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A role for neonatal bacteremia in deaths due to intestinal perforation: spontaneous intestinal perforation compared with perforated necrotizing enterocolitis

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Abstract

Objective: To examine the relationship between intestinal perforations (caused by either spontaneous perforation (SIP) or necrotizing enterocolitis (NEC)) and the outcome “death due to intestinal perforation”.

Methods: Multivariable logistic regression analyses were used to compare infants <28 weeks’ gestation with SIP (n=32) and perforated-NEC (n=45) for the outcome perforation-related death.

Results: In univariate analyses the incidence of death due to perforation was higher among infants with perforated-NEC (36%) than infants with SIP (13%). However, infants with perforated-NEC were more likely to be older than 10 days and have bacteremia/fungemia with non-coagulase negative staphylococci (non-CONS) organisms than infants with SIP. After adjusting for confounding the only variable that was significantly associated with mortality due to perforation was the presence of non-CONS bacteremia/fungemia at the onset of perforation.

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Conclusions: The apparent association between death and perforated-NEC could be explained by the higher incidence of non-CONS bacteremia/fungemia among infants with perforated-NEC.

Introduction:

Acute neonatal intestinal perforations, due to necrotizing enterocolitis (NEC) or spontaneous intestinal perforation (SIP), occur most commonly in preterm infants born before 28 weeks of gestation[1, 2] and are a significant cause of neonatal morbidity and mortality. Although both involve rupture of the intestinal wall, SIP and perforated-NEC appear to be distinct and separate pathologic entities. Preterm perforated-NEC (which usually occurs within 24 hours of NEC presentation)[3] is due to an immature immune, circulatory, and inflammatory response to altered bacterial colonization that leads to ischemic and coagulative necrosis[4, 5]. Preterm perforated-NEC usually occurs between 2–8 weeks after birth (commonly between 30–32 weeks postmenstrual age) and is associated with pneumatosis intestinalis, portal venous gas, thrombocytopenia, systemic illness and focal or widespread intestinal necrosis. SIP, on the other hand, usually presents as an isolated perforation (without evidence of surrounding ischemia, pneumatosis, inflammation or necrosis) within 10 days of birth and appears to be due to aberrant intestinal motility and focal thinning of the muscularis layer of the intestinal wall[6, 7, 8]. Alterations in signaling pathways regulated by nitric oxide, insulin-like growth factor, and epidermal growth factor may contribute to the development of SIP[7, 9]. In contrast with infants with NEC, infants with SIP frequently appear relatively stable, without signs of severe systemic illness immediately prior to the perforation. Infants with SIP are also significantly less likely to die as a result of the perforation event than infants with perforated-NEC[10, 11, 12]. The reason for the difference in mortality is currently unknown.

One feature of NEC, that often occurs at its onset, is bacteremia[1, 2]. Several studies have found an association between the incidence of death in cases of NEC and the presence of bacteremia with non-coagulase negative staphylococci (non-CONS) and Gram-negative organisms[1, 13, 14, 15, 16, 17]. Rather than causing or preceding the onset of NEC, bacteremia appears to be due to the intestine's resident flora opportunistically invading the infant's bloodstream after the loss of mucosal integrity[16, 18]. The stages through which the newborn's resident flora initially becomes established may play a role in this process. Gram-positive cocci, like coagulase negative staphylococci (CONS), are usually the first organisms to colonize the infant's intestine. These are followed by Gram-negative organisms (within the Gammaproteobacteria class)[19, 20, 21, 22, 23, 24, 25]. Although factors like gestational age, Caesarean birth, initiation of enteral feeding, and antibiotics may alter the rate of colonization, they do not appear to alter the tightly regulated sequence of bacterial class progression[19]. At this time, little information exists about the incidence of bacteremia, and especially Gram negative and non-CONS bacteremia, in infants with SIP. Since SIP usually presents within the first 10 days, we hypothesized that SIP might have a decreased incidence of Gram negative and non-CONS bacteremia compared with NEC, and that the decreased incidence of Gram negative and non-CONS bacteremia might contribute to the lower rate of death among infants with SIP.

Methods:

Patient Population:

We performed a retrospective review of infants born before 28 weeks of gestation to determine if infants with SIP have a lower incidence of Gram negative and non-CONS bacteremia at the onset of perforation than those with perforated-NEC, and whether the difference in bacteremia could account for the difference in mortality between infants with SIP and those with perforated-NEC. The study was approved by the Institutional Review Board of the University of California San Francisco. Infants were included in the study population if they delivered before 28 weeks' gestational age and were admitted to the intensive care nursery within 24 hours of birth between January 1995 and December 2018. Infants with known genetic syndromes or major congenital anomalies were excluded from the study, as were infants with intestinal atresia, volvulus, gastroschisis, meconium plug or traumatic perforations.

Detailed descriptions of our consensus driven approaches to respiratory and hemodynamic support, patent ductus arteriosus (PDA) management, and enteral feeding have been previously published[26, 27, 28]. All infants received ampicillin and gentamicin for at least 48 hours after delivery (until cultures were negative). There were no changes to our unit protocol for the volume advance of enteral feeding during the study period. Mother's breast milk was used whenever available. Prior to 2012, premature formula was used in the absence of mother's breast milk; after 2012, donor breast milk was used. Probiotics were not used during the study period. Intestinal perforations were treated with a combination of ampicillin, gentamicin and metronidazole (with fluconazole prophylaxis). The duration of therapy was 7 days which could be extended to 14 days for prolonged clinical illness.

A single neonatologist (RIC) prospectively recorded all of the demographic and outcome measures during the infants' hospitalizations and reviewed all of the abdominal radiographs with the radiologists.

Definitions of SIP and perforated-NEC:

A perforated bowel was diagnosed by either the presence of a pneumoperitoneum on abdominal X-ray (82%) or by the surgeon at the time of laparotomy (18%).

Tissue histology is the gold standard for differentiating SIP from perforated-NEC. However, if tissue is not available, as is often the case when a peritoneal drain is placed instead of performing an open laparotomy, it may be difficult to differentiate SIP from perforated-NEC since their clinical features often overlap[29]. In the absence of a universally accepted clinical definition of NEC and SIP, the International Neonatal Consortium NEC Workgroup recently recommended that research studies use case-based definitions of NEC and SIP that are comprised of the individual components of the definition[30]. Therefore, we defined SIP and perforated-NEC based on the presence or absence of 5 predetermined clinical, radiographic and pathologic criteria (see Table 1): a) duration of clinical deterioration (presence of new onset metabolic acidosis, hypotension requiring dopamine, hyperglycemia, thrombocytopenia, and/or leukopenia) prior to the perforation; b) duration of abnormal abdominal radiographs (fixed isolated or stacked dilated intestinal loops, edematous bowel

wall, or gasless abdomen) prior to the perforation; c) pneumatosis intestinalis and or portal venous gas; d) presence of a small perforation in the intestinal wall, without signs of inflammation, ischemia, or septic necrosis on surgical pathology or observed at the time of laparotomy; and, e) presence of single or multiple perforations with necrosis, inflammation and ischemia on surgical pathology or observed at the time of laparotomy (Table 1).

In 39 of the 77 infants with intestinal perforation an open laparotomy was not performed, and tissue was not available for histologic analysis - either because a peritoneal drain was placed instead of an open laparotomy or the infant deteriorated so rapidly that surgery was contraindicated (Table 1). In these cases, the diagnosis of perforated-NEC or SIP was based on criteria a), b), and c) above. Table 1 shows eight possible combinations of the five criteria and the diagnoses that were assigned to each of the possible combinations. Seven infants (cluster groups 3 and 4, see Table 1) had criteria that could be consistent with either SIP or perforated-NEC. Therefore, we created three different sets of diagnoses of SIP and perforated-NEC for our analyses (a Primary Diagnosis of SIP and perforated-NEC, and two Alternate Diagnoses): the Primary Diagnoses of SIP and perforated-NEC considered the seven infants with conflicting criteria as having perforated-NEC; Alternate Diagnosis #1 considered the seven infants as having SIP; and, Alternate Diagnosis #2 did not include the seven infants in either group (Table 1)

Statistical analysis:

Our primary outcome was death due to intestinal perforation. Perforation-related deaths were defined as those that occurred when an infant died as a direct consequence of the acute perforation (e.g., hypotension, sepsis, bacteremia) within two weeks of onset or of complications of short-gut syndrome. Perforation-related deaths were differentiated from deaths due to respiratory distress, pulmonary hemorrhage, late-onset sepsis or bacteremia (unrelated to the perforation), progressive respiratory insufficiency, chronic lung disease, or intracranial hemorrhage or pathology.

Chi-Square or Fisher’s exact tests were used for comparing categorical variables. Continuous variables were compared with Student’s t-tests when normally distributed, or Mann-Whitney tests if not. Our primary goal was to determine if infants with SIP had both a lower incidence of non-CONS bacteremia or fungemia and a lower incidence of death compared with infants with perforated-NEC. Since the observational period of our study spanned an interval of 24 years, we included demographic variables that examined the effects of birth epoch (when infants were admitted to the nursery) as well as variables that examined the effects of formula or donor breast milk supplementation in our comparisons.

We created multivariable models designed to examine the effects of our primary variable (type of perforation: SIP versus perforated-NEC) on neonatal outcomes. In order to determine if our primary variable was independently related to the model’s outcome, the multivariable models included our primary variable plus any demographic variables that differed significantly between the dichotomous outcome choices.

Results:

887 infants were eligible for our study: 32 had SIP (by the Primary diagnosis), 45 had perforated-NEC (by the Primary diagnosis) (43 also had NEC without a perforation). Infants with SIP and perforated-NEC had similar demographic characteristics except for three neonatal variables: age when the perforation occurred, enteral feeding prior to the perforation, and bacteremia/fungemia within 72 hours of onset of the perforation (Table 2).

Our primary goal was to determine if infants with SIP had a decreased incidence of Gram negative and non-CONS bacteremia/fungemia compared with infants with NEC, and if the decreased incidence of Gram negative and non-CONS bacteremia/fungemia might contribute to SIP's lower rate of death. Despite the fact that infants with SIP and perforated-NEC both had a tear in the intestinal wall allowing intestinal contents access to the peritoneum, infants with perforated-NEC had a significantly higher incidence of non-CONS bacteremia and fungemia at the onset of the perforation than infants with SIP (OR (95% CI)=18.8 (2.3–151), $p<0.01$) (Tables 2 and 3). However, infants with perforated-NEC were also more likely to be older than 10 days when the perforation appeared (OR (95% CI)=51 (6.4–409), $p<0.001$)(Table 2, Figure 1), and also more likely to have started enteral feeding prior to the perforation (OR (95% CI)=3.6 (1.4–9.5), $p<0.01$) (Table 2).

Although the incidence of bacteremia/fungemia was strongly associated with perforated-NEC in our univariate model (Table 2), this relationship was no longer significant after adjusting for the possible confounders (age of onset of the perforation episode and enteral feeding prior to perforation) (OR (95% CI) (for infants with perforated-NEC) = 5.4 (0.5–55.0), p=NS). After adjusting for possible confounding, the only variable that was independently related to the presence of non-CONS bacteremia/fungemia was the variable "age of onset of the perforation episode" (OR (95% CI) (for perforations that occurred >10 days after birth) = 7.7 (1.6–36), $p<0.01$) (see multivariable analyses in Supplemental Information).

Among infants with an intestinal perforation, those who died as a direct consequence of the perforation were similar to those who survived the perforation except for two neonatal variables: the cause of the perforation (SIP or perforated-NEC) and the presence or absence of non-CONS bacteremia/fungemia at the onset of the perforation (Table 4). After adjusting for possible confounding, the only variable that was significantly associated with the outcome "death due to intestinal perforation" was the variable "presence of non-CONS bacteremia/fungemia at the onset of perforation" (OR (95% CI) = 4.4 (1.3–15.4), $p<0.05$) (see multivariable analyses in Supplemental Information). Although mortality appeared to be increased among infants with perforated-NEC in the univariate analysis (Tables 2 and 4), this was no longer seen in the multivariable analysis (OR (95% CI) = 2.2 (0.6–8.4), p=NS) where mortality was most strongly associated with the presence of "non-CONS bacteremia/fungemia" (see multivariable analyses in Supplemental Information).

Consistent with the results of the multivariable analysis, we found that the incidence of death was not significantly different between those who had perforated-NEC and those who had

SIP (OR (95% CI) = 3.1 (0.7–13.5), p=NS) when we examined a subgroup of infants who never developed non-CONS bacteremia/fungemia during the perforation episode (n=59).

The results reported above were the same whether we used the Primary diagnosis of SIP and perforated-NEC (as reported above) or either of the other two Alternate diagnoses (#1 and #2) of SIP and perforated-NEC listed in Table 1 (data not shown).

Discussion:

Intestinal bacterial dysbiosis has been shown to play an important etiologic role in the onset of NEC; conversely, its role in the etiology of SIP appears to be negligible[20, 31, 32, 33, 34]. The presence of bacteremia/fungemia at the onset of NEC also has been hypothesized to play a role in precipitating NEC, however, several studies have concluded that the presence of bacteremia/fungemia at the onset of NEC is a consequence of the disease rather than its cause[16, 18]. Although we observed that the incidence of bacteremia/fungemia was strongly associated with perforated-NEC in our univariate model (Table 2), this relationship was no longer significant after adjusting for possible confounders. When examined in the multivariable model, the apparent association between bacteremia/fungemia and perforated-NEC was likely due to the older postnatal age of the infants with perforated-NEC.

The incidence of death due to intestinal perforation also appeared to be significantly higher among infants with perforated-NEC than infants with SIP in our univariate model (Table 2). This has been observed previously[2, 12]. Although differences in inflammatory and immune-mediated responses between perforated-NEC and SIP may contribute to the different rates of mortality[4, 5, 6, 7, 8, 9], several studies have found an association between the presence of non-CONS bacteremia in cases of NEC and the incidence of death[1, 13, 14, 15, 16, 17]. Since perforated-NEC usually appears after 10 days (Figure 1) and is often accompanied by non-CONS bacteremia/fungemia, we hypothesized that the increased incidence of non-CONS bacteremia/fungemia in infants with perforated-NEC might explain why infants with perforated-NEC have a higher mortality rate than infants with SIP. We found that after adjusting for possible confounding in the multivariable models, the only demographic characteristic that was significantly associated with the outcome “death due to intestinal perforation” was the “presence of non-CONS bacteremia/fungemia at the onset of perforation”. Death was no longer associated with whether the perforation was due to SIP or perforated-NEC when the model was adjusted for “presence of non-CONS bacteremia/fungemia”. The apparent association between death and perforated-NEC that was seen in the univariate model (Table 2) was likely explained by the higher incidence of non-CONS bacteremia/fungemia among infants with perforated-NEC.

Our study has several limitations. As an observational study, it cannot distinguish between causation and association. The study also took place over a 24 years interval. Although we examined the effects of being born during different birth epochs, and having different demographic and treatment variables, unmeasured differences in practice could have affected the rates of mortality. We used data from a single center. Since the rates of SIP and perforated-NEC vary by center our results may not be generalizable to other centers where the rates differ from ours. Although we examined the associations of different protocols for

formula and donor breast milk supplementation on the study outcomes, we did not collect data about the individual infant's daily enteral volume intake or type of feeding and were not able to examine their effects on the study outcomes. In addition, as in other studies of NEC and SIP, tissue was only available for pathology in 50% of the patients; the others had to be diagnosed using clinical and radiographic criteria. However, unlike other studies that used summary clinical and administrative data sets, and lacked consistent case definitions, our study used data collected prospectively by a single investigator, using well defined and consistent criteria for assigning the diagnoses of SIP and perforated-NEC (Table 1).

In conclusion, we found that an infant's postnatal age at the time of an intestinal perforation was strongly associated with the presence of non-CONS bacteremia/fungemia during the perforation event. In our study, death due to the perforation was primarily related to the presence of non-CONS bacteremia/fungemia associated with the onset of the perforation and was independent of whether the perforation was due to SIP or perforated-NEC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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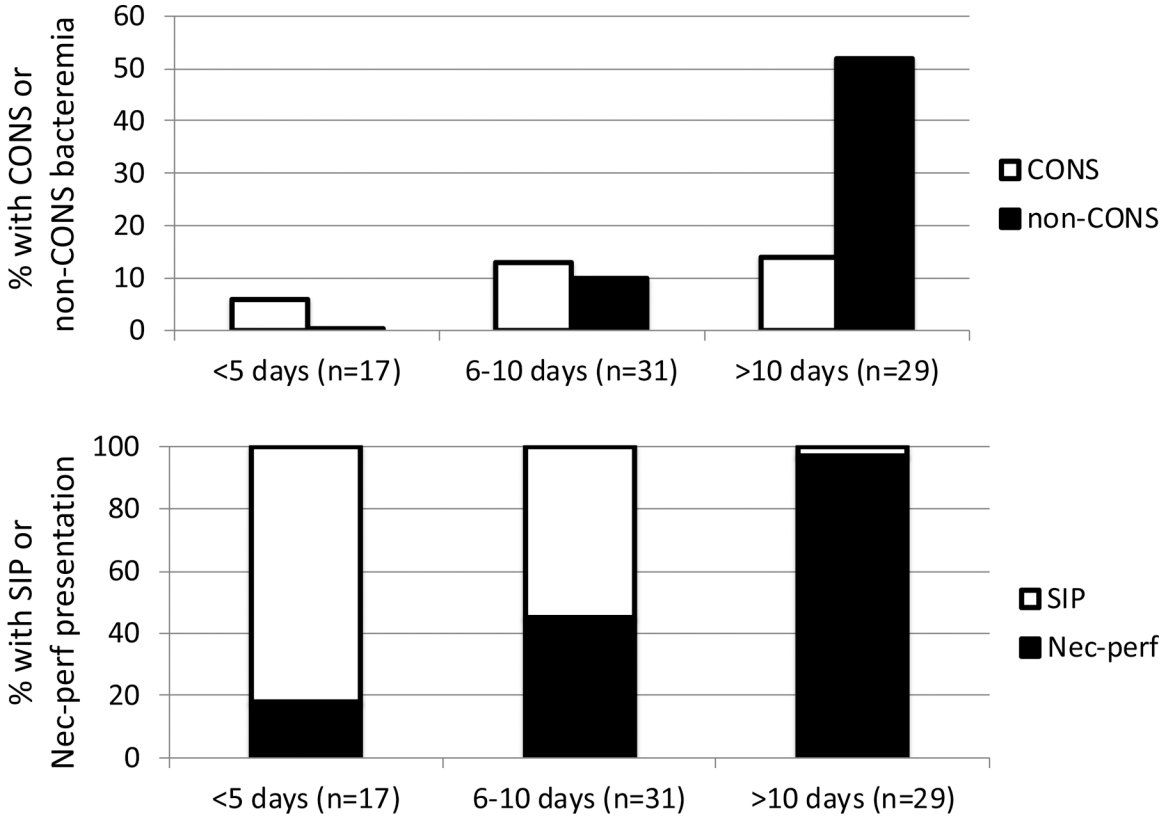

Figure 1:

Relationship between the age of onset of the acute neonatal intestinal disease and the incidence of bacteremia/fungemia, SIP and perforated-NEC among infants with a perforated intestine (n=77).

CONS, coagulase negative staphylococcus

Non-CONS, fungemia or bacteremia (excluding CONS)

Table 1:

Clinical, radiographic and histologic criteria for defining Spontaneous Intestinal Perforation (SIP) and Perforated-Necrotizing Enterocolitis (NEC-perf) among infants (n=77) with intestinal perforations: creating Primary and Alternate diagnoses.

Intestinal Perforation: clinical, radiographic and histologic presentations	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Clinical deterioration prior to perforation (duration - days)	none	none	none	>0.66 & <1	>2	>1	>1	>1
Abnormal abdominal radiograph prior to perforation (duration - days)	none	none	none	>0.66 & <1	>2	>1	>1	>1
Pneumatosis intestinalis ± portal venous gas	none	none	none	none	none	none	yes	yes
SIP diagnosis by histology or at laparotomy	yes	NA	no	NA	NA	no	NA	no
NEC diagnosis by histology or at laparotomy	no	NA	yes	NA	NA	yes	NA	yes
Number of infants in group	7	25	3	4	3	14	7	14
Primary SIP/NEC Diagnosis:	**SIP**	**SIP**	*NEC-perf*	*NEC-perf*	**NEC-perf**	**NEC-perf**	**NEC-perf**	**NEC-perf**
Alternate SIP/NEC Diagnosis #1:	**SIP**	**SIP**	*SIP*	*SIP*	**NEC-perf**	**NEC-perf**	**NEC-perf**	**NEC-perf**
Alternate SIP/NEC Diagnosis #2:	**SIP**	**SIP**	_	_	**NEC-perf**	**NEC-perf**	**NEC-perf**	**NEC-perf**

See Methods for definitions of clinical, radiographic and histologic presentations

NA, not available

Primary SIP/NEC Diagnosis:

The **diagnosis of perforated-NEC (NEC-perf)** was based primarily on surgical pathology, the appearance of the intestine at laparotomy and/or the presence of pneumatosis on >1 abdominal radiograph. Four infants were also considered to have NEC-perf in the absence of pneumatosis, even though a laparotomy had not been performed and surgical pathology was unavailable, since there had been sudden clinical deterioration, with several abnormal abdominal radiographs, for at least 16 hours prior to the detection of the perforation. The **diagnosis of SIP** was based on the appearance of the intestine at laparotomy and surgical pathology. If a laparotomy had not been performed and surgical pathology was unavailable, the diagnosis of SIP was based on the absence of clinical deterioration and the presence of normal abdominal radiographs prior to the detection of the perforation.

Alternate SIP/NEC Diagnosis #1:

The **diagnosis of SIP-alternate #1** was based primarily on the absence of clinical deterioration and the presence of normal abdominal radiographs prior to the detection of the perforation. Three infants were classified as SIP-alternate #1 despite having a small area of inflammation and necrosis (described as consistent with necrotizing enterocolitis by the pathologist) surrounding the perforation. Four infants were classified as SIP-alternate despite having either abnormal clinical symptoms or an abnormal abdominal radiograph because these occurred less than 24 hours prior to detecting the perforation.

The **diagnosis of perforated-NEC (NEC-perf)-alternate #1** was based on the presence of clinical deterioration and abnormal abdominal radiographs for >24 hours prior to detecting the perforation, plus the presence of pneumatosis on >1 abdominal radiograph, and/or on surgical pathology or the appearance of the intestine at laparotomy. Infants who had clinical deterioration and abnormal radiographs longer than 2 days before the perforation were also considered to have NEC-perf even if pneumatosis was not present and even if surgical pathology was unavailable or a laparotomy had not been performed.

Alternate SIP/NEC Diagnosis #2:

The seven infants in cluster groups 3 and 4 who had criteria that were consistent with either SIP or perforated-NEC were not included in the diagnoses.

Table 2:

Demographic characteristics among infants with intestinal perforation (n=77) whose Primary Diagnosis was either spontaneous intestinal perforation (SIP) (n=32) or necrotizing enterocolitis with perforation (Nec-perf) (n=45).

	Intestinal Perforation – Primary Diagnoses (n=77)			Intestinal Perforation – Primary Diagnoses (n=77)	
	SIP (n=32)	**Nec-perf (n=45)**		**SIP (n=32)**	**Nec-perf (n=45)**
Prenatal Variables:			**Neonatal Variables continued:**		
Multiple gestation - %	47	29	Indomethacin prophylaxis - %[6]	69	78
Preeclampsia - %	19	13	Indomethacin prior to perforation - %	75	91
Diabetes - %	16	9	ICH (serious) - %[7]	28	36
Chorioamnionitis - %	22	33	Enteral feeding (prior to perforation) - %	31	62 *
Betamethasone exposure ≥24 hours - %	66	49	Early onset bacteremia - %[8]	0	0
Breech or transverse presentation - %	53	36	Perforation 1st detected ≤10 days - %	97	38 ****
Cesarean section - %	69	60	Age perforation 1st detected - days – median (IQR)	6 (4–7)	15 (8–31) ****
Neonatal Variables:			Late onset bacteremia/fungemia[9] (not related to perforation) (total) - %	31	29
Gestational Age ≤25 weeks - %[1]	59	78	CONS bacteremia - %[10]	16	16
Gestational age (weeks) - (m±sd)	25.4±1.2	25.1±0.9	Non-CONS bacteremia/fungemia - %[11]	16	22
Birthweight (grams) (m±sd)	770±145	740±131	Bacteremia/fungemia related to onset of perforation (total) - %[12]	9	49 **
Small for gestational age - %[2]	13	15	CONS bacteremia - %[10]	6	16
Caucasian race - %	41	29	Non-CONS bacteremia/fungemia - %[11]	3	38 ****
Male gender - %	63	64	Donor breast milk epoch - %[13]	25	22
5 minute Apgar score ≤5 - %	40	33	Early birth epoch (1995 – 2005) [14] - %	41	64
10 minute Apgar score ≤5 - %	13	14			
Respiratory distress syndrome - %	94	89	**Outcomes:**		
Surfactant - %	97	89	BPD - %[15]	57	52

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	Intestinal Perforation – Primary Diagnoses (n=77)			Intestinal Perforation – Primary Diagnoses (n=77)	
	SIP (n=32)	**Nec-perf (n=45)**		**SIP (n=32)**	**Nec-perf (n=45)**
Respiratory Severity Score[3] at 24 hours - median (IQR)	1.7 (1.5–2.6)	2.0 (1.7–3.3)	BPD/Death - %[16]	69	71
Intubated at 24 hours - %	94	87	ROP - %[17]	30	50
Dopamine during first 3 days - %[4]	39	40	Death (all causes) - %	22	40
Hydrocortisone during first 3 days - %[5]	0	5	Death (due to perforation) - %	13	36 *
Daily fluid intake during the first 72 hours (ml/kg/day) - (m ±sd)	158±28	150±36			
Daily urine output during the first 72 hours (ml/kg/day) - (m±sd)	4.3±1.1	4.1±1.3			

m±sd, mean ± standard deviation; IQR, interquartile range

*, p<0.05;

**, p<0.01;

*** p<0.001;

****, p<0.0001; no post-hoc adjustments were made for multiple univariate analyses

[1] *Gestation*, gestational age was determined by the date of last menstrual period and early ultrasounds (before 24 weeks gestation)

[2] *Small for Gestational Age*, birthweight-for-gestational-age z-scores <1.29 [35]

[3] *Respiratory severity score*, mean airway pressure x FiO2

[4] *Dopamine during first 3 days*, required dopamine infusions >6 micrograms/kg/min for more than 20 hours during the first 72 hours after birth

[5] *Hydrocortisone during first 3 days*, required hydrocortisone (in addition to dopamine >15 micrograms/kg/min) to support blood pressure during the first 72 hours after birth

[6] *Indomethacin prophylaxis*, indomethacin administered within 24 hours of birth

[7] *ICH (serious)*, serious intracranial hemorrhage were defined as grades 3 or 4 intracranial hemorrhage (using the four-level grading system) [36] or moderate/large cerebellar hemorrhage [37]

[8] *Early Onset Infection*, number of infants with culture-positive bacteremia prior to 4 days of life

[9] *Late Onset Infection*, number of infants with culture-positive bacteremia/fungemia after 3 days of life

[10] *CONS bacteremia*, number of infants with coagulase negative staphylococci bacteremia (note: some infants had more than one organism - both CONS and non-CONS bacteremia)

[11] *Non-CONS bacteremia/fungemia*, number of infants with non-coagulase negative staphylococci bacteremia or fungemia (note: some infants had more than one organism - both CONS and non-CONS bacteremia)

[12] *Bacteremia/fungemia related to onset of perforation*, number of infants with culture-positive bacteremia/fungemia occurring within 72 hours of perforation presentation

[13] *Donor breast milk epoch*, number of infants admitted after donor breast milk was substituted for infant formula (≥2012)

[14] Early birth epoch (1995 – 2005), number of infants admitted during first half of the study period (between 1995 and 2005)

[15] *BPD*, bronchopulmonary dysplasia was defined using a modified room air challenge test between $36^{0/7}$ and $36^{6/7}$ weeks' corrected age [38]

[16] *BPD/Death*, bronchopulmonary dysplasia or death before 36 weeks

[17] *ROP*, retinopathy of prematurity - maximum stage: stage 2 with plus disease or ≥stage 3

Table 3:

Number of infants with SIP or perforated-NEC who had culture-positive bacteremia or fungemia within 72 hours of their perforation.

Organism	Infants with SIP (n=32)	Infants with perforated-NEC (n=45)
Coagulase-negative staphylococcus (CONS)	2 (6%)	7 (16%)
Non-CONS bacteremia/fungemia - total no.	1 (3%)	17 (38%)
E. coli	1	5
Candida species	0	5
Klebsiella species	0	4
Enterococcus species	0	2
Enterobacter species	0	1
Pseudomonas species	0	1
Serratia species	0	1
S. aureus	0	1

SIP and perforated-NEC were both diagnosed using the Primary Diagnosis of SIP and perforated-NEC (see Methods).

Some infants had more than one organism (e.g., both CONS and non-CONS bacteremia).

Table 4:

Demographic characteristics of infants who died as a result of their intestinal perforation (n=20) with those who survived their perforation (n=57).

	Death due to perforation			Death due to perforation	
	Yes (n=20)	No (n=57)		Yes (n=20)	No (n=57)
Prenatal Variables:			**Neonatal Variables continued:**		
Multiple gestation - %	20	42	Indomethacin prophylaxis - % [6]	65	77
Preeclampsia - %	20	14	Indomethacin prior to perforation - %	85	84
Diabetes - %	0	15	ICH (serious) - % [7]	45	28
Chorioamnionitis - %	30	28	Enteral feeding (prior to perforation) - %	70	42
Betamethasone exposure ≥24 hours - %	55	56	Early onset bacteremia - % [8]	0	0
Breech or transverse presentation - %	45	42	Perforation 1st detected ≤10 days - %	50	67
Cesarean section - %	60	65	Age perforation 1st detected - days – median (IQR)	11 (6–18)	8 (5–16)
Neonatal Variables:			Late onset bacteremia/fungemia [9] (not related to perforation) (total) - %	15	35
Gestational Age ≤25 weeks - % [1]	85	65	CONS bacteremia - % [10]	5	19
Gestational age (weeks) - (m±sd)	25.0±0.8	25.3±1.1	Non-CONS bacteremia/fungemia - % [11]	10	23
Birthweight (grams) (m±sd)	741±129	757±140	Bacteremia/fungemia related to onset of perforation (total) - % [12]	55	25 *
Small for gestational age - % [2]	20	12	CONS bacteremia - % [10]	10	12
Caucasian race - %	25	35	Non-CONS bacteremia/fungemia - % [11]	50	14 **
Male gender - %	45	70	Donor breast milk epoch - % [13]	35	19
5 minute Apgar score ≤5 - %	45	33	Early birth epoch (1995 – 2005) [14] - %	40	60
10 minute Apgar score ≤5 - %	21	11	Primary diagnosis: Nec-perf - %	80	51 *
Respiratory distress syndrome - %	90	91			
Surfactant - %	90	93			
Respiratory Severity Score [3] at 24 hours - median (IQR)	2.4 (1.7–3.3)	1.9 (1.5–2.8)			
Intubated at 24 hours - %	80	93			
Dopamine during first 3 days - % [4]	33	42			
Hydrocortisone during first 3 days - % [5]	0	3			
Daily fluid intake during the first 72 hours (ml/kg/day) - (m±sd)	165±34	150±31			

	Death due to perforation			Death due to perforation	
	Yes (n=20)	**No (n=57)**		**Yes (n=20)**	**No (n=57)**
Daily urine output during the first 72 hours (ml/kg/day) - (m±sd)	4.3±1.5	4.2±1.1			

m±sd, mean ± standard deviation; IQR, interquartile range

*, p<0.05;

**, p<0.01;

*** p<0.001;

****, p<0.0001; no post-hoc adjustments were made for multiple univariate analyses

Footnotes 1 – 14, see Legend of Table 2