Journal of Pediatric Surgery 52 (2017) 993–998

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.elsevier.com/locate/jpedsurg

Basic Science Papers

Independence of gut bacterial content and neonatal necrotizing enterocolitis severity

Lauren K. Barron [a], Barbara B. Warner [b], Phillip I. Tarr [c], William D. Shannon [d], Elena Deych [d], Brad W. Warner [a,*]

[a] Division of Pediatric Surgery, Department of Surgery, Washington University in St. Louis School of Medicine, St. Louis Children's Hospital, St. Louis, MO, USA
[b] Division of Newborn Medicine, Department of Pediatrics, Washington University School of Medicine in St Louis, St Louis, MO, USA
[c] Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, Washington University School of Medicine in St Louis, St Louis, MO, USA
[d] Department of Medicine, Washington University School of Medicine in St Louis, St Louis, MO, USA

ARTICLE INFO

Article history:
Received 16 February 2017
Accepted 9 March 2017

Key words:
Necrotizing enterocolitis
Microbiome
Small bowel resection

ABSTRACT

Introduction: Necrotizing enterocolitis (NEC) is a common and devastating gastrointestinal disease of premature infants. NEC severity varies widely. Recent data have demonstrated a strong link between gut microbial dysbiosis and development of NEC. We tested the hypothesis that alterations in the gut microbiome at the time of diagnosis predict the severity of NEC.
Methods: We used prospectively collected fecal samples from very low birth weight infants who developed NEC, stratifying by NEC severity. Fecal bacterial DNA was sequenced using 16S rRNA pyrosequencing. A generalized Wald-type test based on the Dirichlet multinomial distribution was used to test for differences in microbial communities.
Results: Of 489 infants at risk, 30 NEC cases had 410 fecal samples collected in the 28 days prior to the onset of NEC available for analysis. There were no differences in the pre-NEC gut microbial community between infants treated medically vs. surgically, or those with NEC totalis. Furthermore, neither treatment of NEC significantly changed the gut microbiome post-NEC among the survivors.
Conclusion: We found no evidence that the gut microbiome, prior to the onset of disease, differentiates the clinical course of NEC. These data suggest that factors other than the gut microbiome may dictate disease severity.
Level of evidence: Level 4.

Necrotizing enterocolitis (NEC) is a catastrophic gastrointestinal disease of the newborn affecting 4–13% of premature very low birth weight (VLBW) infants (<1500 g). NEC often results in perforation and irreversible bowel injury requiring resection [1,2]. The overall mortality from NEC remains 20–30%, and disease severity varies from a single segment or discrete segments of bowel, to panintestinal with the preponderance of the bowel being nonviable (NEC totalis) [3]. The management of NEC is guided by multiple factors, but includes Bell's staging [4], infant weight, and clinical condition. Less severe NEC is initially treated medically, while advanced cases require surgical intervention consisting of either percutaneous peritoneal drain (PPD) and/or laparotomy and bowel resection. Mortality in the subset of patients that undergoes surgery ranges from 40 to 60%, decreases inversely with birth weight, and varies with procedure [5]. NEC is notorious for its swift progression and clinicians are often challenged by the minimal time from diagnosis to death [6]. Although many characteristics have been associated with increased risk of developing NEC, no single factor predicts the clinical course of established NEC.

Literature over the past 30 years has articulated a significant role for the gut microbiota in the pathogenesis of NEC [7]. We have previously established an orderly progression of preterm infant gut microbial maturation with changes occurring at predictable postmenstrual ages [8], and subsequently, that NEC is preceded by gut dysbiosis with an increase in Gammaproteobacteria and decreases in Negativicutes and Clostridia [9]. Knowing the orderly progression of the healthy microbiome as well as the dysbiosis that precede NEC, we sought to test the hypothesis that these pre-NEC microbiome changes would predict disease severity, and secondarily, be associated with the effect of medical and surgical treatment on the recovery of the gut microbiome.

1. Methods

1.1. Patient selection

Infants admitted to the St. Louis Children's Hospital NICU between July 2009 and September 2013 weighing ≤1500 g at birth with a predicted survival of >1 week were eligible for inclusion. All samples were collected to 36 weeks postmenstrual age, and frozen (−80 °C) until analyzed, as detailed previously [9]. The study was approved by the Washington University Institutional Review Board (#201503102)

* Corresponding author at: Division of Pediatric Surgery, St. Louis Children's Hospital, One Children's Place Suite 5S40, St. Louis, MO, 63110. Tel.: +1 314 454 6022; fax: +1 314 454 2442.
E-mail address: Brad.Warner@wustl.edu (B.W. Warner).

http://dx.doi.org/10.1016/j.jpedsurg.2017.03.029
0022-3468/

and written consent was obtained prior to processing any of the collected fecal samples of enrolled infants.

Cases were defined as infants whose clinical course and radiographs fulfilled Bell's stage 2 or 3 criteria. Medical NEC was defined as nonsurgical treatment of NEC, in a child who survived >24 h after diagnosis. Surgically treated NEC was defined as infants who underwent laparotomy and resection or peritoneal drain placement with or without subsequent resection. NEC totalis was defined as infants with extensive bowel involvement based on direct visualization at the time of laparotomy or autopsy or infants who died within 24 h of diagnosis before an operation could take place.

For the pre-NEC analysis, samples were selected from 28 days prior to the development of NEC until the day before NEC diagnosis. The week of NEC is designated as week zero and includes samples between seven and one day prior to NEC, samples from days 8 to 14 prior to NEC onset are designated as originating in week −1, and samples from days 15 to 21 prior to NEC onset are designated as originating in week −2, and samples from days 22 to 28 prior to NEC are designated as origination in week –3. If a patient produced more than one specimen within an interval, proportions were collapsed into composite values for that individual for that week. Intervals with <4 subjects having adequate samples were excluded from analysis.

For the post-NEC analysis, we first sought to examine the effect of NEC on the gut microbiota over time. We compared samples from a 14-day interval before diagnosis to a 14-day interval after diagnosis in the same infant. Next, we sought to compare the effect of NEC on the gut microbiota between medically and surgically treated cases, compared to non-NEC controls matched on gestational age (+/− one week) and birth weight (+/−100 g). Comparisons were made in five 15-day intervals based on day of life (DOL) beginning at birth and continuing to DOL 75 or discharge home, whichever came first. Multiple samples from individual subjects were again collapsed into composite values and time intervals with <4 subjects excluded.

1.2. DNA extraction and sequencing

Fecal DNA was extracted using the Qiagen (Hilden, Germany) DNA Stool Mini kit and the automated QIAcube (Qiagen). Sequencing of the bacterial 16S rRNA was performed using amplicons specific to the V3–5 regions at the McDonnell Genome Institute at Washington University. Details regarding methods for DNA sequencing, library construction, and taxonomic grouping have been previously published [9].

1.3. Statistical analysis

Sequences results were analyzed using the generalized Wald-type test based on the Dirichlet multinomial distribution to determine if microbial communities differed between cohorts [10]. Briefly, seven-day intervals were created with day zero being the day of diagnosis and progressing before or after NEC depending on analysis. Bacterial populations at the class level were averaged for all samples collected from each subject during an interval. For cases, samples were included up to the day before NEC diagnosis. If a subject had no sample (or inadequate reads in a sample), no data were entered for that sample.

For demographic comparisons we used X^2 and Fisher's exact to compare categorical variables and Student's t-test and one-way analysis of variance (ANOVA) for continuous variables. Significance was assigned to p values less than 0.05.

2. Results

2.1. Pre NEC analysis

Of 489 infants at risk, 35 developed NEC and 30 of these had sufficient samples for inclusion. No significant differences were evident between the medically treated, surgically treated and the NEC totalis groups with respect to demographics (race, gender), clinical characteristics (gestational age birth weight, APGAR scores, mode of delivery or singleton pregnancy) or age of NEC onset (Table 1).

From these subjects, a total of 410 pre-NEC fecal samples from the 28-day period prior to NEC were used for comparing the microbial community of medical, surgical, and NEC totalis patients. There were 108 samples from infants with medically treated NEC (7–10 infants per analysis interval), 226 samples from infants with surgically treated NEC (6–13 infants per analysis interval), and 150 samples from infants with NEC totalis (6–7 infants per analysis interval).

No statically significant differences in the gut microbiome were found between infants with medically treated NEC, surgically treated NEC and those with NEC totalis at any point during the four week total analysis interval prior to NEC diagnosis (Fig. 1). As previously described, Gammaproteobacteria remained the predominant class at all time points [8]. Likewise, no relationship was evident between the gut microbial community structure and NEC mortality, with no statistical differences between survivors (combined medical and surgical survivors), and those who died from NEC totalis (data not shown).

2.2. Post-NEC analysis

To examine the impact of NEC and its treatment on each subject's gut microbial community we compared samples collected before NEC to samples collected after NEC from the same infant, among the 10 subjects who survived their episode of NEC and who had sufficient pre- and post-NEC samples for study. To account for the patterned progression of community assembly over time that naturally occurs in preterm infants,

Table 1
Characteristics of Infants With NEC Included in Pre-NEC Analyses.

	Surgical (n = 13)	Medical (n = 10)	Totalis (n = 7)	p-value
Gestational age	26.1 (24–30)	26.7 (22–33)	25.7 (23–28)	0.635
Age at NEC onset (in days)	30.9 (16–59)	31.1 (13–61)	31.7 (20–52)	0.994
Birthweight	796 (570–1010)	934 (560–1455)	820 (540–1160)	0.339
APGAR at 1 m	3.8 (0.76)	2.4 (0.65)	3.9 (0.96)	0.362
APGAR at 5 m	5.4 (0.65)	5.1 (0.67))	5.4 (0.80)	0.938
Race				
Black	10 (77%)	8 (80%)	5 (71%)	0.919
White	3 (23%)	2 (20%)	2 (29%)	0.919
Gender				
Boys	8 (62%)	7 (70%)	4 (60%)	0.850
Girls	5 (38%)	3 (30%)	3 (40%)	
Multiples	4 (31%)	2 (20%)	1 (14%)	0.676
Caesarean section	10 (77%)	9 (90%)	3 (43%)	0.089
Length of bowel resected (cm)	16.9 (3–50)	n/a	n/a	
Mortality	2 (15%)	1 (10%)	7 (100%)	0.70*

Data are presented as mean (min–max), (SEM) or n (%) *medical vs. surgical.