Routine Supplementation of *Lactobacillus rhamnosus* GG and Risk of Necrotizing Enterocolitis in Very Low Birth Weight Infants

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Objective To evaluate if routine supplementation of *Lactobacillus rhamnosus* GG ATCC 53103 (LGG) is associated with a decreased risk of necrotizing enterocolitis in very low birth weight (VLBW) infants.

Study design Retrospective observational cohort study of VLBW (<1500 g) infants at a single center from 2008 to 2016. LGG supplementation with Culturelle at a dose of 2.5 to 5 × 10^9 CFU/day began in 2014. We used multivariable logistic regression to evaluate the association between LGG supplementation and necrotizing enterocolitis (modified Bell stage IIA or greater), after adjusting for potential confounders. We also compared changes in necrotizing enterocolitis incidence before and after implementation of LGG using a statistical process control chart.

Results We evaluated 640 VLBW infants with a median gestational age of 28.7 weeks (IQR 26.3-30.6); 78 (12%) developed necrotizing enterocolitis. The median age at first dose of LGG was 6 days (IQR 3-10), and duration of supplementation was 32 days (IQR 18-45). The incidence of necrotizing enterocolitis in the epoch before LGG implementation was 10.2% compared with 16.8% after implementation. In multivariable analysis, LGG supplementation was associated with a higher risk of necrotizing enterocolitis (aOR 2.10, 95 % CI 1.25-3.54, $P = .005$). We found no special cause variation in necrotizing enterocolitis after implementation of LGG supplementation. There were no episodes of *Lactobacillus* sepsis during 5558 infant days of LGG supplementation.

Conclusions In this study, routine LGG supplementation was not associated with a decreased risk of necrotizing enterocolitis. Our findings do not support the use of the most common probiotic preparation currently supplemented to VLBW infants in the US. *(J Pediatr 2018;195:73-9).*

Necrotizing enterocolitis is a major cause of morbidity and mortality in infants born prematurely.[1-3] Between 4% and 7% of very low birth weight (VLBW, <1500 g at birth) infants will develop necrotizing enterocolitis[4] and 15% to 30% of VLBW infants with necrotizing enterocolitis will not survive.[1] Multiple randomized trials have studied the use of probiotics to prevent necrotizing enterocolitis with a variety of probiotic products. A meta-analysis of 25 trials, including 6587 VLBW infants, demonstrate probiotics reduce both severe necrotizing enterocolitis (pooled relative risk [RR] 0.47; 95% CI 0.36-0.61) and all-cause mortality (RR 0.74; 95% CI 0.61-0.90).[5] Despite the heterogeneity in preparations used in these trials, this meta-analysis did not demonstrate a difference in treatment effect for necrotizing enterocolitis by various species of probiotics, including *Lactobacillus, Bifidobacterium,* or multispecies products.

In a phone survey of neonatal intensive care units (NICUs) in the US, 14% of NICUs reported supplementing probiotics to VLBW infants, of which *Lactobacillus rhamnosus* GG (LGG) in the form of Culturelle was the most commonly used product.[6] However, randomized trials demonstrating the effectiveness of this probiotic product in decreasing the risk of necrotizing enterocolitis are lacking. In addition, there is uncertainty as to the appropriate dose and optimal timing of administration of probiotics. Implementation studies may provide data on the treatment effects of specific probiotic products in routine practice. We examined the association of routine supplementation with LGG and the risk of necrotizing enterocolitis in VLBW infants at a single center. We hypothesized that VLBW infants supplemented with LGG would have a lower risk of necrotizing enterocolitis compared with nonsupplemented infants.

Methods

We conducted this retrospective observational cohort study at a single, academically affiliated level III neonatal intensive care unit in Atlanta, Georgia (Emory

LGG	*Lactobacillus rhamnosus* GG
VLBW	Very low birth weight
NICUs	Neonatal intensive care units

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University Hospital Midtown). We included all consecutively admitted infants with a birth weight <1500 g who were admitted to the NICU between August 1, 2008 and July 31, 2016. We excluded infants with major congenital anomalies, those with a length of stay ≤3 days or those admitted after 1 week of age, as they would not have been eligible to initiate probiotic supplementation within the first week of life. We reviewed routinely collected clinical data, including physician and nursing documentation, laboratory results from hematology and microbiology, pediatric radiologists' interpretations of radiographic studies, and the medication administration record. The study was reported according to the Strengthening the Reporting of Observational studies in Epidemiology statement.[7] This study was approved by the Emory University Institutional Review Board.

Definitions

The primary exposure was LGG supplementation, which we defined as the receipt of at least a single dose of LGG. LGG supplementation was implemented in February of 2014 through a standard protocol. LGG was supplemented once daily at a dose of 2.5×10^9 colony forming units per day and then increased to 5×10^9 colony forming units per day once feedings were advanced using a single sachet of LGG powder (Culturelle, i-Health, Cromwell, Conneticut). For infants feeding 1-2 mL every 3 hours (or an equivalent hourly volume), LGG was mixed in sterile water. For infants feeding 3 mL every 3 hours or greater (or an equivalent hourly volume), LGG was mixed in either breast milk or formula. Supplementation was initiated once an infant was tolerating enteral feeding and continued until 35 weeks postmenstrual age. The primary outcome was necrotizing enterocolitis, defined as modified Bell stage IIA or greater.[8] Isolated pneumoperitoneum without other radiographic or clinical evidence of necrotizing enterocolitis was considered to be a spontaneous intestinal perforation and not necrotizing enterocolitis. The modified Bell staging of all cases of necrotizing enterocolitis were adjudicated by the study team through unblinded review of clinical notes and abdominal radiograph reports interpreted by pediatric radiologists to identify staging criteria. Infants with possible necrotizing enterocolitis who underwent staging were identified through 1 of 3 methods: (1) Identifying all infants with necrotizing enterocolitis listed as a diagnosis (eg, *International Classification of Diseases, Ninth Revision*); (2) Concern for necrotizing enterocolitis noted anywhere in the summary of the infant's hospitalization; and (3) review of all abdominal radiographs taken for each infant. If there was uncertainty regarding the staging, the study team reviewed the cases to reach consensus. Staging and adjudication was performed before statistical analysis and radiographic characteristics of staged cases were summarized. We specified denominators to indicate any missing data; no imputation was performed. We ascertained race and ethnicity based on documentation in the medical record. We defined small for gestational age as birth weight <10% percentile for gestational age using published sex-specific intrauterine growth curves.[9] We defined indomethacin prophylaxis as receipt of indomethacin within 1 day of birth.

Statistical Analyses

We used SPSS v 23 (IBM, Armonk, New York) for all statistical analysis. We acquired data and performed statistical analysis from September 16, 2015 to October 4, 2017. We compared baseline maternal and neonatal characteristics between infants exposed and unexposed to LGG and with and without necrotizing enterocolitis. We described continuous variables using medians with IQRs reported as 25th-75th percentiles with comparisons using a Wilcoxon rank-sum test. We compared categorical variables using χ^2 or Fisher exact tests.

For the primary analysis, we evaluated the association between exposure to LGG and the risk of necrotizing enterocolitis using multivariable logistic regression. We evaluated for potential confounding from differences in case-mix over time by including variables in the model based on available knowledge[10] or variables associated with necrotizing enterocolitis in bivariable analysis at $P < .1$ with either the exposure or outcome. We included only exposures that occurred before the onset of necrotizing enterocolitis in multivariable models. We retained confounders for inclusion in the model by determining the change in the estimate of the association between LGG and necrotizing enterocolitis between full and reduced models with and without the potential confounder of interest. Collinearity was assessed using correlation matrices, and variables with high collinearity (eg, birth weight and gestational age) were not included in the models. We adjusted for gestational age, small for gestational age, multiple gestation, prolonged rupture of membranes >18 hours, receipt of initial empiric antibiotics, and receipt of indomethacin prophylaxis. Additional variables that were individually evaluated but not included in the final model because they were not associated with necrotizing enterocolitis or inclusion did not change the point estimate of the association between LGG and necrotizing enterocolitis by more than 10% included: maternal race, maternal age, maternal receipt of tocolytic therapy, maternal receipt of antibiotics, maternal receipt of antenatal steroids, Apgar at 1 minute, Apgar at 5 minutes, receipt of initial empiric antibiotics for >2 days, receipt of inotropes, age at first feed, admission hemoglobin, and lowest hemoglobin in first month. We did not adjust for human milk feeding given the overall high number of infants receiving any human milk (ie, small number of unexposed). No tests for interaction were performed given the relatively low number of outcome events.

We performed four sensitivity analyses: (1) including only baseline covariates with complete data in the multivariable model to limit the effect of covariates with missing data; (2) evaluating necrotizing enterocolitis or death as a composite outcome to account for the competing outcome of death; (3) restricting analysis to a more contemporaneous cohort of infants born from 2011 onward to limit the effect of changes in practice over time; and (4) comparing infant characteristics and the incidence of necrotizing enterocolitis between the pre- and post-LGG implementation epochs and assess-