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Serum Unconjugated Bile Acids and Small bowel bacterial overgrowth in Pediatric Intestinal failure: A Pilot Study

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Abstract

OBJECTIVE—We determined qualitative and quantitative serum unconjugated bile acid (SUBA) levels among children with history of intestinal failure (IF) and suspected small bowel bacterial overgrowth (SBBO).

METHODS—This was a single center case-control pilot study conducted at Cincinnati Children's Hospital Medical Center. Children with history of IF and suspected SBBO were enrolled as subjects. Age matched children without IF or suspected SBBO served as controls. All participants underwent small bowel fluid sampling for microbial culture analysis. Additionally, serum fractionated and total bile acids were measured by liquid chromatography-mass spectrometry (LC-MS) at enrollment and following treatment for SBBO.

RESULTS—SUBA concentrations were elevated in IF subjects (1.16 μ M, range 0.43-10.65) compared to controls (0.10 μ M, range 0.05-0.18), $p=0.001$. Among SUBA, chenodeoxycholic acid (CDCA) was significantly elevated in subjects (0.8 μ M, range 0-7.08) compared to controls (0 μ M, range 0-0.03), $p=0.012$. When controls were excluded from analysis, IF subjects with positive aspirates for SBBO demonstrated higher concentration of CDCA (median 7.36 μ M, range 1.1-8.28) compared to IF subjects with negative aspirates (median 0.18 μ M, range 0-1.06), $p=0.017$. Treatment for SBBO did not alter SUBA concentration.

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CONCLUSIONS—SUBA concentrations are elevated in children with history of IF and presumed SBBO compared to non-IF controls. CDCA was more prevalent in IF subjects with positive aspirates for SBBO compared to IF subjects with negative aspirates. The determination of SUBA concentration may be a useful surrogate to small bowel fluid aspiration in the diagnosis of SBBO in children with history of IF.

Keywords

bile acids; bacteria; overgrowth; short gut; intestinal failure; pediatric

INTRODUCTION

Small bowel bacterial overgrowth (SBBO), defined as the presence of greater than 10^5 colony forming units (cfu) of colonic type bacterial flora per milliliter (mL) of sampled duodenal fluid¹, is commonly encountered in children with short bowel syndrome and intestinal failure.^{2,3} SBBO can lead to diarrhea, abdominal distension, feeding intolerance, nutrient malabsorption, D-lactic acidosis, elevated serum ethanol levels, and/or hyperammonemia.^{4–6} Furthermore, SBBO may increase the risk of central line infections.^{7,8}

Currently, the “gold standard” for the diagnosis of SBBO is quantification of colony forming units present in a duodenal aspirate. This method of diagnosis is invasive, expensive and requires sedation or general anesthesia with pediatric endoscopy, rendering this diagnostic tool clinically impractical. Other, less invasive methods of diagnosing SBBO such as breath tests and urine tests have varying sensitivity, specificity, and lack standardization making them problematic.^{1,9} As a result, the accurate diagnosis and management of SBBO in infants and children remains a challenge.

Serum unconjugated bile acid (SUBA) measurement may provide an alternative method to diagnose SBBO in children with IF since overgrowth leads to deconjugation of bile acids in the proximal intestine with reabsorption and appearance of unconjugated bile acids in the peripheral circulation.^{10–12} Although elevated SUBA have been well-described in adults with SBBO^{13–17}, we have yet to identify any studies demonstrating the diagnostic value and clinical utility of this method in the pediatric population. This pilot study was designed to determine if SUBA concentrations in children with a history of IF suspected of having SBBO (study subjects) were increased compared to healthy controls (age matched children without IF or symptoms suggestive of SBBO). We also sought to determine if antimicrobial treatment of SBBO would impact SUBA concentrations in children with history of IF.

MATERIALS AND METHODS

Enrollment

This was a prospective case-control pilot study performed at Cincinnati Children’s Hospital Medical Center (CCHMC) between October 2014 and August 2015. After obtaining informed consent from parents/guardians, IF subjects and controls between the ages of nine months to 17 years were enrolled in the study. Additional assent was required from IF subjects/controls above age 11 years per institutional review board guidelines. Inclusion

criteria for IF subjects were as follows: 1) Planned upper endoscopy for clinical indications (i.e. abdominal pain, dysphagia, vomiting, and eosinophilic esophagitis), 2) history of extensive intestinal resection resulting in impaired nutrient absorption and a need for either parenteral or supplementary non-oral enteric feeding, and 3) clinical suspicion for SBBO defined only by the presence of symptoms (not quantified) exceeding subject's baseline (abdominal pain, abdominal distension, feeding intolerance, excessive stooling, excessive ostomy output, emesis). Exclusion criteria included: 1) current febrile illness, 2) antibiotic use within 14 days of enrollment, 3) cholestatic liver disease (direct bilirubin > 1 mg/dL), 4) history of organ transplantation, or 5) parent refusal. Control group inclusion criteria included: 1) Planned upper endoscopy for diagnostic evaluation and 2) age matched to within six months of study subjects. Exclusion criteria included: 1) current febrile illness, 2) antibiotic use within 14 days of enrollment, 3) history of intestinal resection, 4) current diarrheal illness, 5) diagnosis of irritable bowel syndrome, 6) presence of any chronic medical condition, 7) history of parenteral nutrition requirement, and 8) parent refusal.

Procedures

Enrolled IF subjects and controls underwent the following research procedures: small bowel aspirate and fasting blood collections. All sample collection was performed on the day of scheduled upper endoscopy. IF subjects found to have SBBO by small bowel aspirate ($>10^5$ colony forming units/milliliter of cultured fluid (cfu/mL)) had a repeat blood sample collected within one week after completion of antimicrobial treatment for SBBO during the study period. Antimicrobial treatment was not controlled for in this study with agent, dose and frequency determined by primary provider. No further research participation was required for controls. For all IF subjects, a thorough chart review was performed to collect the following data: age (months), diagnoses, intestinal length (cm), medications, antibiotic exposures, probiotic exposures, recent lab results (six months), and feeding modality (enteral vs parenteral). Additionally, all IF subjects and controls were assessed for presence of symptoms on the day of procedures and following treatment (in IF subjects) including diarrhea, ostomy output, abdominal distension, feeding intolerance, fussiness, abdominal pain, emesis, fever, and flatulence.

Intestinal Aspirate

Aspirates were collected in the following manner after: 1) endoscope was positioned in the distal duodenum (second, third portion), a sterile catheter was passed via endoscope with subsequent intraluminal aspiration of two milliliters of fluid by hand with care being taken to avoid aeration of the sample. The fluid sample was analyzed for pH (acidic fluid samples, pH <7, may inhibit growth of bacterial cultures). Fluid samples with pH ≥ 7 were considered adequate and then placed in two separate sterile 1.5 mL micro centrifuge tubes for analysis in the CLIA-certified CCHMC Microbiology Laboratory. Fluid samples were cultured for aerobic and anaerobic bacteria and colony counts. Positive aspirates for small bowel bacterial overgrowth were defined by colony counts of $> 10^5$ cfu/mL. Identification of predominant genera and species of bacteria was performed on all positive aspirates using matrix-assisted laser desorption ionization-time of flight mass spectrometer (MALDI-ToF).

Serum bile acid measurement

Serum collected after induction of anesthesia was analyzed for total bile acid concentration, qualitative distribution of 15 different unconjugated and conjugated bile acids using a validated LC-MS/MS method.¹⁸ Proportion measurements for SUBA and CDCA were taken as a percent of total bile acid concentration measured in each research participant. Serum was analyzed from subjects with IF and confirmed SBBO within one week of completion of an antimicrobial course. All samples were collected following a minimum of a four hour fast.

DATA ANALYSIS

The study was powered to determine differences in total SUBA between IF subjects with SBBO and controls. Power analysis for a two-group independent sample t-test performed prior to study initiation indicated that a sample size of eight in each group would be sufficient to attain a power of 0.8 with an alpha of 5% to detect an expected difference in total SUBA concentrations between controls and IF subjects with SBBO (control mean of 1.2 $\mu\text{mol/L}$ vs. subject mean of 10 $\mu\text{mol/L}$) based upon previous studies in adults.¹⁴ Therefore, a sample size of 10 in each group was selected to determine correlation between study variables. Variables analyzed included total SUBA concentrations (μM), total bile acid concentrations, individual conjugated and unconjugated bile acid concentrations. Statistical analysis was performed using SAS® software (SAS Institute Inc. 2013. Version 9.4. Cary, NC). Exact Wilcoxon two-sample test was used to compare control versus cases. To compare outcomes pre- and post treatment and between age-matched cases and controls, Wilcoxon Rank Sum test was used. Statistical significance was based on a p value of <0.05 .

RESULTS

Demographics

Fourteen subjects with history of IF were enrolled in the study between October 2014 and August 2015. Table 1. The median age was 57 months. The most common diagnoses for IF among subjects at study enrollment were complicated gastroschisis and congenital intestinal atresias (57%). Other diagnoses included necrotizing enterocolitis, cloacal extrophy (OEIS syndrome), midgut volvulus and Hirschsprung's disease. With respect to intestinal anatomy, the majority of IF subjects had a history of both small and large bowel resections (8 of 14). Additionally, the small and large intestine were in continuity in the majority (12 of 14). The ileocecal valve (ICV) was present in three subjects (21%) and five (36%) were receiving partial or total parenteral nutrition at the time of study. Approximately one third of subjects (36%) had received a regimen of cycled antibiotics (defined as 1-2 weeks of use monthly) for prevention of SBBO in the months prior to enrollment (chart review went back 6 months prior to enrollment), while six (43%) were using enteral proton pump inhibitors (PPI) at time of enrollment. Only two subjects were on probiotics (*Lactobacillus acidophilus*, *Lactobacillus rhamnosus* GG) prior to enrollment. Symptoms of SBBO reported at time of enrollment (exceeding subject's baseline) were abdominal pain, abdominal distension and feeding intolerance (defined as excessive stooling and/or emesis with feeding advancement).

Eleven controls were enrolled in the study. Table 1. The median age was 54 months. The most common diagnosis among controls was eosinophilic esophagitis (5, 46%), followed by gastroesophageal reflux disease, dysphagia and emesis. Only one control subject had antibiotic exposure during the month prior to study enrollment, while PPI use was common (73%). The most prevalent symptoms among controls in the week preceding enrollment were abdominal pain, emesis and diarrhea.

Serum bile acid comparison among all IF subjects and controls

Total serum bile acids measured (sum of all 15 bile acid species assessed) was higher in IF subjects (median 2.73 μ M, range 0.9-11.25) compared to controls (median 0.45 μ M, range 0.18-1.44), $p=0.010$. Table 2. The median SUBA concentration was elevated in IF subjects (1.16 μ M, range 0.43-10.65) compared to controls (0.10 μ M, range 0.05-0.18), $p=0.001$. Among SUBA analyzed, the median concentration of chenodeoxycholic acid (CDCA) was found to be significantly elevated in IF subjects (0.8 μ M, range 0-7.08) compared to controls (0 μ M, range 0-0.03), $p=0.012$. No other primary or secondary bile acids were significantly different.

SUBA concentrations were also compared as a fraction of total serum bile acids measured in each cohort and found to be present in greater proportion among IF subjects (87.4%, range 29.7-92.6) compared to controls (22.2%, range 9.2-42.5), $p=0.002$. Table 1. Several individual bile acids also differed significantly between IF subjects and controls. Free chenodeoxycholic acid (CDCA) was present in higher proportions in IF subjects (40%, range 0-56.6), compared to controls (0%, range 0-2.1), $p=0.004$, while glycodeoxycholic acid (GCDCA) was less prevalent among IF subjects (6.1%, range 3.8-24.6) compared to controls (35.4%, range 23.5-51.1), $p=0.042$.

Bile acid profile comparisons among subjects with history of IF

Additional bile acid comparisons were made among subjects with history of IF only with respect to the duodenal aspirate result ($n = 6$ for positive aspirate, $n = 8$ for negative aspirate, Table 2). Total bile acids, SUBA and CDCA were compared. IF subjects with positive aspirates for SBBO had a higher concentration of total bile acids (median 10.53 μ M, range 2.49-12.36) compared to IF subjects with negative aspirates (median 1.63 μ M, range 0.55-4.02), $p=0.04$. Additionally, CDCA concentrations were increased in IF subjects with positive aspirates (median 7.36 μ M, range 1.1-8.28) compared to IF subjects with negative aspirates (median 0.18 μ M, range 0-1.06), $p=0.017$. SUBA concentration was higher in IF subjects with positive aspirates. However, this difference trended toward, but failed to reach significance. No significant difference was found with respect to proportion of SUBA and CDCA.

Serum Unconjugated Bile Acids following treatment for Positive Aspirate SBBO

Among the six IF subjects who underwent antimicrobial treatment for duodenal aspirate-proven SBBO, four provided post treatment samples. Monotherapy was used to treat SBBO in five of six subjects with one of the following: ciprofloxacin, rifaximin, trimethoprim-sulfamethoxazole, amoxicillin-clavulanate, and colistin. The remaining subject was treated with trimethoprim-sulfamethoxazole and amoxicillin-clavulanate concurrently. There was no

significant difference in total serum bile acids, SUBA, SUBA percentage, or serum CDCA acid concentrations between pre- and post-treatment samples among subjects with aspirate-proven SBBO. Table 2.

Organisms cultured for SBBO

Aspirates for SBBO were positive in six subjects with history of IF. Table 3. Half of these subjects were on cycled antibiotics for SBBO prior to study enrollment (one on a combination of ciprofloxacin/polymixin E alternating two weeks each monthly, the other two on metronidazole for two weeks each month). The majority of aspirates (5 of 6), grew both gram positive and gram negative bacteria. The final aspirate was comprised of only gram negative organisms. Genera of gram positive organisms cultured were *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus* and *Gemella* while *Veillonella*, *Morganella*, *Klebsiella* and *Escherichia* comprised the gram negative genera cultured. *Enterococcus* and *Klebsiella* were the predominant genera cultured among all aspirates. Duodenal aspirates for both IF subjects on probiotics prior to enrollment were negative for bacterial overgrowth.

DISCUSSION

Our study showed that SUBA were significantly increased in children with history of IF and suspected SBBO compared to diseased control children in both concentration and proportion of bile acids measured. Additionally, chenodeoxycholic acid (CDCA) was the most predominant SUBA identified among children with history of IF and suspected SBBO. IF subjects with positive aspirates for SBBO had a higher concentration of CDCA compared to IF subjects with negative aspirates. Treatment of positive aspirates for SBBO during the study period did not alter SUBA concentration or proportion of bile acids measured.

Elevated SUBA has been previously reported in adults with SBBO and is attributed to increased intestinal bacterial deconjugation of intraluminal bile acids in the presence of SBBO. Bolt et al. found elevated post prandial SUBA concentrations in adults with SBBO diagnosed by [¹⁴C] glycocholate breath test.¹⁴ Additionally, Masclee found SUBA to be elevated in adults with SBBO diagnosed by jejunal fluid aspirate.¹⁵ The majority (8 of 10) had SUBA concentrations greater than 4 µM/L. In contrast, none of the controls without SBBO had SUBA concentrations above this value. Therefore, Masclee et al. proposed that a threshold SUBA level of 4 µM/L would be a clinically useful marker for the diagnosis of SBBO in adults.

Bile acid profiles for SBBO in children have not previously been described. However, norms for serum bile acid measurements in healthy children have been described. Nijima described normal serum bile acid measurements in 98 healthy children using high performance liquid chromatography (HP-LC). In 98 children, aged 4-6 years, the mean total serum bile acid concentration was 3.68 ± 1.57 µM/L with a mean SUBA concentration of 0.53 ± 0.36 µM/L.¹⁹ SUBA were found to comprise approximately $16.33 \pm 9.79\%$ of the bile acids analyzed in healthy children.¹⁹ In previous unpublished work using LC-MS methodology, we observed that healthy children had a mean concentration of overall bile acid measured of <8 µM/L. The mean fraction of SUBA among bile acids measured was found to be approximately 18%.

When we compare our results to these previously determined thresholds, we see that the proportion of SUBA in our controls (22%) does approximate the expected value of 18% in healthy children, whereas children with history of IF and suspected SBBO had a much higher fraction of SUBA at 87%. Absolute concentration of SUBA was higher as well in IF subjects compared to controls but only five of 14 IF subjects exceeded the proposed cutoff of 4 $\mu\text{M/L}$ for SBBO described in adults by Masclee¹⁵ (1 of 11 controls did surpass 4 $\mu\text{M/L}$). Among these five IF subjects, however, four did have positive aspirates for SBBO. Therefore, as an attempt to determine if bile acid changes observed were due to the presence of SBBO versus the IF itself, we determined to exclude controls and analyze bile acid profiles among IF subjects only, comparing those with positive aspirates to those with negative aspirates for SBBO.

In this comparison, IF subjects with positive aspirates were found to have higher absolute concentrations of total bile acids measured and CDCA without difference in relative proportion. The majority of IF subjects with positive aspirates for SBBO (4 of 6) did exceed the proposed SUBA cutoff of 4 $\mu\text{M/L}$ for SBBO. However proportions of SUBA were comparable between IF subjects with and without positive aspirates. Therefore, it is possible that the difference in concentration of SUBA among IF subjects is attributable to the total bile acid pool measured, which was almost 10 times higher in the positive aspirate group, and not the presence of SBBO. Possible explanations for the increased bile acids measured in subjects with positive aspirates may be attributed to alterations in fat absorption patterns and the enterohepatic circulation process, which can be influenced by age differences and anatomical variations, both of which were present among IF subjects in this study.

CDCA, one of the main primary bile acids synthesized by the liver, was found in greater concentration and proportion among IF subjects suspected of SBBO compared to controls. This observation remained unchanged when comparing IF subjects with positive and negative aspirates for SBBO. It is possible that these differences are attributable to the global increased bile acid concentration measured in patients with SBBO. Other possibilities include alterations in the interaction between intraluminal bile acids, Farnesoid X receptor, Takeda G-protein coupled receptor 5 (TGR5) and downstream effects driven by changes to intestinal anatomy, the intestinal microbiome and nutritional delivery, which has been described in animals.²⁰

Gut microbiota are able to execute modification of bile acids and deconjugation through the use of bile salt hydrolases. Bile salt hydrolase activity has been identified among various phyla of intestinal flora in humans. Namely, *Firmicutes*, *Bacteroidetes* and *Actinobacteria*.^{21,22} With the exception of one aspirate, all of the bacteria identified in positive SBBO aspirates (*Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus* and *Escherichia*) belonged to these phyla, which may explain the differences in SUBA concentrations measured. We acknowledge the inherent challenges and limitations with obtaining small bowel aspirates through duodenal sampling for SBBO such as the preference for more distal aspirates (jejunal) when possible and difficulty with yield of bacterial colonies in both aerobic and anaerobic laboratory environments. Furthermore, we recognize that bacterial species cultured from the proximal small bowel do not always correspond to the flora present distally in the intestinal lumen.

Although there was subjective improvement clinically following treatment for SBBO among IF subjects with positive aspirates, SUBA concentrations did not decrease as would be expected. This may be due to the use of antimicrobials that did not target the correct flora responsible for SBBO and the deconjugation of bile acids. Other possibilities include small sample size and finally, the possibility that the presence of large intestinal resections may contribute to increase SUBA among IF subjects. As an exploratory aim, we analyzed SUBA concentrations with respect to exposure to cycled antibiotics prior to enrollment. There was a significant difference in median SUBA concentrations (data not shown). This is particularly interesting given that the metronidazole, used most often in a monthly cycling regimen, targets anaerobes, which are primarily attributed to the development of SBBO.^{23,24} Among the five IF subjects with cycled antibiotic exposure, three had positive aspirates although all had elevated SUBA. Given the small number of IF subjects being compared, it is difficult to draw any definitive conclusions. It is possible that prior exposure to prophylactic antibiotic use in a cyclical pattern leads to dysbiosis in the gut microbial community, which may alter the balance of microbes that are able to deconjugate luminal bile acids. We have previously published on some of these possible influences.^{8,25}

The synthesis, regulation and enterohepatic recycling of bile acids is a complex process that relies partially on multiple anatomical checkpoints, liver function, gut microbiota composition and available receptors.^{22,26} Furthermore, intestinal resection, namely of the ileum, does not appear to have the same effect on bile acid synthesis in children as has been found in adults.²⁷ In this study we hypothesized that SUBA concentration would be able to distinguish pediatric subjects with history of IF and SBBO from those that did not. We chose to enroll pediatric IF subjects with symptoms suggestive of SBBO rather than confirmed SBBO due to frequency of SBBO in this population. We acknowledge the inherent bias with respect to the subject population due to possible alterations in bile acid levels that may already be present secondary to anatomy. Although we controlled for the presence of liver disease in study subjects, the study cohort of IF subjects was inherently heterogeneous with respect to anatomy, exposure to cycled antibiotics and parenteral nutrition dependence, all of which may influence bile acid physiology and serve as potential confounders to our study results. Additionally, the initial power calculation for our study required eight subjects in each group for comparison. However, we were only able to identify six subjects with culture proven SBBO. Therefore, these study results are limited in their interpretation.

In conclusion, in this pilot study, SUBA concentrations do vary among pediatric IF subjects with suspected SBBO in comparison to non-IF children. However, follow up studies are needed that include various types of pediatric subjects with aspirate proven SBBO in order to confirm that SUBA elevation coincides with SBBO and not just pediatric IF. Additionally, larger, more longitudinal studies are needed to establish bile acid profiles in children with IF at baseline and during SBBO in order to categorize these profiles based on variations in anatomy and microbiome patterns and verify the clinical utility of SUBA measurements for SBBO in this population.

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Abbreviations

SUBA	serum unconjugated bile acids
IF	intestinal failure
SBS	short bowel syndrome
SBBO	small bowel bacterial overgrowth

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Clinical Relevancy Statement

Small bowel bacterial overgrowth remains a poorly understood entity among the pediatric intestinal failure population and poses many challenges to the healthcare provider, from recognition of symptoms to proper diagnosis and management. Often, healthcare providers forego invasive testing and empirically treat with antimicrobials, sometimes in a continuous, monthly, cycled fashion. Less invasive and more reliable methods for diagnosing bacterial overgrowth in this population are needed. This study provides a framework for further investigation to determine the potential utility of serum unconjugated bile acids for the diagnosis of small bowel bacterial overgrowth in pediatric patients with a history of intestinal failure.

Table 1

Demographics.

	Subjects (n = 14)	Controls (n = 11)
Median age (months)	57	54
Diagnosis (n, %)		
<i>gastroschisis</i>	4 (29)	<i>EoE</i> 5 (46)
<i>atresias</i>	4 (29)	<i>GERD</i> 3 (27)
<i>necrotizing enterocolitis</i>	2 (14)	<i>dysphagia</i> 2 (18)
<i>OEIS</i>	2 (14)	<i>emesis</i> 1 (9)
<i>midgut volvulus</i>	1 (7)	
<i>Hirschsprung's disease</i>	1 (7)	
Anatomy (n, %)		
<i>short bowel (small, large)</i>	8 (57)	0 (0)
<i>short bowel (small only)</i>	3 (21)	0 (0)
<i>ileocecal valve</i>	3 (21)	11 (100)
<i>continuity (small, large bowel)</i>	12 (85)	11 (100)
<i>ostomy</i>	5 (36)	0 (0)
<i>normal</i>	0 (0)	11 (100)
TPN (n, %)	5 (35)	0 (0)
Cycled antibiotics (n, %)	5 (36)	1 (9)
<i>metronidazole</i>	3	
<i>ciprofloxacin</i>	1	
<i>alinia</i>	1	
<i>amoxicillin</i>		1
PPI exposure (n, %)	6 (43)	8 (73)

Antibiotic exposure is defined as cycled antibiotic use monthly prior to enrollment (subjects) and use within 3 weeks of enrollment (controls). EoE = eosinophilic esophagitis; GERD = gastroesophageal reflux disease; OEIS = omphalocele, extrophy of bladder/rectum, imperforate anus, spinal defects; PPI = proton pump inhibitor; TPN = total parenteral nutrition

Table 2**Bile Acid Comparisons**

IF subjects vs. Controls (n = 14 vs 11)					
Bile acid	IF subjects	IQR	Controls	IQR	p-value
Total BA	2.73	0.9–11.25	0.45	0.18–1.44	0.01
SUBA	1.16	0.43–10.65	0.10	0.05–0.18	0.001
SUBA%	87.4	29.7–92.6	22.2	9.2–42.5	0.002
CDCA	0.8	0–7.08	0.00	0–0.03	0.012
CDCA%	40	0–56.6	0.0	0–2.1	0.004
GCDCA	0.46	0.11–0.78	0.25	0.06–0.74	0.637
GCDCA%	6.1	3.8–24.6	35.4	23.5–51.1	0.042
IF subjects only (positive vs negative aspirates, n= 6 vs 8)					
Bile acid	Positive	IQR	Negative	IQR	p-value
Total BA	10.53	2.49–12.36	1.63	0.55–4.02	0.04
SUBA	9.71	1.42–11.81	0.46	0.33–1.82	0.059
SUBA%	91.3	56.7–92.6	81.6	23.5–93.4	0.573
CDCA	7.36	1.1–8.28	0.18	0–1.06	0.017
CDCA%	57.0	35.8–74.2	7.6	0–51.9	0.051
IF subjects with positive aspirates (pre vs post treatment, n = 4)					
Bile acid	Median difference	IQR	p-value		
Total BA	3.74	14.14	0.250		
SUBA	2.76	16.43	0.375		
SUBA%	13.43	54.65	0.625		
CDCA	1.49	6.74	0.375		

BA = bile acids; CDCA = chenodeoxycholic acid; GCDCA = glycochenodeoxycholic acid; IF = intestinal failure; IQR = interquartile range; SUBA = serum unconjugated bile acids

Table 3

Demographics, Aspirate description, and Bile acid profiles among IF subjects

Positive aspirates	Age (mo)	Diagnosis	SB%	ICV	colon%	Ileostomy	TPN	PPI	Cycled	SUBA (μM)	CDCA (μM)
Subject 2	39	gastroschisis	13	yes	100	no	yes	no	yes	8.50	7.64
Subject 5	34	atresias	10	no	<50	no	yes	no	yes	10.92	8.28
Subject 8	89	NEC	15	no	<50	no	no	no	yes	17.87	7.08
Subject 9	52	atresias	37	no	<50	no	yes	yes	no	0.30	0.20
Subject 10	53	atresias	37	no	<50	no	yes	yes	no	1.42	1.10
Subject 12	78	gastroschisis	55	no	100	no	no	yes	no	11.81	9.17

Organisms cultured (cfu/mL)											
Subject 2	<i>Morganella morganii</i> (50×10^6)										
Subject 5	<i>Veillonella parvula</i> (5×10^6), <i>Bifidobacterium</i> species (3×10^6), <i>Lactobacillus</i> species (2×10^6)										
Subject 8	<i>Escherichia coli</i> (310×10^6), <i>Enterococcus faecium</i> (50×10^6)										
Subject 9	<i>Enterococcus faecalis</i> (25×10^6), <i>Klebsiella oxytoca</i> (2×10^6)										
Subject 10	<i>Enterococcus faecium</i> (50×10^6), <i>Klebsiella oxytoca</i> (330×10^6)										
Subject 12	Gram positive cocci in pairs, chains (10×10^6), Oxidase negative gram negative rods (5×10^5)										

Negative aspirates	Age (mo)	Diagnosis	SB%	ICV	colon%	Ileostomy	TPN	PPI	Cycled	SUBA (μM)	CDCA (μM)
Subject 1	23	atresias	37	no	100	no	no	no	no	0.23	0.00
Subject 3	84	NEC	15	yes	100	no	no	no	yes	10.65	5.60
Subject 4	38	volvulus	26	no	50	no	yes	yes	no	0.46	0.36
Subject 6	143	HD	42	no	0	yes	no	yes	yes	2.73	1.60
Subject 7	97	gastroschisis	52	no	<50	no	no	no	no	0.90	0.51
Subject 11	89	gastroschisis	74	no	<50	no	no	no	no	0.43	0.00
Subject 13	58	OEIS	100	no	0	yes	no	yes	no	0.46	0.00
Subject 14	92	OEIS	100	yes	<50	no	no	no	no	0.13	0.00

CDCA = chenodeoxycholic acid; Cycled= defined as cycled antibiotic use monthly prior to enrollment; HD= Hirschsprung's disease; ICV= ileocecal valve; IF = intestinal failure; NEC = necrotizing enterocolitis; OEIS= omphalocele, extrophy of bladder/rectum, imperforate anus, spinal defects; PPI= proton pump inhibitor; SB= small bowel; SUBA = serum unconjugated bile acids; TPN= total parenteral nutrition; 10^6 = 1 million; 10^5 = 100,000