

Antibiotic Exposure in the Newborn Intensive Care Unit and the Risk of Necrotizing Enterocolitis

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Objective To determine whether duration of antibiotic exposure is an independent risk factor for necrotizing enterocolitis (NEC).

Study design A retrospective, 2:1 control-case analysis was conducted comparing neonates with NEC to those without from 2000 through 2008. Control subjects were matched on gestational age, birth weight, and birth year. In each matched triad, demographic and risk factor data were collected from birth until the diagnosis of NEC in the case subject. Bivariate and multivariate analyses were used to assess associations between risk factors and NEC.

Results One hundred twenty-four cases of NEC were matched with 248 control subjects. Cases were less likely to have respiratory distress syndrome ($P = .018$) and more likely to reach full enteral feeding ($P = .028$) than control subjects. Cases were more likely to have culture-proven sepsis ($P < .0001$). Given the association between sepsis and antibiotic use, we tested for and found a significant interaction between the two variables ($P = .001$). When neonates with sepsis were removed from the cohort, the risk of NEC increased significantly with duration of antibiotic exposure. Exposure for >10 days resulted in a nearly threefold increase in the risk of developing NEC.

Conclusions Duration of antibiotic exposure is associated with an increased risk of NEC among neonates without prior sepsis. (*J Pediatr* 2011;159:392-7).

Necrotizing enterocolitis (NEC) is a disease process of the gastrointestinal tract that occurs primarily in premature neonates. Approximately 1% to 5% of very low birth weight infants (<1500 g) will develop NEC,^{1,2} with an associated mortality rate of 25% to 33%.³ NEC is believed to be a multifactorial disease process with risk factors including prematurity,⁴ low birth weight,⁵ enteral feeding,⁶ and alterations in bacterial colonization of the gastrointestinal tract.⁷

Healthy term breast-fed infants are typically colonized with several microbial species by 1 week of age, including anaerobic species of *Bifidobacterium* and *Lactobacillus*. Normal intestinal flora may provide resistance to colonization by highly pathogenic bacteria and stimulate antiinflammatory cytokine production.⁸ In contrast, the hospitalized preterm neonate has less microbial diversity and fewer anaerobes, typically becoming colonized with *Escherichia coli*, species of *Enterococcus* and *Klebsiella pneumoniae*.⁹⁻¹¹ Proliferation of these organisms can result in invasion of the intestinal wall, translocation, and inflammatory cytokine production.¹²

The widespread use of antibiotics in the neonatal intensive care unit (NICU) population may contribute to aberrant gut colonization. Antibiotic exposure may reduce the biodiversity of the fetal microbiota, delay beneficial colonization with normal gastrointestinal flora, and promote proliferation of pathogenic and antibiotic-resistant organisms.¹³⁻¹⁵ The increase in potentially pathogenic organisms and decrease in normal gut flora coupled with impairment in the intestinal epithelial barrier may predispose preterm neonates to NEC.⁷ We hypothesize that an increased duration of antimicrobial exposure in neonates will increase the probability for the development of NEC.

Methods

A retrospective, 2:1 control/case analysis was performed to investigate the association between the duration of antimicrobial therapy and subsequent diagnosis of NEC among neonates born from January 1, 2000 to December 31, 2008, and admitted to the Newborn Special Care Unit (NBSCU) at Yale-New Haven Children's Hospital. The NBSCU is a 54-bed, level IIIc¹⁶ NICU for infants with complex medical and surgical conditions.

BPD	Bronchopulmonary dysplasia
NBSCU	Newborn special care unit
NEC	Necrotizing enterocolitis
NICU	Newborn intensive care unit
PDA	Patent ductus arteriosus
RDS	Respiratory distress syndrome
SGA	Small for gestational age
TPN	Total parenteral nutrition

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Cases included neonates diagnosed with NEC modified Bell's stage \geq IIA.¹⁷ Each identified case was matched to two control subjects. The control population included neonates without NEC with matching performed on age (number of hospital days before NEC in matched cases), gestational age (± 1 week), birth weight (± 200 g) and year of hospital admission (± 1 year). Neonates with major congenital abnormalities (including those of the gastrointestinal tract) were excluded from the study, as well as neonates transferred from outside hospitals.

Cases and control subjects were identified by review of an electronic database, which maintains demographic, hospital course, and outcome data for all admissions to the NBSCU. Additional information not available from the database was obtained by chart review. Data collection on all subjects included demographics, potential risk factors for NEC, and outcomes. Risk factor data were collected for each case and its two matched control subjects from birth up to and including the day before NEC in the case subject. For example, if the case patient had development of NEC on day of life 15, data pertaining to the hospital course in the case and two assigned control subjects were collected from birth through day of life 14.

Potential risk factors for NEC included antenatal corticosteroid exposure, 5-minute Apgar score, small for gestational age (SGA), respiratory distress syndrome (RDS), the presence of a patent ductus arteriosus (PDA), laboratory-confirmed bloodstream infection, feeding practices, antibiotic exposure, and umbilical catheter use. More specifically, data regarding feeding practices included the day of first enteral feeding, type of feeding (breast milk only, formula only, or a combination), maximum enteral feeding volume achieved (milliliters per kilogram per day) and, if applicable, day of life full enteral feeds were achieved (ie, when total parenteral nutrition [TPN] was discontinued). The duration of antibiotic exposure included the cumulative number of days a neonate was on any antibiotic. These data were initially collected in hours of exposure, which was then converted into days. Data regarding the specific antimicrobial regimen used were also collected. Standard empiric antimicrobial therapy in the NBSCU during the study period included ampicillin and gentamicin for suspected early-onset sepsis (≤ 72 hours of life), vancomycin and gentamicin for suspected late-onset sepsis

(>72 hours of life), and ampicillin plus gentamicin and clindamycin for suspected or confirmed NEC.

Outcome data were also collected and included the presence or absence of bronchopulmonary dysplasia (BPD), duration of mechanical ventilation, duration of TPN, length of hospital stay, and death. Unlike the risk factor data, continuous outcome data (ie, duration of mechanical ventilation and TPN and length of stay) were reflective of the entire NBSCU stay.

Definitions

RDS was defined as respiratory distress and an oxygen requirement necessitating intubation and the administration of surfactant. BPD was defined as the need for supplemental oxygen at 36 weeks postmenstrual age in association with characteristic radiographic changes.¹⁸ A PDA was defined by the presence of clinical signs (ie, continuous murmur, hyperdynamic precordium, bounding pulses, widened pulse pressure, increased pulmonary vascular markings or cardiomegaly on chest x-ray films, increased oxygen requirement), echocardiographic confirmation by a pediatric cardiologist, and subsequent treatment with either indomethacin or ibuprofen. Sepsis was defined as a laboratory confirmed bloodstream infection according to criteria from the Centers for Disease Control and Prevention that were current during the study period.¹⁹ Blood cultures were assessed during this time period using a fluorescent-detection system for the presence of carbon dioxide (Bactec II or 9240; Becton Dickinson, Franklin Lakes, New Jersey).

Statistical Analysis

Comparisons between cases and control subjects on demographic and clinical characteristics, as well as established risk factors for NEC, were first made via unadjusted conditional logistic regression analysis. Patient characteristics showing significant trends ($P \leq .10$) in association with NEC diagnosis were evaluated in a multivariate conditional logistic regression. The interaction between the diagnosis of sepsis and cumulative exposure to antimicrobial therapy was also evaluated. Data are presented as unadjusted and adjusted OR. All data were analyzed by use of SAS 9.2 (SAS, Cary, North Carolina). Significance in the adjusted and unadjusted analyses was established with alpha of less than 0.05. This study was approved by the Human Investigation Committee of the Yale University School of Medicine.

Table I. A comparison of demographic and outcome data in cases and control subjects

Variable	Case (n = 124)	Control subject (n = 248)	Unadjusted OR (95% CI)*
Male sex	74 (59.7%)	135 (54.4%)	1.28 (0.80, 2.00)
RDS	70 (56.5%)	167 (67.3%)	0.48 (0.27, 0.85)
BPD	53 (42.7%)	111 (44.8%)	0.87 (0.49, 1.54)
Days on ventilator	32.2 \pm 32.7	18.8 \pm 27.2	1.03 (1.02, 1.05)
Days on TPN	50.9 \pm 34.8	24.3 \pm 18.7	1.06 (1.04, 1.07)
Hospital days	38.3 \pm 39.7	69.0 \pm 48.2	1.005 (1.00, 1.011)
Death	29 (23.4%)	10 (4.0%)	10.17 (3.91, 26.45)

*Conditional logistic regression.

Results

The 124 cases of NEC were matched with 248 control subjects. Neonates with NEC were diagnosed, on average, at day of life 22 ± 15 , with 51% receiving only medical treatment and 49% undergoing surgical intervention. Cases and control subjects were well matched with respect to gestational age (28.2 ± 3.4 weeks in cases vs 28.1 ± 3.4 weeks in control subjects; $P = .849$), birth weight (1162 ± 573 g in cases vs 1169 ± 584 g in control subjects; $P = .902$), and year of

admission to the NBSCU ($P = 1.000$). Matching was also effective in the subgroups of cases with birth weight < 750 g (647 ± 60 g in cases vs 660 ± 77 g in control subjects; $P = .290$) and ≥ 750 g (1319 ± 567 g in cases vs 1324 ± 583 g in control subjects; $P = .479$). No significant differences were observed between cases and control subjects with respect to sex, the proportion of small for gestational age infants, prenatal corticosteroid exposure, 5-minute Apgar score, umbilical catheter use, the timing of first enteral feeding, BPD, and length of NBSCU stay (Tables I and II).

A few notable differences, however, were observed between the case and control populations. A significantly higher proportion of control subjects were diagnosed with RDS and PDA as compared with cases (Tables I and II). Also, neonates with NEC were more likely to obtain a higher enteral feeding volume or reach full enteral feeding as compared with controls (Table II). Neonates diagnosed with NEC required a significantly longer duration of mechanical ventilation and TPN over their entire hospital stay and had a higher rate of death before discharge than control subjects (Table I).

A significantly higher proportion of neonates with NEC were diagnosed with culture-proven sepsis than those without (Table II). Timing of sepsis varied between populations, with neonates in the control group being diagnosed with sepsis, on average, 1 week earlier than the case group (Table II). Most sepsis (65%) in the case

population occurred within 24 hours of or concurrent with the diagnosis of NEC with 71% of these patients then undergoing surgery for NEC. The type of antibiotic exposure differed only with respect to a significantly higher proportion of clindamycin use in the case population as compared with control subjects. However, the total duration of exposure to any and all antibiotics before our study cutoff point (ie, the day of life NEC was diagnosed in the case subject) did not differ significantly between cases and control subjects in the unadjusted analysis (Table II). When duration of exposure to each individual antibiotic was analyzed, no significant differences were noted between cases and controls with respect to ampicillin (5.2 vs 4.5 days; $P = .149$), gentamicin (5.9 vs 5.0 days; $P = .238$), vancomycin (5.3 vs 4.6 days; $P = .893$), clindamycin (5.8 vs 6.0 days; $P = .967$), and cefotaxime (5.0 vs 6.6 days, $P = .337$).

In the adjusted analysis, attainment of full enteral feeding ($P = .028$) remained a significant predictor of NEC, and RDS ($P = .018$) was protective. Near-significant trends included SGA as a risk factor and breast milk as a protective factor for NEC (Table III). PDA was not significant ($P = .220$). Also in the adjusted analysis, we found that the interaction between sepsis and antibiotic use was significant (Table III). Given discrepancies in the proportion of neonates with sepsis in the case and control populations and the strong interaction of sepsis with antibiotic use, the 59 neonates with sepsis were removed from the cohort and

Table II. A comparison of risk factors for NEC in cases and control subjects

Variable	Case (n = 124)	Control subject (n = 248)	Unadjusted OR (95% CI)*
Prenatal corticosteroids	110 (88.7%)	213 (85.9%)	0.73 (0.34, 1.53)
SGA	16 (12.9%)	21 (8.5%)	3.23 (0.98, 10.63)
5-minute Apgar	7.8 ± 1.6	7.5 ± 1.8	1.16 (0.98, 1.37)
PDA	15 (12.1%)	51 (20.6%)	0.52 (0.28, 0.98)
UAC	13 (10.5%)	18 (7.3%)	3.17 (0.80, 12.63)
UVC	9 (7.3%)	19 (7.7%)	0.90 (0.30, 2.73)
Type of Feed			Reference
Formula	42 (33.9%)	61 (24.6%)	
Breast milk	32 (25.8%)	94 (37.9%)	0.44 (0.24, 0.81)
Both	40 (32.3%)	74 (29.8%)	0.75 (0.42, 1.33)
Did not feed	10 (8.1%)	19 (7.7%)	0.79 (0.27, 2.35)
Day of first feed	5.3 ± 3.9	5.2 ± 3.0	1.02 (0.94, 1.11)
Maximum enteral feeding volume (cc/kg/day)	112.3 ± 41.1	96.3 ± 49.7	1.01 (1.00, 1.02)
Full feeds achieved	81 (65.3%)	135 (54.4%)	2.03 (1.15, 3.59)
Day of life achieved	16.6 ± 8.9	15.1 ± 9.4	1.05 (0.99, 1.11)
Sepsis	31 (25%)	28 (11.3%)	2.62 (1.48, 4.66)
DOL sepsis	20.8 ± 14.5	14.0 ± 11.4	
Multiple episodes	3 (9.7%)	4 (14.3%)	
Type of sepsis organism			
Gram-positive	12 (9.7%)	18 (7.3%)	1.54 (0.72, 3.30)
Gram-negative	14 (11.3%)	8 (3.2%)	4.78 (1.78, 12.8)
Fungal	5 (4.0%)	2 (0.8%)	5.96 (1.12, 31.82)
Days on antibiotics	6.8 ± 6.8	6.4 ± 6.3	1.02 (0.98, 1.07)
Type of antibiotic			
Ampicillin	115 (92.7%)	231 (93.2%)	0.93 (0.37, 2.33)
Gentamicin	115 (92.7%)	233 (94.0%)	0.79 (0.31, 2.01)
Vancomycin	33 (26.6%)	77 (31.0%)	0.71 (0.38, 1.31)
Cefotaxime	16 (12.9%)	37 (14.9%)	0.84 (0.44, 1.59)
Clindamycin	11 (8.9%)	8 (3.2%)	4.16 (1.29, 13.44)

UAC, umbilical arterial catheter; UVC, umbilical venous catheter; DOL, day of life.
*Conditional logistic regression.

Table III. Multivariate regression analysis of risk factors for NEC

Characteristic	Adjusted OR (95% CI)*	P value*
SGA	3.40 (0.96, 12.05)	.058
RDS	0.46 (0.24, 0.88)	.018
Type of feed		
Breast milk	0.57 (0.30, 1.10)	.093
Breast milk & formula	0.74 (0.39, 1.41)	.360
No feeds	0.90 (0.28, 2.85)	.854
Formula	Reference	
Full feeds reached	2.11 (1.08, 4.10)	.028
Sepsis	11.20 (3.44, 36.45)	<.0001
Antibiotic exposure	1.10 (1.02, 1.19)	.015
Sepsis by antibiotic exposure	0.85 (0.78, 0.94)	.001

*Conditional logistic regression.

separate analyses performed. When the cohort with sepsis was analyzed, we observed a decreased risk of NEC as the cumulative duration of antibiotic therapy lengthened. On day 1 to 2 of prior antibiotic exposure, neonates with sepsis had a 24.54-fold increased risk for development of NEC. This risk decreased with each day of antibiotics to 15.59 for 3 to 4 days, 9.90 for 5 to 6 days, 6.29 for 7 to 8 days, 4.00 for 9 to 10 days, and 2.54 for >10 days.

In those without sepsis, which represented 84% (313 of 373) of the study population, antibiotic exposure was determined to be a significant independent risk factor for NEC (Table III). We also observed a steadily increasing risk of NEC as duration of cumulative antibiotic exposure increased (Figure). After 1 to 2 days of antibiotic exposure, the risk for development of NEC in this population increased by 1.19 times and continued to increase with each additional day of antibiotic exposure to 1.43 for 3 to 4 days, 1.71 for 5 to 6 days, 2.05 for 7 to 8 days, 2.45 for 9 to 10 days, and 2.94 for >10 days of exposure (Figure).

Discussion

The findings support our hypothesis that increased duration of antibiotic exposure in NICU patients may increase their risk of NEC although, because of the confounding relationship between sepsis and antibiotic use, this was only found in neonates without a prior documented bloodstream infection. Antibiotic use in the neonatal population has been previously described as a potential contributing factor in the development of NEC. Antibiotic administration may result in suppression or eradication of protective anaerobic bacteria which normally colonize the gastrointestinal tract and result in overgrowth of potentially pathogenic enteric aerobic Gram-negative rods.^{20,21} Previous investigations have determined that overgrowth of pathogenic species is greatly increased after 3 days of antimicrobial exposure,²² as is the risk of NEC.²³ Our study suggests that even longer durations of antibiotic exposure to neonates may further increase this risk.

Our findings support the retrospective cohort analysis performed by Cotten et al,²³ which included 4039 extremely

low birth weight (<1000 g) neonates born between September 1, 1998, and December 31, 2001. Maternal and neonatal data were collected for the first 3 postnatal days and prolonged (ie, ≥ 5 days) initial duration of antibiotic exposure analyzed as a potential risk factor for NEC. A multivariate analysis determined that duration of initial antibiotic exposure was associated with an increased risk of NEC (adjusted OR: 1.07; 95% CI: 1.04 to 1.10) and prolonged antibiotic exposure was associated with an increased risk of NEC or death (adjusted OR: 1.30; 95% CI: 1.10 to 1.54).²³ Although our sample size was much smaller, we also determined duration of antibiotic exposure to be associated with an increased risk of NEC among infants without sepsis. Unlike the prior investigation, we performed a matched case-control analysis and included the cumulative duration of antimicrobial therapy until the diagnosis of NEC. We also considered other potential risk factors for NEC in our analysis, such as type of enteral feeding and the presence of a PDA.

In our initial bivariate analysis comparing cases and control subjects, no differences were observed with respect to duration of antibiotic exposure. However, the associations between NEC, sepsis, and antibiotic use presented a challenge to this analysis. An infectious cause for NEC has been previously suggested given the periodic occurrence of NEC in clusters²⁴ and associations between NEC and infection caused by species of bacteria including the Enterobacteriaceae, *Clostridia*, and coagulase-negative staphylococci²⁵⁻²⁷ have been described. The more common accepted belief is that infection in patients with NEC is not causative but instead results from impairment in the intestinal barrier leading to translocation of intestinal flora and bacteremia.²⁸ In our cohort, most sepsis occurred in the case population and concurrently with the diagnosis of NEC. As a result, we chose to separate these infants from most of the cohort. When we analyzed the effect of antibiotic exposure on the risk of NEC in infants with sepsis, we determined that their risk of NEC was greatest at antibiotic exposure days 1 and 2 and decreased as duration of exposure increased. This supports the idea that sepsis and NEC are closely associated and, in many cases, may be diagnosed simultaneously.

When we eliminated the potential confounding effects of sepsis from our analysis, we determined that antibiotic duration increased the probability of NEC by approximately 20% per day of exposure (OR = 1.2). Furthermore, exposure to more than 10 days of antimicrobial therapy resulted in nearly a threefold increase in the risk of development of NEC. Approximately 93% of our entire cohort received at least 5 days of empiric antimicrobial therapy, which, given our findings and those presented by Cotten et al²³ is concerning. Given the widespread use of antibiotics in our and other NICUs and their potential negative effects, antibiotic stewardship programs for the NICU have been suggested. A recent retrospective observational study applied the Center for Disease Control and Prevention's 12-Step guidelines for assessing antimicrobial prescribing in four NICUs.²⁹ Approximately 25% of antibiotic courses and antibiotic days,

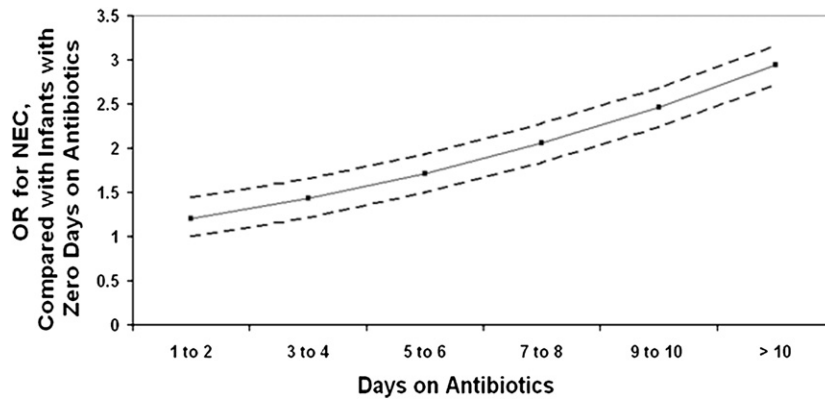


Figure. OR of developing necrotizing enterocolitis (Y-axis) as the cumulative duration of antibiotic exposure increases (X-axis) in neonates without a prior diagnosis of culture-proven sepsis. The comparison group is neonates with zero days of antibiotic exposure. The *dotted lines* represent 95% CIs. A statistically significant increase is observed.

on the basis of these guidelines, were determined to be “inappropriate.” It was noted that most inappropriate antibiotic use occurred with continuation, not initiation, of antibiotics.²⁹ Implementation of an antibiotic control program in the NICU may help avoid excess antibiotic use and potentially decrease the risk of associated morbidities.

There are several limitations with our investigation that mainly stem from its retrospective nature. Although several potential confounding variables for NEC were collected, additional maternal risk factors may have been helpful (if available), including mode of delivery, chorioamnionitis, maternal antibiotic therapy, hypertension, and placental abruption. We did not collect data on the use of H₂-blockers in our cohort, a known independent risk factor for NEC.³⁰ Also, it is possible that duration of antibiotic exposure is merely a marker of illness severity, and the inclusion of an illness severity score may have been helpful in differentiating this from the true effect of antimicrobial exposure. Lastly, inclusion of a larger control population and more precise criteria for matching, although not possible given the sample size, would have likely improved the validity of our results.

Increased antibiotic exposure in infants in the NICU is associated with an increased risk of development of NEC. A cautious approach to the initiation and continuation of antibiotics, especially in neonates with sterile cultures, should be considered given this association. ■

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