neonatal intensive care units (NICUs). Infants of 500- to 1,500-g birth weight (BW) are randomized 1:1 to a single daily enteral dose of IBP-9414 (*Limosilactobacillus reuteri*) or sterile water placebo within 48 hours of birth until a PMA of $34^{6/7}$ weeks. Follow-up is conducted at PMA 40 weeks ± 7 days. The primary endpoints of the "Connection Trial" are the incidence of NEC confirmed by independent adjudication of abdominal X-rays, laparotomy, or autopsy (confirmed NEC) and the time to reach a sustained feeding tolerance.[17] Recruitment ends and randomization codes will be broken at study exit of 2,158 VLBW infants.

The "Connection Trial" examines the new biological drug candidate IBP-9414 under the U.S. Investigative New Drug (IND) application and European Union (EU) Clinical Trial Exemptions (CTX). IBP-9414 is of pharmaceutical grade with quality standards equivalent to drug products. Manufacturing requires full compliance with pharmaceutical manufacturing from cell banking to final product, which includes continuous controls of raw materials, ingredients, and excipients, as well as verified absence of a range of potential contaminants, batch control, shelf-life determination, and validated dosing procedures. The design, conduct,

and results evaluation of the "Connection Trial" are compliant with Good Clinical Practice and International Council of Harmonization guidelines.[18] These circumstances contrast to probiotics that are classified as food additives and administered to preterm infants, despite the rising concern and warnings by the U.S. Food and Drug Administration and others on their lack of verified quality, efficacy, and safety characteristics.[19–23]

Analyzed Clinical Variables

The investigated clinical variables follow the study protocol stipulating a select daily collection to interfere as little as possible with the routines of the involved centers. Examples in this respect include daily registration of any feeds with human milk as well as parenteral nutrients, but not their amounts nor any specifics as to micronutrient additives. All the data relied on reports from the investigators managing the infants and were gathered with maintained blinding as to treatment group assignment of the infants. Reported study safety events comprised investigators' assessments, which included the classification of an AE as a serious adverse event (SAE) or not. The events analyzed herein were selected on the basis of reported frequency and previous event analyses of the infant material.[17,24,25] They were selected from nearly 500 reported event types and some were gathered into groups for ease of interpretation. Criteria for diagnosing safety events were not provided in the trial protocol. The diagnosis of confirmed NEC, however, relied on independent adjudication of abdominal radiographs taken for clinical signs of NEC (as defined by the investigator) and demonstrating PI/PVG and/or on the diagnosis of NEC at laparotomy or autopsy. The independent radiographic adjudication involved three pediatric radiologists. Whenever the first two of them agreed on the presence of PI/PVG, the diagnosis of confirmed NEC was registered. Should they disagree on the presence of radiological signs of NEC, the third adjudicator was utilized in almost half of the examinations to determine the presence or absence of confirmed NEC. The duration of the adjudication process prohibited consideration of the findings in the treatment of the infants, which was based on the interpretation of clinical signs and local diagnostics at each center.

Descriptive and Quantitative Statistics

Subject characteristics are described as median with interquartile range (IQR) and mean with standard deviation for numerical variables and as absolute and relative frequencies for categorical variables. Comparison between groups utilized *t*-test for continuous variables and Fisher's exact test for the categorical ones. Notable is that no AE or SAE reports of NEC were included into the statistical analyses of confirmed NEC. The association of the clinical variables with the risk of confirmed NEC was analyzed with logistic regression models. Variables that were statistically significant ($p < 0.05$) on univariable analysis were subsequently incorporated into multivariable analyses, where the optimal model was determined using the Akaike Information Criteria (AIC) with a stepwise approach.[26] AIC balances the trade-off between accuracy of the fit and the complexity of the model. It quantifies the quality of the model by considering the data fit, while penalizing models using a greater number of parameters. The output is presented as the odds ratio (OR) with 95% confidence intervals (CI), and nominal *p*-values. OR is the relative difference in the risk of developing confirmed NEC related to a one-unit change in the specific variable.

A time-dependent Cox regression model was used to explore further those events associated with confirmed NEC. Among infants with confirmed NEC, the events were updated from birth until NEC was confirmed, and among infants without NEC, the events were updated to study discontinuation or postnatal day 53, whichever came first. Each factor was computed to be zero for each day until the first day of occurrence, and one from the day thereafter. The univariable analyses were performed first followed by the multivariable stepwise model using AIC. Results are presented as hazard ratio (HR) with 95% CI and nominal *p*-values. HR is the relative difference in the risk of developing confirmed NEC related to a one-unit change in the specific variable. Adjustment for multiplicity was not performed due to the exploratory character of the analyses.

Results

Recruitment into the "Connection Trial" was initiated in infants with BW 750 to 1,000 g and expanded down to BW 500 g after an initial, independent safety evaluation. The cohort of the current study underwent a second, independent safety evaluation prior to extending the recruitment up to BW of 1,500 g. The investigated cohort consists of 1,382 infants (►**Table 1**) with median BW and gestational age (GA) at birth of 840 g and 27 weeks (evaluated as complete weeks). Altogether 361 infants (26%) had BW 500 to 749 g and 1,021 infants (74%) had BW 750 to 1,000 g. The frequency of the explored clinical variables is shown in ►**Table 2**. The infants were hospitalized for a median of 79 days, and the clinical variables were collected daily for a median of 53 days.

Confirmed NEC was recognized in 119 infants (8.6%) based on abdominal radiographs ($n = 111$ infants) and surgery/autopsy ($n = 21$ infants). A total of 118 infants (8.5%) were reported by site investigators with NEC as AE, and this included 52% of the infants with confirmed NEC. Fifty-six infants (4.1%) had NEC diagnosed by abdominal radiography and laparotomy/autopsy prior to adjudication. The median age at the diagnosis of confirmed NEC was 14 days (IQR, 6–25 days), and the cumulative incidence over postnatal age is shown in ►**Fig. 1**. The common abdominal signs leading to radiographic evaluation were abdominal distension and signs of gastric retention (in 92 and 54% of infants, respectively), whereas hypoxia, bradycardia, acidosis, hypotension, shock, and/or anuria occurred in 13% of infants. BW and GA at birth of the infants with confirmed NEC were significantly lower than in those without confirmed NEC (►**Table 2**). Other highly significant differences between these groups included the incidences of any SAE, gastrointestinal (GI) AEs including perforation and intestinal obstruction as well as hypotension, hyponatremia, hypokalemia,

Table 1 Baseline infant clinical characteristics for the study cohort

Characteristic	Number	Median (IQR)	Mean (SD)
BW, g all	1,382	840 (740–930)	821 (128)
500–749	361	650 (590–700)	643 (66)
750–1,000	1,021	883 (825–950)	884 (74)
GA at birth, wk, all	1,382	27 (25–28)	27 (2)
500–749 g	361	25 (24–26)	27 (2)
750–1,000 g	1,021	27 (26–29)	27 (2)
Gender, female, all	674	–	–
500–749 g	190	–	–
750–1,000 g	484	–	–
Male, all	708	–	–
510–749 g	171	–	–
750–1,000 g	537	–	–
Apgar 5-min score, points	1,382	7 (6–8)	7 (2)
Race, Caucasian	789	–	–
African American	392	–	–
Other/multiple/unknown	201	–	–
Study duration[a], d	1,382	53 (40–63)	50 (18)
In-hospital stay[b], d	1,382	79 (61–95)	87 (74)

Abbreviations: BW, birth weight; GA, gestational age at birth; IQR, interquartile range; PMA, postmenstrual age; SD, standard deviation.
[a]The period of daily recordings up to PMA $34^{6/7}$ weeks.
[b]Measured to follow up at PMA 40 weeks ± 7 days.

Table 2 Descriptive statistics with color-coded significance levels[a] for the infants with and without confirmed necrotizing enterocolitis

Variable	cNEC ($n = 119$)	No cNEC ($n = 1,263$)	Total ($n = 1,382$)
BW, g, median (IQR)	810 (680–900)	845 (740–935)	840 (740–930)
GA wk, median (IQR)	26 (25–28)	27 (25–28)	27 (25–28)
Study duration[b], d	55 (39–66)	53 (41–63)	53 (40–63)
Days in hospital[c], median (IQR)	83 (57–99)	79 (61–95)	79 (61–95)
Any SAE, *n* (%)	47 (40)	305 (24)	352 (26)
SAE GI, *n* (%)	9 (7.6)	58 (4.6)	67 (4.8)
AE abdominal sign e.g., distension, discoloration, vomiting, reflux, hematochezia, *n* (%)	62 (52)	366 (29)	428 (31)
AE GI, *n* (%)	76 (64)	552 (44)	628 (45)
AE GI perforation, *n* (%)	10 (8.4)	32 (2.5)	42 (3.0)
AE intestinal obstruction, *n* (%)	6 (5.0)	14 (1.1)	20 (1.4)
AE feeding intolerance, *n* (%)	29 (24)	197 (16)	226 (16)
AE cholestasis, *n* (%)	43 (36)	424 (34)	467 (34)
SAE non-GI, *n* (%)	45 (38)	281 (22)	326 (24)
SAE respiratory, *n* (%)	16 (13)	132 (10.5)	148 (10.7)
AE respiratory depression (e.g., apnea, respiratory failure, pneumothorax, pulmonary hemorrhage/ hypertension, BPD), *n* (%)	70 (59)	727 (58)	797 (58)
AE pneumonia including tracheitis, pneumonitis, bronchiolitis, *n* (%)	12 (10.1)	137 (10.8)	149 (10.8)
AE BPD including chronic respiratory insufficiency, *n* (%)	39 (33)	376 (30)	415 (30)

Table 2 (*Continued*)

Variable	cNEC ($n = 119$)	No cNEC ($n = 1,263$)	Total ($n = 1,382$)
SAE cardiac, *n* (%)	2 (1.7)	21 (1.7)	23 (1.7)
AE PDA, *n* (%)	42 (35)	489 (39)	531 (38)
AE bradycardia, *n* (%)	19 (16)	128 (10.1)	147 (10.6)
AE hypotension, *n* (%)	23 (19)	96 (7.6)	119 (8.6)
AE ROP, *n* (%)	40 (34)	333 (26)	373 (27)
AE intracranial bleeding, *n* (%)	31 (26)	279 (22)	310 (22)
AE hyponatremia, *n* (%)	53 (45)	376 (30)	429 (31)
AE hypokalemia, *n* (%)	26 (22)	104 (8.2)	130 (9.4)
AE hypophosphatemia, *n* (%)	11 (9.2)	49 (3.9)	60 (4.3)
AE hyperglycemia, *n* (%)	27 (23)	137 (10.8)	164 (12)
AE metabolic acidosis/alkalosis, *n* (%)	21 (18)	180 (14)	201 (14)
AE anemia, *n* (%)	75 (63)	748 (59)	823 (60)
AE renal, *n* (%)	25 (21)	125 (9.9)	150 (10.9)
AE LOS, *n* (%)	28 (24)	139 (11)	167 (12)
Antibiotics treatment days, median (IQR)	18 (8–34)	7 (1–19)	8 (2–20)
Human milk, % days of study duration, median (IQR)	81 (64–94)	97 (83–100)	97 (81–100)
Parenteral feeding, % days of study duration, median (IQR)	64 (39–97)	35 (17–69)	38 (19–72)
Weight change (g/kg body weight/week), median (IQR)	153 (117–182)	154 (124–184)	154 (124–184)
Death, *n* (%)	19 (16)	86 (6.8)	105 (7.6)

Abbreviations: AE, adverse event independent of seriousness; BPD, bronchopulmonary dysplasia; BW, birth weight; GA, gestational age at birth; GI, gastrointestinal; IQR, interquartile range; LOS, late-onset, culture-positive sepsis (>72 hours of age); *n*, number of infants; NEC, necrotizing enterocolitis; PDA, persistent ductus arteriosus; PMA, postmenstrual age; ROP, retinopathy of prematurity; SAE, serious adverse event defined as e.g., life-threatening, prolonging hospitalization.

[a]Color-coded *p*-values < 0.001 | $0.001 - < 0.01$ | $0.01 - < 0.025$ | $0.025 - < 0.05$ | $0.05 - < 0.10$ | ≥ 0.10.
[b]Measured during the period of daily recordings up to PMA $34^{6/7}$ weeks.
[c]Measured up to follow up at PMA 40 weeks ± 7 days.

hyperglycemia, and renal events. Altogether 24% of the infants with confirmed NEC had late-onset sepsis (culture-positive, LOS). They were treated with intravenous antibiotics for a median 33% of the study days and the highest use occurred early postnatally (►**Fig. 2**). Any human milk was given for a median 81% of the study days (►**Table 2**). Nineteen (16%) of the infants with confirmed NEC died, and NEC was a cause of death in each of them. Contributory causes of death included LOS (two infants), multiple organ failure (two infants), as well as intestinal perforation, pulmonary hemorrhage, and metabolic acidosis (one infant each).

Univariable association analysis showed that confirmed NEC decreased by 18 and 14% for each increment of 100 g in

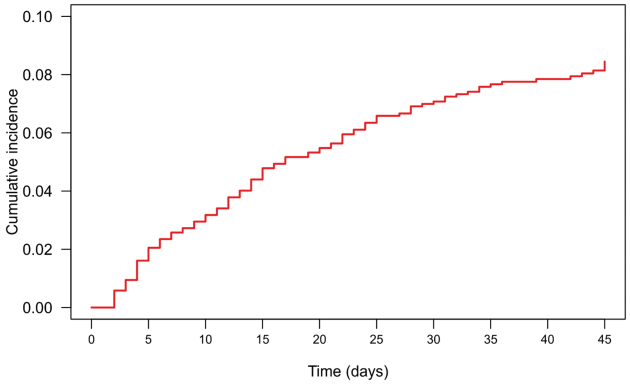

Fig. 1 Cumulative incidence of confirmed NEC over age at the day of diagnosis of confirmed NEC. NEC, necrotizing enterocolitis.

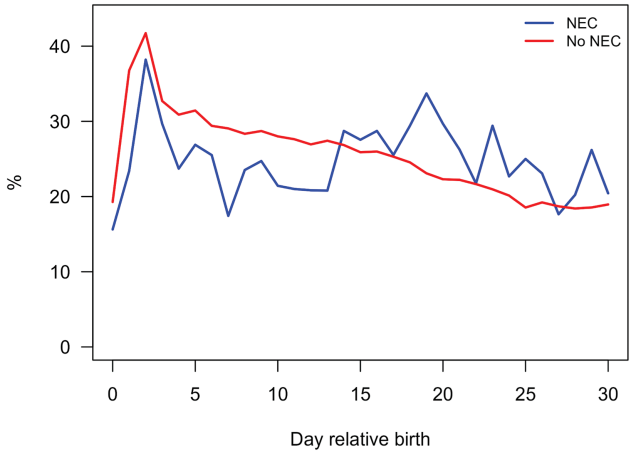

Fig. 2 Proportion per day of infants with and without confirmed NEC given any intravenous antibiotics over age in days. NEC, necrotizing enterocolitis.

BW and 1 week in GA at birth, respectively (►Table 3). Furthermore, confirmed NEC associated with OR of at least 2.5 for GI perforation, intestinal obstruction, hypotension, hypokalemia, hypophosphatemia, and death. The multivariable model showed the strongest association of confirmed NEC with abdominal signs, hypokalemia, hyponatremia, and duration of parenteral feeding.

When the associations of confirmed NEC were analyzed in infants with BW 500 to 749 g ($n = 39$ infants) and those with BW 750 to 1,000 g ($n = 80$ infants), similar reductions in the

Table 3 Univariable regression and multivariable regression[a] with a stepwise approach and color-coded significance levels[b] for the associations of clinical variables to confirmed necrotizing enterocolitis in the 119 infants

Variable	Univariable		Multivariable	
	OR	95% CI	OR	95% CI
BW, 100-g interval	0.82	0.71–0.94	–	–
GA, wk	0.86	(0.78–0.95	–	–
Days in hospital	1.00	0.99–1.00	–	–
Any SAE	2.05	1.39–3.03	–	–
SAE GI	1.70	0.82–3.52	–	–
AE abdominal sign	2.67	1.82–3.90	2.77	1.81–4.23
AE GI	2.28	1.54–3.36	–	–
AE GI perforation	3.53	1.69–7.37	–	–
AE intestinal obstruction	4.74	1.79–12.57	2.66	0.91–7.74
AE feeding intolerance	1.74	1.12–2.72	–	–
AE cholestasis	1.12	0.76–1.66	–	–
SAE non-GI	2.13	1.43–3.15	1.52	0.93–2.49
SAE respiratory	1.33	0.76–2.32	–	–
AE respiratory depression	1.05	0.72–1.54	–	–
AE pneumonia	0.92	0.49–1.72	–	–
AE BPD	1.15	0.77–1.72	–	–
AE PDA	0.86	0.58–1.28	–	–
AE bradycardia	1.68	1.00–2.84	–	–
AE hypotension	2.91	1.77–4.80	–	–
AE ROP	1.41	0.95–2.11	–	–
AE intracranial bleeding	1.24	0.81–1.91	–	–
AE hyponatremia	1.89	1.29–2.77	1.64	1.06–2.55
AE hypokalemia	3.12	1.93–5.03	1.98	1.15–3.42
AE hypophosphatemia	2.52	1.27–5.00	–	–
AE hyperglycemia	2.41	1.52–3.84	1.56	0.93–2.62
AE acidosis	1.29	0.78–2.12	–	–
AE anemia	1.17	0.80–1.73	–	–
AE renal	2.42	1.50–3.91	–	–
AE LOS	2.49	1.57–3.94	1.52	0.92–2.53
Antibiotics treatment days	1.01	1.00–1.01	–	–
Human milk, % days of study duration	0.99	0.99–1.00	–	–
Parenteral feeding, % days of study duration	1.02	1.01–1.02	1.02	1.01–1.02
Weight change (g/kg body weight/week)	1.00	1.00–1.00	–	–
Death	2.60	1.52–4.45	2.04	0.99–4.22

Abbreviations: AE, adverse event independent of seriousness; BPD, bronchopulmonary dysplasia; BW, birth weight; CI, confidence interval; GA, gestational age at birth; GI, gastrointestinal; LOS, late-onset, culture-positive sepsis (>72 hours of age); OR, odds ratio i.e., the risk of confirmed NEC related to a one-unit change in the specific variable; PDA, persistent ductus arteriosus; ROP, retinopathy of prematurity; SAE, serious adverse event defined as e.g., life-threatening, prolonging hospitalization.

[a]Displayed variables are those selected by the model.

[b]Color-coded *p*-values: < 0.001 | $0.001 - < 0.01$ | $0.01 - < 0.025$ | $0.025 - < 0.05$ | $0.05 - < 0.10$ | ≥ 0.10.

Table 4 Univariable regression[a] and color-coded significance levels[b] for the association of select clinical variables in the infants with confirmed necrotizing enterocolitis divided according to birthweight <750 g ($n = 39$ out of 361 infants) and ≥750 g ($n = 80$ out of 1,021 infants)

Variable	Infants < 750-g BW		Infants ≥ 750-g BW	
	OR	95% CI	OR	95% CI
BW, 100-g interval	0.70	0.42–1.14	0.72	0.53–0.99
GA, wk	0.82	0.65–1.02	0.89	0.78–1.02
Any SAE	1.19	0.60–2.34	2.57	1.59–4.14
AE abdominal sign	2.15	1.09–4.25	3.02	1.90–4.80
AE GI	2.00	1.01–3.96	2.42	1.50–3.89
AE GI perforation	4.32	1.54–12.13	2.55	0.85–7.70
AE intestinal obstruction	4.30	0.76–24.27	4.90	1.50–15.99
AW feeding intolerance	0.76	0.26–2.25	2.30	1.39–3.86
SAE non-GI	1.32	0.67–2.61	2.58	1.59–4.19
AE bradycardia	1.91	0.74–4.95	1.63	0.87–3.06
AE hypotension	3.61	1.79–7.30	1.95	0.85–4.49
AE ROP	1.72	0.87–3.37	1.23	0.74–2.03
AE hyponatremia	2.39	1.22–4.69	1.63	1.02–2.61
AE hypokalemia	4.12	1.86–9.16	2.63	1.43–4.83
AE hypophosphatemia	2.61	0.98–6.92	2.17	0.82–5.79
AE hyperglycemia	2.06	1.00–4.22	2.49	1.33–4.64
AE renal	2.85	1.37–5.94	1.98	1.03–3.82
AE LOS	1.95	0.92–4.15	2.72	1.52–4.85
Antibiotics treatment days	1.01	1.00–1.02	1.01	1.00–1.01
Human milk, % days of study duration	0.99	0.98–1.00	0.99	0.99–1.00
Parenteral feeding, % days of study duration	1.01	1.00–1.02	1.02	1.01–1.03
Death	1.80	0.80–4.04	3.22	1.54–6.71

Abbreviations: AE, adverse event independent of seriousness; BW, birth weight; CI, confidence interval; GA, gestational age at birth; GI, gastrointestinal; LOS, late-onset, culture-positive sepsis (>72 hours of age); OR, odds ratio i.e., the risk of confirmed necrotizing enterocolitis related to a one-unit change in the specific variable; ROP, retinopathy of prematurity; SAE, serious adverse event defined as e.g., life-threatening, prolonging hospitalization.

[a]Displayed variables are restricted to those showing a statistically significant association (defined as $p < 0.05$) with confirmed necrotizing enterocolitis in the 119 infants (cf. ►Table 3).

[b]Color-coded *p*-values: <0.001 | 0.001–<0.01 | 0.01–<0.025 | 0.025–<0.05 | 0.05–<0.10 | ≥0.10.

risk of confirmed NEC (28–30%) was found for each 100-g increment in BW and 1 week in GA at birth (11–30%, ►Table 4). Furthermore, the associations of confirmed NEC with e.g., any SAE, non-GI SAE, LOS, and death were stronger in the higher BW group, whereas the associations of confirmed NEC with particularly GI perforation, hypotension, hypokalemia, and renal complications were stronger in the lower BW group. Some of these differences were also found in the multivariable analyses (►Table 5).

Comparison of events occurring after the day of diagnosing confirmed NEC (median observation, 31 days) with any time during the study duration of the infants without confirmed NEC (median observation, 53 days) is shown in ►Table 6. Significant overrepresentation after confirmed NEC was recorded for any SAE, GI events including perforation and obstruction, LOS, hypotension, and hypokalemia. In contrast, events like respiratory depression, cholestasis, persistent ductus arteriosus, intracranial bleeding, and anemia occurred less frequently after confirmed NEC. The temporal relationship to the day of diagnosing confirmed NEC is shown in ►Fig. 3 for GI perforation (A), non-GI SAEs (B), LOS (C), hypokalemia (D), hyponatremia (E), hyperglycemia (F), cholestasis (G), and intravenous antibiotics use (H).

Univariable regression analyses of events that occurred prior to the day of diagnosing confirmed NEC (median observation, 14 days) and those that occurred at any time in the infants without confirmed NEC (median observation, 53 days) showed significantly increased risk (HR, 1.68–1.93) for non-GI SAEs (consisting of 55% infections including LOS, 33% respiratory depression including apnea and respiratory distress), hyponatremia, and hyperglycemia (all $p \leq 0.013$–0.01). Conversely, HR 0.54 and 0.56 were found for cholestasis ($p < 0.02$) and for any use of intravenous antibiotics ($p = 0.003$). These HR and significance levels persisted upon multivariable analysis with a stepwise approach. The antibiotic treatments prior to confirmed NEC (►Fig. 2) were

Table 5 Multivariable regression with a stepwise procedure and color-coded significance levels[a] for the association of clinical variables with confirmed necrotizing enterocolitis in the infants divided according to birthweight <750 g and ≥750 g

Variable	OR[b]	95% CI
Birth weight <750 g ($n = 39$ infants)		
AE abdominal sign	2.05	0.97–4.34
AE hypotension	2.73	1.25–5.95
AE hypokalemia	3.37	1.40–8.12
Antibiotics treatment days	1.01	1.00–1.01
Parenteral feeding, % days of study duration	1.01	1.00–1.02
Birth weight ≥750 g $n = 80$ infants		
AE abdominal sign	3.19	1.92–5.30
SAE non-GI	2.32	1.36–3.95
AE hyponatremia	1.73	1.01–2.95
AE hyperglycemia	1.75	0.86–3.55
AE LOS	1.81	0.96–3.42
Parenteral feeding, % days of study duration	1.02	1.01–1.03

Abbreviations: AE, adverse event independent of seriousness; CI, confidence interval; GI, gastrointestinal; LOS, late-onset, culture-positive sepsis >72 hours of age); SAE, serious adverse event defined as e.g., life-threatening, prolonging hospitalization.

[a]Color-coded *p*-values < 0.001 | 0.001– < 0.01 | 0.01– < 0.025 | 0.025– < 0.05 | 0.05– < 0.10 | ≥0.10.

[b]OR (odds ratio) is the risk of confirmed necrotizing enterocolitis related to a 1-unit change in the specific variable.

Table 6 Clinical variables occurring after the day of diagnosing confirmed necrotizing enterocolitis and at any time in the treatment of infants without confirmed necrotizing enterocolitis

	After cNEC $n = 119$	No NEC $n = 1263$
Any SAE, *n* (%)	39 (33)	305 (24)
SAE GI, *n* (%)	8 (6.7)	58 (4.6)
AE abdominal sign, *n* (%)	50 (42)	366 (29)
AE GI, *n* (%)	66 (56)	552 (44)
AE GI perforation, *n* (%)	8 (6.7)	32 (2.5)
AE intestinal obstruction, *n* (%)	5 (4.2)	14 (1.1)
AE cholestasis, *n* (%)	25 (2)	424 (34)
SAE non-GI, *n* (%)	38 (32)	281 (22)
SAE respiratory, *n* (%)	12 (10.1)	132 (10.5)
AE respiratory depression, *n* (%)	49 (41)	727 (58)
AE pneumonia, *n* (%)	8 (6.7)	137 (10.8)
AE BPD, *n* (%)	30 (25)	376 (30)
AE LOS, *n* (%)	23 (19)	139 (11)
AE PDA, *n* (%)	19 (16)	489 (39)
AE bradycardia, (%)	10 (8.4)	128 (10.1)
AE hypotension, *n* (%)	17 (14)	96 (7.6)
AE ROP	37 (31)	333 (26)
AE intracranial bleeding, *n* (%)	10 (8.4)	279 (22)
AE hyponatremia, *n* (%)	32 (27)	376 (30)
AE acidosis, *n* (%)	17 (14)	180 (14)
AE hyperglycemia, *n* (%)	12 (10.1)	137 (10.8)
AE hypokalemia, *n* (%)	23 (19)	104 (8.2)
AE hypophosphatemia,(%)	7 (5.9)	49 (3.9)
AE anemia, *n* (%)	35 (29)	748 (59)
AE renal, *n* (%)	18 (15)	125 (9.9)

Table 6 (*Continued*)

Abbreviations: AE, adverse event independent of seriousness; BPD, bronchopulmonary dysplasia; GI, gastrointestinal; LOS, late-onset, culture-positive sepsis (>72 hours of age); *n*, number of infants; %, proportion of infants; PDA, persistent ductus arteriosus; ROP, retinopathy of prematurity; SAE, serious adverse event defined as e.g., life-threatening, prolonging hospitalization.

Note: Significance levels are color-coded. Color-coded *p*-values.

< 0.001 | 0.001– < 0.01 | 0.01– < 0.025 | 0.025– < 0.05 | 0.05– < 0.10 | ≥0.10.

given to 73 infants (63%) during median 4 days (range: 1–27 days). These treatments consisted of 105 independent courses, defined as nonoverlapping drug type or treatment date, and included ampicillin (78% of infants), gentamicin (62%), vancomycin (32%), cefepime (16%), and others (48%) alone or in various combinations. Sixty-one of these courses were given during the first 3 days after birth and the recorded indication was "empirical" or prophylactic in 44 of them.

Discussion

The "Connection Trial" is a pivotal study under U.S. IND and EU CTX on the safety and efficacy of a new biological drug

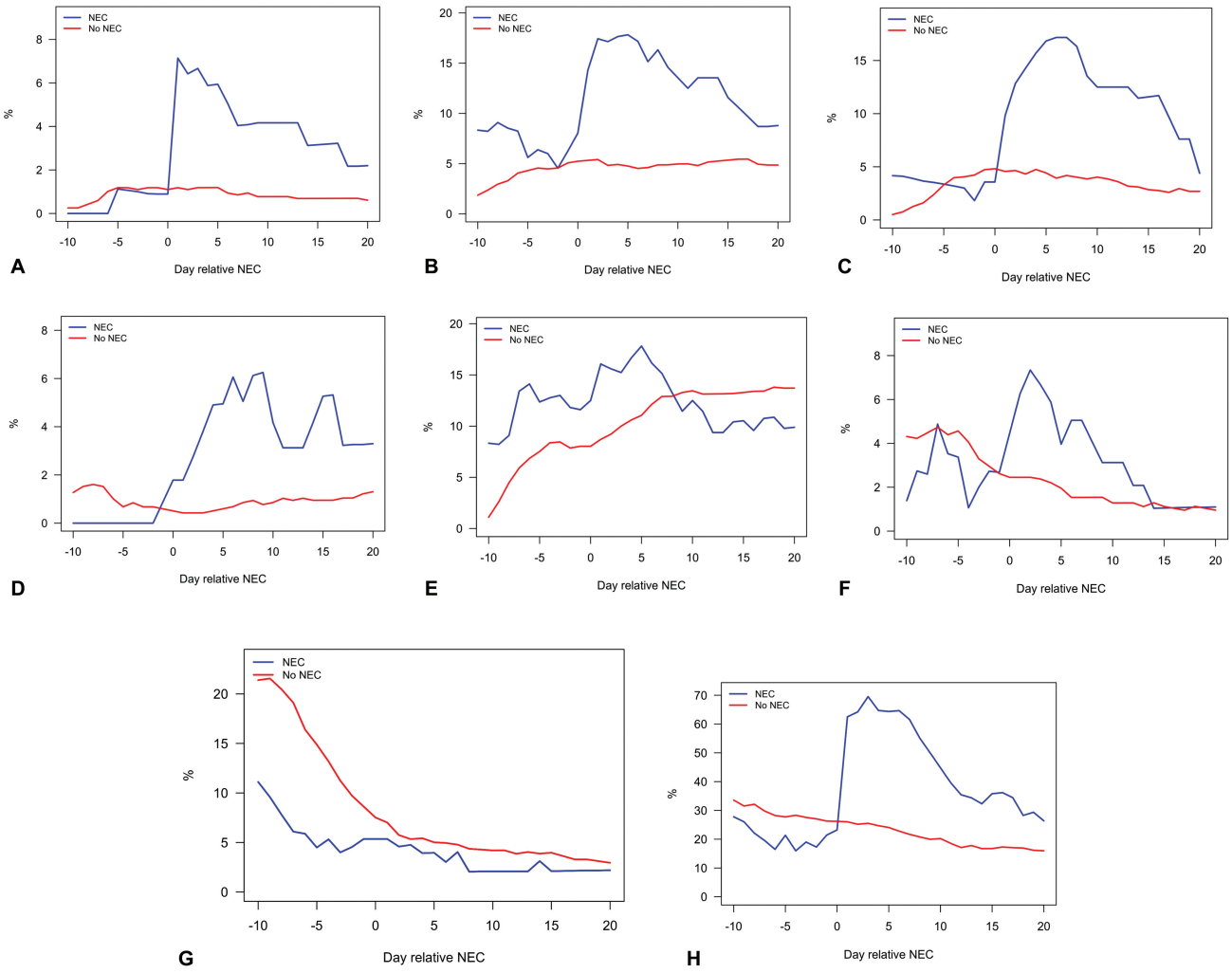

Fig. 3 (**A**–**H**). Proportional incidence over time in days for some clinical variables associating with confirmed NEC. The events are analyzed day-by-day based on the start and stop dates in each infant. Time zero is set at the day of diagnosing confirmed NEC (median age, 14 days), and arbitrarily at age 14 days in those without confirmed NEC. (**A**) GI perforation; (**B**), non-GI SAEs; (**C**), LOS (culture positive); (**D**) hypokalemia; (**E**) hyponatremia; (**F**) hyperglycemia; (**G**) cholestasis; (**H**) intravenous antibiotic use. Note different ordinate scales. NEC, necrotizing enterocolitis.

candidate in the high-risk population of preterm infants. In the design of the trial, it was critical to use a standardized methodology across the entire study population for the recognition of the primary efficacy endpoint of confirmed NEC. We standardized the diagnostic algorithm for confirmed NEC based on a synthesis of central interpretation of plain abdominal radiographs, surgical reports, and pathology findings. Although ultrasonography is used to diagnose NEC with increasing frequency,[27–29] abdominal radiography is universally available in NICUs worldwide and well suited to independent, external adjudication.[12,13] Interaction with the relevant health authorities on the study design resulted in the selective use of this imaging modality for the radiological diagnosis of confirmed NEC. Due to significant interobserver variability,[15,16] all the abdominal X-rays obtained for clinical signs of NEC, as defined by the responsible investigator, were reviewed for the presence of PI/PVG by independent pediatric radiology experts. The three utilized experts were not involved in the care of the infants and were blinded to their clinical course as well as to the reading outcome of each other. Based on agreements and disagreements between the first two adjudicators, the third adjudicator was utilized in almost half of examinations that provided confirmation of NEC. The diagnostic process led to the identification of confirmed NEC in 8.6% of the infants, which was similar to the rate of NEC from investigators' AE reports, but more than twice the rate of NEC diagnosed by abdominal X-ray, laparotomy/autopsy at the treating centers. This suggests that an NEC diagnosis relying on plain abdominal radiography may be underdiagnosed in contemporary ELBW infants and that the attempts of refining its criteria of recognition are important.[1,30–32]

Multiple risk factors for and complications of NEC have been described.[10,33–38] When the associations with confirmed NEC were explored with regression models, confirmed NEC showed a stronger association with BW than previously reported with approximately 20 to 30% reduced incidence for every 100-g increment and approximately 10 to

20% for every GA week at birth.[35] Overall, confirmed NEC associated with up to 4.7 times increased risks, e.g., GI perforation, intestinal obstruction, hypotension, hypokalemia, hyponatremia, and LOS. Furthermore, confirmed NEC associated with a 2% increase in the daily use of intravenous antibiotics, whereas a corresponding reduction was found for any additional 1-day feeding with human milk. The risk of death was more than doubled in the infants with confirmed NEC, which is lower than in previous analyses of ELBW infants with NEC.[5,8,9,33] This difference may relate to the study exclusion criteria of infants "in extremis," and those with, e.g., early-onset sepsis, recognized malformations and/or born to mothers with chorioamnionitis. It also is notable that *L. reuteri* has been claimed to reduce the incidence of NEC and the mortality of VLBW infants.[39]

Dichotomization of the analyzed cohort by BW 750 g followed the staged recruitment of infants into the study. The differences between these groups included median BWs of 630 and 870 g, and median GAs at birth of 24 and 27 weeks, respectively. As expected,[5,33,35,40,41] the smaller infants had a generally higher absolute risk of complications with at least twice the rate of GI SAEs, including perforation, as well as for pneumonia, hypotension, metabolic acidosis, hypophosphatemia, and renal complications (not shown). They also had a higher overall mortality (15.2%) compared with those in the heavier BW group (4.9%). However, the subgrouping aimed at exploring characteristics of confirmed NEC rather than those of the BW subgroups per se. Confirmed NEC was found in 10.8 and 7.8% of the infants in the lower and higher BW groups, respectively, and the diagnosis was recognized at a median 14 days of age in both of them. Noting the discrepant statistical power between the subgroups, the associations of confirmed NEC were regarded as similar for BW and GA at birth as well for GE AEs, intestinal obstruction, hyperglycemia, antibiotic treatment days, and the duration of parenteral nutrition. However, confirmed NEC in the smaller infants had weaker associations with non-GI SAEs, LOS, and stronger associations with GI perforation, hypotension, hypokalemia, hyponatremia, and renal AEs. In addition, the risk of dying in the infants with confirmed NEC was 1.8 times higher in the infants born at 750 to 1,000 g (OR, 3.2) compared with the lower BW group (OR, 1.8). These quantifications have, to our knowledge, not been displayed previously.

The examined clinical variables associated with confirmed NEC may be risk factors for confirmed NEC or consequences of the disorder. Several complications were overrepresented after the day of diagnosing confirmed NEC, including GI complications as perforation and obstruction, LOS, hypotension, and hypokalemia. This overrepresentation and the time for recognizing some of them support a strong coupling to the pathophysiology of confirmed NEC. Subsequent analyses involved linear and logistic regressions for clinical variables predicting confirmed NEC. Such variables with HR $>$ 1.6 included non-GI SAEs, hyponatremia, and hyperglycemia. Almost halved risk of confirmed NEC involved any use of intravenous antibiotics. The infants with confirmed NEC received antibiotics during one-third of the study days with the expected peak at the time of diagnosis of confirmed NEC. However, the infants without confirmed NEC had higher exposures to such antibiotics during the first 2 weeks of the study. This time corresponded to the median postnatal age of the diagnosis of confirmed NEC, which may explain the recorded reduction in the risk of developing confirmed NEC. These observations should be regarded as to reflect current NICU practices as recent consensus advocates strong restriction with respect to the indication and duration of early antibiotic use.[7,42,43]

The present analysis of treatment blind data from the "Connection Trial" was conducted to explore the characteristics of confirmed NEC diagnosed with a standardized algorithm involving central interpretation of abdominal radiographs, surgery, and pathology. The observational findings should not be regarded as evidence for causal relationships to confirmed NEC. The findings nevertheless underline the clinical relevance of confirmed NEC and support feasibility of using independent adjudication of abdominal radiographs for the recognition of NEC across multiple countries and NICUs. These outcomes provide a suggestion for future endeavors in the critical area of identifying prophylactics as well as specific treatments for NEC.

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Conflict of Interest

J.N. is the global coordinating investigator of the Connection Trial sponsored by Infant Bacterial Therapeutics. R.S., M.D., J.F.-T., M.H., and J.A.Z. are principal investigators of the Connection Trial sponsored by Infant Bacterial Therapeutics. A.K., J.R., and S.S. are employees of the sponsor Infant Bacterial Therapeutics. M.T. is a consultant statistician employed by the sponsor Infant Bacterial Therapeutics.

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