

Timing of Radiographic Necrotizing Enterocolitis Diagnosis Relative to Empiric Antibiotic Initiation During Late-Onset Infection Episodes

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Keywords: late-onset infection, necrotizing enterocolitis, empiric antibiotic therapy, radiographic diagnosis, timing

Posted Date: June 11th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9644627/v1>

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Additional Declarations: There is **NO** conflict of interest to disclose.

Abstract

Background

Among preterm infants who develop Necrotizing Enterocolitis during treatment for late-onset infection, the interval between empiric antibiotic initiation and radiographic confirmation is clinically recognized but poorly characterized. This variability may influence clinical trial design and interpretation.

Methods

Multicenter retrospective cohort study of very low birth weight infants (< 1500 g) born at < 33 weeks' gestation treated for late-onset infection who were subsequently diagnosed with NEC (modified Bell stage ≥ 2). The interval from the first antibiotic dose to the first pathognomonic abdominal radiographic finding was measured and stratified by quartiles.

Results

Among 271 infants, radiographic recognition occurred ≥ 4 hours after antibiotic initiation in 33% and ≥ 12 hours in 25%. Quartiles differed in mortality, surgical intervention, and survivor length of stay (all $p < 0.05$).

Conclusions

The interval between empiric antibiotic initiation and radiographic recognition of NEC varied widely and NEC may influence the design and interpretation of clinical trials and epidemiologic studies.

Introduction

Late-onset infection concerns frequently prompt evaluation and empiric antibiotic treatment in preterm infants. During these episodes, it is clinically recognized that the diagnosis of necrotizing enterocolitis (NEC) may not be present or apparent at the time empiric therapy is initiated. Although NEC is increasingly recognized as a heterogeneous clinical condition rather than a single disease entity,^{1, 2, 3} the interval between empiric antibiotic initiation for infection concerns and recognition of pathognomonic radiographic findings may vary substantially and is not well documented. Understanding the temporal relationship between empiric antibiotic initiation and subsequent radiographic recognition of NEC may influence the design and interpretation of clinical trials and epidemiologic studies.

Preventive and interventional trials cannot succeed without recognizing and integrating disease heterogeneity into their design and implementation¹. In clinical practice, heterogeneity may also arise from variation in the timing of disease recognition during routine care. In NEC, classification based on the interval between empiric antibiotic initiation and radiographic recognition may reveal important differences in clinical trajectory, including risks of mortality and surgical intervention^{4, 5}. Greater precision in characterizing this temporal dimension may therefore inform clinical investigation and trial design, ultimately contributing to improved care for preterm infants with acute abdominal pathology³. We therefore sought to characterize the interval between empiric antibiotic initiation and radiographic recognition of NEC during late-onset infection treatment episodes in a multicenter cohort of very low birth weight, preterm infants.

Methods

Patients

We performed a multicenter retrospective observational cohort study approved by the Institutional Review Board (IRB) at each participating center prior to data collection. Because of the retrospective nature of the study, the requirement for informed consent was waived. Eligible infants were inborn preterm infants (< 33 weeks gestation) with very low birth weight (< 1500 g) admitted to the neonatal intensive care units at the University of Florida Health (UF) (January 2012–April 2023), University of North Carolina (UNC) (May 2022–September 2025), Texas Children's Hospital (TCH) (January 2015–December 2024), or Johns Hopkins University (JHU)

(July 2016–January 2023). Infants were included if they developed NEC during a late-onset infection treatment episode and received ≥ 5 days of parenteral antibiotics (or died before completing 5 days). If a patient experienced more than one NEC episode, only the first episode was included in the analysis. Infants with major congenital anomalies were excluded.

A late-onset infection treatment episode was defined as initiation of parenteral antibiotics > 72 hours after birth and following ≥ 48 hours without prior antibiotic exposure. Infants with NEC (modified Bell stage $\geq 2^6$) were identified using problem lists, International Classification of Diseases (ICD) codes, and review of electronic health record (EHR) documentation, including operative reports, pathology reports, daily progress notes, consultation notes, and discharge summaries. All parenteral antibiotic administrations were curated for included patients, and the timing (start and stop) and duration of treatment episodes were determined. The examination time of the earliest radiology report documenting a pathognomonic abdominal radiographic abnormality of NEC (pneumatosis intestinalis, portal venous gas, pneumoperitoneum⁶) for each episode was identified through chart review. The interval between the first antibiotic dose of the treatment episode and the earliest radiographic documentation of NEC was calculated. Bacteremia and fungemia were defined as recovery of pathogenic bacteria or fungus from the blood, respectively, during the episode. Severity of illness was quantified using the neonatal sequential organ failure assessment (nSOFA)⁷. The nSOFA score ranges from 0 (least severe) to 15 (most severe) and is calculated using the presence of mechanical ventilation and SpO₂/FiO₂ ratio for respiratory dysfunction, presence of vasoactive medications and/or corticosteroids for cardiovascular dysfunction, and platelet count for hematologic dysfunction. The maximum nSOFA during the episode was calculated. Vasoactive-inotropic medication exposures during the episode were identified. Medications considered vasoactive-inotropic included dopamine, dobutamine, epinephrine, milrinone, vasopressin, and norepinephrine. For infants with vasoactive-inotropic medication exposures, the maximum vasoactive-inotropic score (VIS) was calculated as follows: dopamine dose ($\mu\text{g/kg/min}$) + dobutamine dose ($\mu\text{g/kg/min}$) + $100 \times$ epinephrine dose ($\mu\text{g/kg/min}$) + $10 \times$ milrinone dose ($\mu\text{g/kg/min}$) + $10 \times$ vasopressin dose (mU/kg/min) + $100 \times$ norepinephrine dose ($\mu\text{g/kg/min}$)⁸.

Analytical methods

Parametric continuous variables were summarized as mean with standard deviation and compared using ANOVA with Tukey's multiple comparisons test. Non-parametric continuous variables were summarized as medians with the interquartile range (IQR) and compared between groups using Kruskal-Wallis with Dunn's multiple comparisons test. Categorical variables were presented as percentages and compared using the Chi-square test. The threshold for statistical significance was less than 0.05 for 2-sided tests. Analyses were performed using GraphPad Prism (version 10.3; group comparisons and graph productions), and R (version 4.2.2; calculation of nSOFA from raw EHR data).

Results

Patients

We identified the first episode of NEC during late-onset infection treatment episodes in 271 patients (JHU = 47; TCH = 98; UNC = 37; UF = 89) that met our inclusion criteria (median GA 27 weeks (IQR 25,29); median BW 815g (650, 1070) during the center-specific study periods. Data were presented and compared by quartiles for the interval between empiric antibiotic initiation and radiographic recognition of NEC during late-onset infection treatment episodes (Table 1). Significant differences in episode characteristics were found for the frequency of episode mortality, overall mortality, surgical intervention, survivor length of treatment, and survivor length of stay (all $p < 0.05$). Pneumatosis intestinalis ($n = 185$; 68%) and the combination of pneumatosis intestinalis and portal venous gas ($n = 37$) were the most common documented first noted abdominal radiographic abnormalities. The frequency of pneumatosis intestinalis and portal venous gas was different between groups by timing of the discovery of radiographic signs. Radiographic findings were documented within ± 4 hours of empiric antibiotic initiation in 148 of 271 episodes (55%) and within ± 6 hours in 174 episodes (64%). However, 19 episodes (7%) had radiographic findings documented more than 6 hours prior to antibiotic initiation and 78 (29%) more than 6 hours after initiation (Fig. 1). Consistent with this distribution, the interval from the first dose of empiric antibiotics to the first documented abdominal radiographic abnormality was ≥ 4 hours in 33% of episodes ($n = 89$) and ≥ 12 hours in 25% ($n = 68$).

Discussion

In this multicenter, multiyear study of 271 infants with NEC during late-onset infection treatment episodes, we observed marked heterogeneity in the timing of radiographic findings relative to empiric antibiotic initiation. Because NEC occurs in the late neonatal period and not during the early-onset sepsis window (≤ 72 hours after birth), affected infants almost invariably present during late-

onset infection evaluations that prompt empiric antibiotic therapy. While approximately half of episodes had radiographic findings documented near the time antibiotics were started, 33% were documented ≥ 4 hours after initiation and 25% ≥ 12 hours after initiation. To our knowledge, this study provides the first detailed characterization of the real-world temporal relationship between empiric clinical action during late-onset infection treatment episodes and the subsequent documentation of pathognomonic abdominal radiographic findings. This variability in timing has important implications for clinical research, particularly for improving the precision of patient classification among those who meet diagnostic criteria for NEC^{1, 2}.

The aim and findings of our study are distinct from works that have described the impact of prolonged antecedent antibiotic exposure and the risk of NEC development⁹ or the impact of delays in receipt of antibiotics after order entry in patients with NEC¹⁰. Although a diagnosis of NEC is often assumed to occur very near the initiation of empiric antibiotic therapy, our data demonstrate substantial variability in this temporal relationship. While radiographic findings clustered near empiric antibiotic initiation in many episodes (55% within ± 4 hours), nearly half occurred outside this window. Notably, delayed documentation of radiographic findings after empiric treatment began was far more common than earlier documentation, with more than one-third of episodes showing radiographic findings ≥ 4 hours after treatment initiation and nearly one-third occurring more than 6 hours later (Fig. 1). These findings suggest that the temporal alignment often assumed between empiric infection treatment and radiographic NEC identification may oversimplify the real-world clinical course of these episodes.

Systemic, sepsis-like instability frequently precedes the radiographic diagnosis of NEC¹¹. Although the precise onset of NEC cannot be determined retrospectively, the timing of empiric antibiotic initiation relative to radiographic documentation reflects real-world clinical workflow during late-onset infection evaluations. Radiology report timestamps reflect the time of documentation rather than the moment when findings are incorporated into clinical decision-making. Accordingly, the interval between radiographic documentation and empiric antibiotic initiation should be interpreted as reflecting timing within the clinical workflow rather than the precise moment of diagnostic recognition. NEC diagnoses in this study were established using modified Bell staging criteria (stage ≥ 2) and clinical documentation, whereas radiology report timestamps were used only to identify the earliest recorded imaging evidence. This distinction is particularly relevant for episodes in which radiographic abnormalities were documented hours before empiric antibiotic initiation. In our cohort, variation in the interval between empiric treatment and radiographic documentation was associated with differences in clinical trajectory, including mortality, surgical intervention, and survivor length of treatment and hospitalization. Notably, gestational age, birth weight, age at episode onset, and illness severity were not significantly different across interval groups, suggesting that commonly measured baseline characteristics did not account for these differences. These findings suggest that the timing of radiographic NEC documentation during infection treatment episodes may represent an underrecognized dimension of clinical heterogeneity with potential implications for patient classification and clinical investigation¹².

The temporal variability of radiographic NEC documentation relative to empiric antibiotic initiation observed in this multicenter, multiyear cohort has important implications for NEC classification and prospective research studies. Reliance on radiograph-based frameworks, including modified Bell staging, may contribute to phenotypic heterogeneity because infants meet diagnostic criteria at different times relative to clinical recognition and treatment initiation. Radiographic findings represent documentation of disease rather than the moment of disease onset. Therefore, studies that define NEC onset or severity solely by radiographic confirmation will necessarily enroll infants at heterogeneous stages of illness. Classic staging systems such as the Bell criteria continue to guide NEC diagnosis and management but were not designed to address this temporal relationship^{6, 13}.

Limitations

We acknowledge these results cannot inform care. Although the multicenter design strengthens the study, patient enrollment periods differed across centers and clinical decision-making, including the justification for empiric antibiotic initiation and the timing of diagnostic evaluation, could not be standardized due to the retrospective design. The inclusion of 271 cases from four academic centers spanning more than a decade provides a broad representation of clinical practice, and the similar timing patterns observed across centers suggest that these findings reflect generalizable clinician behavior. Radiology report timestamps reflect the time of documentation rather than the moment when findings were incorporated into clinical decision-making, and radiology reports often include language indicating that abnormalities are possible or cannot be excluded. However, using the earliest documented radiographic abnormality provides a consistent and reproducible reference point for measuring timing across episodes. Finally, abdominal radiographs were obtained based on clinical judgment rather than a standardized surveillance protocol, so variation in

imaging frequency may influence the recorded timing of radiographic findings; however, this variability reflects real-world NICU practice across multiple institutions and over more than a decade, strengthening the external validity of the findings.

Conclusion

In this multicenter, multiyear cohort of preterm very low birth weight infants with necrotizing enterocolitis occurring during late-onset infection treatment episodes, we observed substantial variability in the interval between empiric antibiotic initiation and documentation of pathognomonic abdominal radiographic findings. The timing of radiographic documentation relative to empiric antibiotic initiation was associated with differences in clinical trajectory, including mortality, surgical intervention, survivor length of treatment, and length of stay. These findings highlight that radiographic confirmation of NEC often occurs at variable times relative to empiric infection treatment and suggest that incorporating temporal context into patient classification may improve phenotypic precision and strengthen the design and interpretation of observational studies and clinical trials.

Abbreviations

ANOVA
Analysis of variance
BW
birth weight
EHR
electronic health record
g
grams
ICD
international classification of diseases
IQR
interquartile range
IRB
institutional review board
Min
minute
NEC
necrotizing enterocolitis
NICU
neonatal intensive care unit
nSOFA
neonatal sequential organ failure assessment
VIS
vasoactive–inotropic score
VLBW
very low birth weight

Declarations

Author Contributions: Conceptualization: D.C, J.W, K.A, Methodology, Validation, Formal Analysis, Investigation, Writing: D.C and J.W, Data Curation: D.C, J.W, E.I., D.S., C.R, S.K, A.H, M.G., and K.A.. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This project was reviewed and approved by the institutional review board at all participating institutions prior to data collection and analysis.

Informed Consent Statement: This retrospective study was deemed exempt from patient consent by the Institutional Review Boards at each institution because it posed minimal risk.

Data Availability Statement: The datasets presented in this work are not readily available because of the vulnerable patient population. Request to access the datasets should be addressed to: ddelacruz@ufl.edu

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kim JH, Sampath V, Canvasser J. Challenges in diagnosing necrotizing enterocolitis. *Pediatr Res* 2020, 88(Suppl 1): 16–20.
2. Patel RM, Ferguson J, McElroy SJ, Khashu M, Caplan MS. Defining necrotizing enterocolitis: current difficulties and future opportunities. *Pediatr Res* 2020, 88(Suppl 1): 10–15.
3. Neu J, Modi N, Caplan M. Necrotizing enterocolitis comes in different forms: Historical perspectives and defining the disease. *Semin Fetal Neonatal Med* 2018, 23(6): 370–373.
4. Berrington JE, Embleton ND. Time of Onset of Necrotizing Enterocolitis and Focal Perforation in Preterm Infants: Impact on Clinical, Surgical, and Histological Features. *Front Pediatr* 2021, 9: 724280.
5. Dermyshe E, Granger C, Chmelova K, Embleton N, Berrington J. Age of onset of necrotising enterocolitis (NEC) and focal intestinal perforation (FIP) in very preterm and low birthweight infants: a systematic review. *BMJ Open* 2023, 13(7): e070638.
6. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin North Am* 1986, 33(1): 179–201.
7. Lewis AN, de la Cruz D, Wynn JL, Frazer LC, Yakah W, Martin CR, et al. Evaluation of the Neonatal Sequential Organ Failure Assessment and Mortality Risk in Preterm Infants with Necrotizing Enterocolitis. *Neonatology* 2022, 119(3): 334–344.
8. Aziz KB, Lavilla OC, Wynn JL, Lure AC, Gipson D, de la Cruz D. Maximum vasoactive-inotropic score and mortality in extremely premature, extremely low birth weight infants. *J Perinatol* 2021.
9. Cotten CM, Taylor S, Stoll B, Goldberg RN, Hansen NI, Sanchez PJ, et al. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. *Pediatrics* 2009, 123(1): 58–66.
10. Chetta KE, Vincent KG, Fanning B, Klumb AB, Chetta JA, Rohrer AM, et al. Impact of Delayed Time to Antibiotics in Medical and Surgical Necrotizing Enterocolitis. *Children (Basel)* 2023, 10(1).
11. Nandakumar C, Angadi C, Ramachandrin B, Adhisivam B, Devi U. Sepsis Associated Necrotizing Enterocolitis (SA-NEC) in Preterm Infants: Risk Factors and Clinical Outcomes. *Indian J Pediatr* 2026, 93(2): 185–187.
12. Cuna A, Kumar N, Sampath V. Understanding necrotizing enterocolitis endotypes and acquired intestinal injury phenotypes from a historical and artificial intelligence perspective. *Front Pediatr* 2024, 12: 1432808.
13. Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg* 1978, 187(1): 1–7.

Tables

Table 1 is available in the Supplementary Files section.

Figures

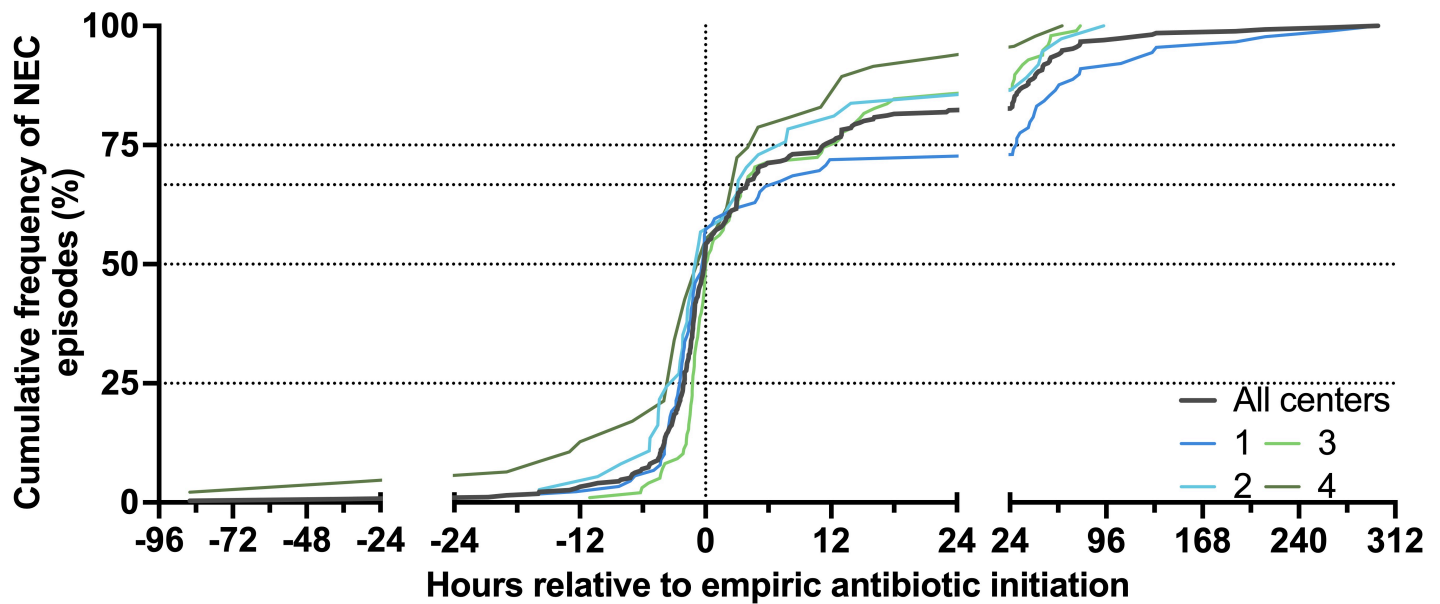


Figure 1

Legend: Relationship between empiric antibiotic initiation and radiographic recognition of necrotizing enterocolitis (NEC) during late-onset infection treatment episodes. The graph shows the cumulative frequency of NEC episodes (0–100%) plotted by time (hours) between the first dose of empiric antibiotics and the first documented pathognomonic abdominal radiographic findings (pneumatosis intestinalis, portal venous gas, or pneumoperitoneum). Negative values on the x-axis represent cases in which radiographic findings preceded empiric antibiotic initiation. Positive values represent cases in which radiographic findings occurred after empiric antibiotic initiation. Dotted lines on the y-axis indicate cumulative frequencies at the 25th, 50th, 67th, and 75th percentiles. The vertical dashed line indicates the time of empiric antibiotic initiation. Breaks in the x-axis at -24 and 24 hours allow visualization of the range of both early and delayed radiographic identification. Data are shown by center and in aggregate.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.Cohortandepisodecharacteristicsbyradiographicnecrotizingenterocolitisdiscoverytimingrelativetofirstdoseofantibiotics.pdf](#)