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Original Research

Evaluation of caffeine and the development of necrotizing enterocolitis

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Abstract.
OBJECTIVE: To test the association between medical or surgical necrotizing enterocolitis (NEC) and caffeine administration in premature infants.
STUDY DESIGN: This single-center, retrospective study evaluated patients admitted to a level 3 neonatal intensive care unit (NICU) over an 18-month period. All patients were evaluated for factors associated with the development of NEC including exposure to caffeine (dosing and duration), gestational age, birth weight, vasoactive medications and maternal illicit drug use.
RESULTS: There were 615 subjects included in the study; among these subjects, 7.3% ($n = 45$) developed NEC (35 subjects receiving caffeine and 10 subjects not receiving caffeine). The administration of caffeine ($p = 0.008$), birth weight ($p = 0.014$) and the use of vasopressors ($p = 0.033$) were associated with the development of NEC. When considering only infants with a birth weight less than 1500 g and less than 32 weeks gestation, the effects of caffeine and vasopressor use remained statistically significant ($p = 0.047$ and $p = 0.045$, respectively). The time to development of NEC did not differ statistically between patients receiving caffeine and those not receiving caffeine ($p = 0.129$).
CONCLUSION: A potential association between the administration of caffeine and the development of medical or surgical necrotizing enterocolitis in premature infants exists. Further investigation of dose-dependent effects and loading doses is warranted.

Keywords: Necrotizing enterocolitis, caffeine citrate, premature infants

1. Introduction

Necrotizing enterocolitis (NEC) is commonly seen in premature, low birth weight infants and is generally characterized by partial or full-thickness intestinal ischemia and resulting bowel tissue necrosis leading to inflammation, infiltration of gas-producing enteric organisms, and invasion into the portal venous system [1, 2]. The incidence of NEC in neonatal intensive care units (NICU) in the United States ranges from 1 to 8% [1–4]. In neonates weighing less than 1500 grams, the incidence is approximately 7% [4]. Significant morbidity and mortality can result from NEC, with the reported average mortality rate ranging from 20 to 30%, increasing with severity of illness and those requiring surgery [5, 6]. Standard therapeutic approaches to NEC management include

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broad-spectrum antibiotic therapy, bowel rest, use of intravenous fluids (including parenteral nutrition), and nasogastric bowel decompression, termed medical NEC, and surgical removal of the necrotic intestinal tissues in the intestines, known as surgical NEC [1–3]. Medical NEC constitutes approximately 60–70% of NEC cases [6]. Long-term adverse effects resulting from surgical treatment include developmental delays, cognitive delays, parenteral associated liver disease, and short bowel syndrome [4]. With prevention being the ultimate goal, there have been extensive efforts to identify potential modifiable risk factors that could precipitate NEC.

The exact etiology of NEC remains unknown despite considerable research over the past several decades [1–4, 7]. Current evidence has identified both modifiable and non-modifiable risk factors [7]. Non-modifiable risk factors include prematurity, placental abruption, intestinal ischemia related to absent or reversed end diastolic flow velocity, and maternal hypertensive disease of pregnancy. Proposed modifiable risk factors including type of enteral feeding, intestinal bacterial colonization changes, transfusions, and drug-induced intestinal ischemia, continue to be at the forefront of prevention research [3, 7–16]. The use of breast milk has been widely shown to decrease the incidence of NEC, while other feeding-related strategies, including the use of feeding protocols, more aggressive feeding advancement, and avoidance of hypertonic medications and/or solutions (including formulas and fortification agents) have been used despite more limited data [17, 18]. Conflicting evidence also suggests the use of probiotics, immunoglobulins, and nutritional supplements, such as lactoferrin, in the prevention of NEC [19–24]. When examining the role of intestinal ischemia in the development of NEC, various conditions including birth asphyxia, exchange transfusions, and congenital heart diseases have been implicated [3, 7]. Additionally, co-morbidities associated with prematurity may necessitate the use of medications associated with alteration in mesenteric hemodynamics that may lead to intestinal ischemia. Multiple medications, including caffeine, histamine-2 blockers, indomethacin, epinephrine, and maternal use of cocaine or marijuana have been implicated in decreasing mesenteric blood flow, thus precipitating NEC [3, 4, 6–8]. Targeting medications used in the premature population poses one avenue for risk reduction.

Caffeine is traditionally used in neonates to treat apnea of prematurity and prevention of bronchopulmonary dysplasia, respiratory distress syndrome, and extubation failure [25–30]. Currently, caffeine citrate is US Food and Drug Administration approved to treat apnea of prematurity in infants with gestational age of 28–33 weeks [31]. Caffeine citrate is usually administered at a loading dose of 20–25 mg/kg/dose (up to 40 mg/kg/dose) and a maintenance dose of 5–10 mg/kg/dose [30, 31]. Higher doses of 15–30 mg/kg/dose have been studied in intubation reduction, earlier extubation, and prophylaxis against apnea post-extubation [32, 33]. Methylxanthines, including caffeine, have been shown to be associated with decreased cerebral and intestinal blood flow due to vasoconstriction properties, thereby potentiating ischemia [7, 25, 27, 28, 34]. Earlier studies, including *in vitro* data, suggest higher caffeine doses lead to greater decreases in mesenteric blood flow [28, 34–36]. Dose dependent blood flow changes and the correlation with serum caffeine trough levels have not been studied.

Safety and efficacy trials have evaluated both short- and long-term use of caffeine with no reports of NEC. The Caffeine Therapy for Apnea of Prematurity (CAP) trial was the largest study evaluating long-term effects of caffeine on premature infants, but the primary focus was long-term neurodevelopmental outcomes and survival. The CAP trial demonstrated caffeine was associated with improved survival rates in premature infants and that NEC rates did not differ between caffeine or placebo groups [25, 26]. Although no difference was found in these studies, neither NEC nor gastrointestinal adverse events were a primary safety focus. Another study evaluating the association of early caffeine administration and neonatal outcomes in preterm neonates showed a reduction in rates of death, bronchopulmonary dysplasia, and patent ductus arteriosus (PDA) without any adverse impact on NEC [37]. In a recent retrospective case-control study of patients with NEC, Lampkin and colleagues reported that NEC was not associated with cumulative caffeine exposure, although the study used a relatively small sample size and did not control for co-administration of specific confounding medications [38]. This study will investigate the association between dose-dependent exposure to caffeine and development of medical or surgical NEC in a large neonatal population at an academic medical center.

2. Methods

2.1. Study cohort

This study was approved by the Palmetto Health Institutional Review Board. All neonates admitted to Palmetto Health Children's Hospital Level 3 NICU from July 1, 2008 through December 31, 2009 were included. Patients were screened from NICU admissions by utilizing the Pediatrix® database. All patients admitted to the NICU were available for inclusion provided laboratory, radiographic and medication data were available. The medical record number listed in the Pediatrix® database was used to access information. Patients were stratified into two groups based on caffeine administration (yes or no) and each group was assessed for development of NEC according to the following study definition: having received any surgical and/or medical intervention following an ICD-9 code for NEC (777.50, 777.51, 777.52, 777.53), confirmed by positive labs for suspected NEC and Bell Staging criteria [39]. Patients with suspected NEC, but who had no surgical or medical treatment, were included in the group without NEC. Of note, patients with a spontaneous intestinal perforation (SIP) were included in the NEC group only if a diagnosis of NEC was also made. If a patient developed surgical NEC after medical NEC, they were counted only as a surgical case. For patients with NEC, caffeine exposure prior to diagnosis was verified. At our institution, caffeine is used for both prevention and treatment of apnea of prematurity and is not utilized to facilitate extubation or for neuroprotection.

Information obtained from electronic medical records included patient demographics, relevant patient laboratory information, caffeine dosing information, and serum trough concentrations, when available. Serum caffeine concentrations are not performed in-house and thus are not routinely ordered at our institution. Chest/abdominal x-ray transcriptions, concurrent and recent medications, NEC management data (if applicable), and transfer information from other institutions on outborn infants were also collected. To examine the impact of additional confounding factors, information on vasopressor, maternal illicit drug, and medical PDA treatment use was recorded. The presence of hepatic or renal dysfunction and acquired infections was also recorded. Patients with significant missing information were excluded.

2.2. Outcomes

The primary objective of this study was to assess the association between caffeine administration and development of medical or surgical NEC. Secondary objectives include assessing other confounders potentially associated with NEC (use of other vasoconstricting agents, gestational age, birth weight, and maternal illicit drug use), the evaluation of caffeine cumulative dose on the development of NEC, and the time to NEC relative to caffeine administration. Caffeine dosing was initiated at the discretion of the provider and no standard institutional guidelines existed during the study period. Adjustments to dosing throughout therapy would be for clinical efficacy or for weight changes. Cumulative dosing was recorded in milligrams until the time to diagnosis of NEC, discharge, or death.

2.3. Statistical analysis

All statistical analyses were carried out using R (Version 2.12.2). Logistic regression was used to model the probability of developing NEC as a function of gestational age (in weeks), birth weight (in grams), vasopressor use (yes/no), maternal use of cocaine, marijuana, tobacco, or methadone (yes/no), PDA medical treatment (yes/no), and caffeine (yes/no). Cox proportional hazards regression was used to model the time to developing NEC as a function of the same factors. Additional logistic and Cox models were fit to examine the effect of cumulative caffeine dosing. All models were also refit using a high risk subsample (neonates less than or equal to 32 weeks gestation and less than 1500 grams) for each outcome.

3. Results

A total of 615 patients were included in the study. Demographic data are shown in Table 1. The average gestational age was 33.5 weeks and 29.4% had a birth weight less than 1500 grams. One hundred thirty-seven patients (22.3%) received caffeine, 9 of whom were greater than 32 weeks gestation. There were 45 cases (7.3%) of NEC, 14 of which were surgical cases. The average day of life for NEC diagnosis was 27.9 days. Prior to the development of NEC, 77.8% ($n = 35$) received caffeine, 44.4% ($n = 20$) received a vasopressor, and 20 patients (44.4%) received PDA treatment (8 received ibuprofen lysine, 12 received

Table 1
Patient demographics

Characteristic	No caffeine ($n=478$)	Caffeine ($n=137$)
Gestational age, median	35 weeks (range: 22 – 44)	28 weeks (range 23 – 40)
<26 weeks (%)	15 (3.1)	30 (21.9)
26 – 28 weeks (%)	3 (0.6)	53 (38.7)
29 – 31 weeks (%)	43 (9)	45 (32.8)
32 – 35 weeks (%)	191 (40)	7 (5.1)
≥36 weeks (%)	226 (47.3)	2 (1.5)
Birth weight (g), median	2361 (range: 265 – 5775)	1033 (range: 390 – 2858)
<500 (%)	5 (1)	5 (3.6)
500 – 999 (%)	18 (3.8)	61 (44.5)
1000 – 1499 (%)	38 (7.9)	54 (39.4)
1500 – 1999 (%)	100 (20.9)	11 (8)
2000 – 2500 (%)	101 (21.1)	3 (2.2)
>2500 (%)	216 (45.2)	3 (2.2)
Race, *n* (%)		
African – American	237 (49.6)	91 (66.4)
Caucasian	171 (35.8)	39 (28.5)
Hispanic	45 (9.4)	2 (1.5)
Other/unknown	25 (5.2)	5 (3.6)
No. male (%)	222 (46.4)	69 (50.4)
No. outborn (%)	174 (36.4)	28 (20.4)
Maternal drug use, *n* (%)	33 (6.9)	20 (14.6)
Multiple substances, *n* (%)	7 (1.5)	7 (5.1)
Vasopressor use, *n* (%)	40 (8.4)	48 (35)
NSAID use[a], *n* (%)	8 (1.7)	61 (44.5)
Vasopressor + NSAID[a], *n* (%)	5 (1)	35 (25.5)
Medical NEC, *n* (%)	5 (1)	26 (19)
Surgical NEC, *n* (%)	5 (1)	9 (6.6)

[a]NSAID use either ibuprofen lysine or indomethacin.

Table 2
Characteristics of patients administered caffeine

	NEC ($n=35$)	No NEC ($n=102$)
Gestational age in weeks, mean (SD)	26.9 (2.67)	28.2 (2.66)
Birth weight in grams, mean (SD)	918.9 (437.7)	1146.1 (424.2)
Loading dose >25 mg/kg/dose, *n* (%)	6 (17.1)	26 (25.5)
Maintenance dose >10 mg/kg/day, *n* (%)	5 (14.3)	9 (8.8)
Cumulative dose, median (mg/kg)	290	178
Vasopressor use, *n* (%)	13 (37.1)	26 (25.5)
Treatment of PDA[a], *n* (%)	13 (37.1)	39 (38.2)
Vasopressor + PDA treatment [a], *n* (%)	11 (31.4)	20 (19.6)
Surgical NEC, *n* (%)	14 (40)	n/a

[a]Indomethacin or ibuprofen lysine.

indomethacin). Table 2 describes the characteristics of patients who received caffeine. All-cause mortality was 4.6% ($n=28$) for the study cohort. All patients who died ($n=3$) in the NEC group received caffeine.

3.1. Logistic regression results

Table 3 shows regression estimates, *p*-values, and odds ratio estimates from fitting a first-order logistic regression model for the entire sample ($n=615$) and also for the subsample of high risk patients ($n=169$). When a factor is viewed individually, its effect is judged to be statistically significant if its corresponding *p*-value is less than 0.05. To adjust for multiple comparisons using a Bonferroni correction (because the first-order model contains six factor estimates), an effect is judged to be significant if its *p*-value is $<0.05/6 \approx 0.0083$. Standard model adequacy checks did not suggest lack of fit with the first-order model.

The results from the analysis on the entire sample show that the probability of developing NEC is positively related to caffeine ($p=0.008$) and that this effect is significant even after a multiple comparison adjustment. The odds of developing NEC for those receiving caffeine is estimated to be 3.49 times greater than the odds of developing NEC for those not receiving caffeine (individual 95% CI: 1.42–9.23). Two additional factors were found to be individually significant

Table 3
First-order logistic regression results. Regression estimates (p-values) were obtained using the glm function in R. Odds ratios are the exponentiated estimates; 95 percent Wald confidence intervals for the true odds ratios are also given

	Entire sample ($n = 615$)		High risk[a] ($n = 169$)	
Factor	Estimate (p-value)	Odds ratio (95% CI)	Estimate (p-value)	Odds ratio (95% CI)
Gestational age	0.021 (0.823)	1.02 (0.84, 1.21)	0.099 (0.465)	1.10 (0.85, 1.44)
Birth weight	–0.001 (0.014)	0.998 (0.997, 0.999)	–0.002 (0.090)	0.998 (0.996, 1.00)
Vasopressor use				
Yes	0.899 (0.033)	2.45 (1.07, 5.63)	0.938 (0.045)	2.55 (1.03, 6.54)
No		(1.0 reference)		(1.0 reference)
Drug use				
Yes	0.591 (0.223)	1.81 (0.67, 4.54)	0.007 (0.991)	1.01 (0.29, 3.10)
No		1.0 (reference)		(1.0 reference)
PDA				
Yes	0.104 (0.816)	1.11 (0.46, 2.68)	–0.030 (0.950)	0.97 (0.38, 2.48)
No		1.0 (reference)		(1.0 reference)
Caffeine				
Yes	1.249 (0.008)	3.49 (1.42, 9.23)	1.123 (0.047)	3.08 (1.09, 10.32)
No		1.0 (reference)		(1.0 reference)

[a]High risk patients are identified as weighing <1500 g and with gestational age ≤32 weeks.

for the development of NEC: birth weight and vasopressor use. After adjusting for the other factors as confounders, the probability of developing NEC is inversely related with birth weight ($p = 0.014$) and positively related to vasopressor use ($p = 0.033$). The effects due to gestational age, drug use, and PDA were not statistically significant.

When considering the high risk subsample (infants less than 1500 grams and gestational age 32 weeks or less; $n = 169$), the logistic regression analysis shows that the effect of caffeine is still significant ($p = 0.047$) although not after a multiple comparisons adjustment. The identical conclusion is reached regarding vasopressor use. No other covariate effects are significant in explaining the probability of developing NEC for the high risk subsample.

3.2. Time-to-event analysis results

Cox proportional hazards regression was used to estimate the time to developing NEC with the same factors listed in Table 3 for both the entire sample and the high risk subsample. For the entire sample, birth weight was nearly significant ($p = 0.074$) and vasopressor use was significant ($p = 0.005$) even after adjusting for multiple comparisons. It is worth noting that caffeine was not significant ($p = 0.129$); i.e., administering caffeine did not significantly impact the time to developing NEC. Additionally, gestational age, drug use, and PDA treatment were not statistically significant. For the high risk subsample, vasopressor use remains statistically significant ($p = 0.015$), but not after adjustment, and the effect of caffeine is not significant ($p = 0.234$).

3.3. Dosing analysis

Cumulative dose was not significant in either the logistic regression analysis ($p = 0.25$) or the time-to-event analysis ($p = 0.659$). For patients receiving a loading dose of ≥25 mg/kg/dose ($n = 38$), 17% developed NEC. Twenty-nine percent of the patients receiving <25 mg/kg/dose ($n = 90$) developed NEC. Nine patients received no loading dose and three developed NEC. Fourteen percent of patients receiving maintenance doses ≥10 mg/kg/day ($n = 27$) developed NEC, and 27% developed NEC in the <10 mg/kg/day group ($n = 105$). Table 4 details caffeine loading and maintenance doses.

Table 4
Caffeine dosing

Loading doses (mg/kg)[a]	No. of patients ($n = 128$)
5 to 10	1
10 to 19.9	11
20 to 24.9	78
25 to 30	33
Greater than 30	5
Maintenance dose average over course[b] *(mg/kg)*	No. of patients ($n = 132$)
1 to 5	9
5 to 9.9	96
10 to 14.9	21
15 to 20	6

[a]Nine patients were not administered a loading dose. [b]Five patients were not administered a maintenance dose; dose was averaged over course of therapy.

4. Discussion

This represents one of the largest studies assessing the association between caffeine administration and development of NEC. Although studies have evaluated both short- and long-term sequelae of caffeine use for apnea of prematurity, they lack reporting of gastrointestinal effects (e.g. NEC), and primarily describe neurodevelopmental outcomes [25, 26]. Pharmacodynamic studies, however, have shown caffeine to have significant effects on cerebral and intestinal blood flow velocities (BFV). It has been postulated that both endothelium-dependent and endothelium-independent vasoconstriction allow for caffeine's reduction in intestinal blood flow and ischemia, ultimately increasing the risk of NEC [35, 36].

Our study is among the first to show a clinical correlation with caffeine and the development of NEC [28, 34, 35]. In the entire sample of patients, the odds of developing either medical or surgical NEC was nearly 3.5 times greater for those receiving caffeine, and it was nearly 3.1 times greater when considering the high-risk subgroup. Patients outside the 'high-risk' group of less than 1500 grams and a gestational age less than or equal to 32 weeks were included to assess the relationship between caffeine and NEC in what may be considered to be a low- to moderate-risk population [8]. Since limited data exist evaluating the development of NEC in this low- to moderate-risk group, identification of potential risk factors for this population could lead to more prompt identification and treatment of NEC and ultimately prevent more devastating clinical outcomes.

Both Lane and Hoeker and colleagues describe dose-dependent changes in mesenteric blood flow following administration of a caffeine loading dose [34, 35]. These data suggest dividing the loading dose as an alternative dosing strategy, causing a decrease in cerebral blood flow without a significant change in intestinal blood flow and left ventricular output [34, 35]. To our knowledge, no data exist describing the relationship between caffeine maintenance dosing and development of NEC. Results of our study cannot provide further implications of caffeine loading or maintenance dosing in the development of NEC. Our study did evaluate the effect of cumulative dose on the development of NEC, but it was not found to be significant, a result also observed in Lampkin et al. [38]. At our institution, intravenous caffeine loading doses are administered over 30 minutes and maintenance doses over 10 minutes. During the study period, Alaris® (Carefusion Corporation) 'smart pump' technology was implemented, and the infusion time prior to pump technology could not be verified. Like dosing, infusion time of caffeine may play a role in acute changes in BFV and deserves further investigation.

The utility of therapeutic drug monitoring (TDM) of caffeine in preterm neonates with apnea of prematurity has also been debated. When evaluating TDM in our patients, five patients had a serum level of >20 mg/L. Of these, 60% developed NEC, but it should be noted that these patients were on varying doses, ranging from 5 to 20 mg/kg/dose. Natarajan and colleagues found patients had comparable serum concentrations whether obtained for lack of efficacy or routine monitoring [40].

Another mechanism for caffeine's potential association with NEC may result from recent findings of its impairment on gastrointestinal function, including decreases in muscle tone, gastric and intestinal muscle contractions, and gastric emptying time [41]. While feeding intolerance would not directly impact mesenteric blood flow, a delay in feeding and inability to provide gastric nutrition can impair overall intestinal function, nutrition, and require the use of gut pH-altering medications, each potentially increasing the risk of NEC. The use of concomitant pH-altering medications and the relationship of caffeine and feeding may provide an interesting area for future research.

Other medications, including the vasopressors epinephrine and dopamine, impact intestinal blood flow in neonates potentially increasing the risk of NEC [42]. Many premature neonates have immature development of the enzymes protecting the mucosal layer, and when combined with medication-induced decreases in intestinal blood flow, significant intestinal ischemia may occur. Anecdotally, a large number (31%) of patients who received caffeine and developed NEC were also on other medications that potentially cause intestinal vasoconstriction (e.g. epinephrine). For the entire sample, the odds of developing NEC was 2.45 times greater for patients who were exposed to vasopressors when compared to those who were not exposed ($p = 0.033$). Of the 14 patients administered caffeine who developed surgical NEC, approximately 43% ($n = 6$) were also on vasopressors prior to diagnosis. Decreases in blood pressure in the absence of adequate vasopressor treatment may also worsen intestinal blood flow, thus increasing the risk of NEC [42, 43]. For this reason, prudent attention to balancing clinical status with additional medication-related

risks is warranted. Regarding these patients as having an increased risk of NEC may lead to a lower threshold for initiating NEC treatment, and ultimately benefit this population.

The use of NSAIDs has also been implicated in the development of NEC and is more likely to be agent specific [44, 45]. Like caffeine studies, a significant change in mesenteric artery BFV occurs with rapid administration (infused over 20 to 35 minutes) of indomethacin [46, 47]. Ibuprofen did not cause a significant change in BFV [46]. In the present study, the use of either indomethacin or ibuprofen lysine was not significant ($p = 0.816$). During the study period, indomethacin was utilized for intraventricular hemorrhage prevention per our institution protocol, and providers selected either ibuprofen or indomethacin for PDA closure. Of note, the default infusion time of indomethacin at our institution is one hour. Although mixed, the literature has shown an increase in NEC with indomethacin use, while spontaneous intestinal perforations are more closely associated with ibuprofen lysine use [44, 45].

While our analysis included both indomethacin and ibuprofen lysine for PDA closure ($n = 69$), only 6 patients received both due to PDA closure failure, and 41 (59.4%) patients received indomethacin only. These data support close observation of patients receiving caffeine and other vasoconstricting agents, potentially lowering the threshold for aggressive management of NEC for these higher-risk patients.

Until recently, the investigation of medications as NEC risk factors has been limited. A newly proposed NEC risk assessment tool, GutCheck^{NEC}, created by Gephart and colleagues, does account for both vasopressor and indomethacin use in infants weighing less than 1500 grams, but it does not include other vasoactive medications including caffeine [8]. More studies are needed to confirm caffeine's additive role in this NEC risk assessment.

Limitations to our study include its retrospective nature which makes it difficult to detect other potential confounders that may have increased the prevalence of NEC in the caffeine group. For example, nutrition is an important factor that has been studied particularly in the prevention of NEC. Current literature supports the use of a standardized approach, particularly initiation with breast milk versus formula results in decreased risk of NEC [48]. Because nutrition status changed throughout the patient's stay and practice varied among the providers, the study investigators chose ultimately not to include nutrition in the analysis. Also, during the study period, our hospital began utilization of donor milk for qualifying patients. Many of the patients developing NEC and/or receiving caffeine could have been influenced by the change in nutrition protocol. Additionally, the absence of transfusion data is a limitation of this study. Because many of our infants required vasopressors, there is increased likelihood they also required transfusions. However, several recent studies have suggested an absence of increased NEC risk with transfusions [49, 50]. The lack of caffeine TDM in our patient population may also be a limitation; however, the measurement of serum caffeine concentrations has not been shown to correlate with clinical efficacy or toxicity [40].

In conclusion, there is a potential association between administration of caffeine and the development of medical or surgical NEC in premature infants. Despite the limitations of this study, our results do support the addition of caffeine into considerations for NEC risk, especially in combination with other medications or factors that cause vasoconstriction. Future studies should target cumulative risk evaluation of concomitant host and medication factors to confirm these results.

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Conflict of interest

The authors declare no conflict of interest.

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