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Alterations in intestinal microbiota relate to intestinal failure-associated liver disease and central line infections

Panliang Wang [a], Ying Wang [b], Lina Lu [b], Weihui Yan [b], Yijing Tao [b], Kejun Zhou [b], Jie Jia [b,c,*], Wei Cai [a,b,d,*]

[a] Department of Pediatric Surgery, Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
[b] Shanghai Key Laboratory of Pediatric Gastroenterology and Nutrition, Shanghai, China
[c] Department of Nutrition, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[d] Shanghai Institute of Pediatric Research, Shanghai, China

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ABSTRACT

Background: The gut microbiota plays a vital role in modulating the metabolic and immune functions of the intestines. We aimed to analyze the dysbiosis of microbiota in infants with short bowel syndrome (SBS) with different complications.
Procedure: We included 26 fecal samples from 18 infants with SBS during parenteral nutrition. The samples were categorized into three groups: asymptomatic, parenteral nutrition-associated liver disease (PNALD), and central line-associated bloodstream infection (CLABSI). Seven healthy infants were enrolled as controls. Fecal microbiota, secretory IgA, calprotectin, bile acids, and short chain fatty acids were detected.
Results: The bacterial diversity of the Asymptomatic and Control Groups was significantly higher than that in the PNALD and CLABSI Groups. Proteobacteria was the most pronounced phylum in the PNALD and CLABSI Groups. Decreased acetate was observed in all SBS samples; however, fecal secretory IgA and calprotectin and the proportion of primary and secondary bile acids did not differ from those in healthy controls.
Conclusions: Marked alterations of the intestinal microbiota with decreased level of acetate were shown in SBS patients compared with healthy controls. Over-abundance of Proteobacteria (especially *Enterobacteriaceae*) was found in the samples from the PNALD and CLABSI Groups.
Level of evidence: Prognosis Study, Level I.

The consensus definition of short bowel syndrome (SBS), more specifically, short bowel syndrome–intestinal failure, is that it results from surgical resection, congenital defect, or disease-associated loss of absorption [1]. It is characterized by the inability to maintain protein–energy, fluid, electrolyte or micronutrient balances when on a conventionally accepted, normal diet. The reported overall incidence of SBS was 22.1 per 1000 infants in the neonatal intensive care unit and the case fatality rate was 37.5% [2]. For severe cases of SBS, long-term parenteral nutrition (PN) is unavoidable. Microbiota–host interactions play a key role in maintenance of normal metabolic, nutritional and immunological functions in humans, which can be affected by age, race, diet and drugs, and are involved in development of several diseases [3]. Alterations of the microbiota in SBS patients are associated with weaning from PN, and complications of SBS, both within and beyond the gastrointestinal tract, such as mucosal inflammation, infection, hepatobiliary disease, and D-lactic acidosis [4]. Consequently, the metabolism of short chain fatty acids (SCFAs) and bile acids (BAs), as well as colonic immune function may also change [5]. Recent advances in high-throughput sequencing with in-depth bioinformatics analysis provide more microbiome information compared with traditional culture-based techniques, which cannot characterize nonculturable or fastidious microorganisms that colonize the human gut. The few studies that have focused on microbiota in children with SBS, using high-throughput sequencing techniques, have been restricted to the late stage of SBS (patients with good tolerance of enteral nutrition) [6,7].

Studies in murine models have found that PN dependence leads to altered intestinal microbiota, characterized by increased Proteobacteria and Bacteroidetes, which in turn are attributed to PN-associated complications [8]. There have been few human studies, but their results seem comparable to those of animal studies. These studies have shown marked loss of fecal bacterial diversity and increased abundance of *Enterobacteriaceae* in children with PN-dependent SBS compared with their healthy siblings [7]. PN-associated liver disease (PNALD) and

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BA, bile acid; CLABSI, central line-associated bloodstream infection; ELISA, enzyme-linked immunosorbent assay; ICV, ileocaecal valve; LPS, lipopolysaccharide; OTU, operational taxonomic unit; PN, parenteral nutrition; PNALD, parenteral nutrition-associated liver disease; SBS, short bowel syndrome; SCFA, short chain fatty acid; sIgA, secretory IgA.

* Corresponding author at: Department of Pediatric Surgery, Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. No. 1665 Kongjiang Road, Yangpu District, Shanghai 200092, China.
E-mail addresses: panliang133000@126.com (P. Wang), jie.jia@shsmu.edu.cn (J. Jia), caiw1978@163.com (W. Cai).

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central line-associated bloodstream infection (CLABSI) are the two most common and intractable complications in SBS [9,10]. Gut microbiota is known to participate in the metabolism of BAs [11]. In a piglet model of SBS, decreased BA-biotransforming bacteria, specifically *Bacteroides*, were of particular relevance [12]. These changes result in a reduced proportion of secondary BAs and increased proportion of primary BAs. The altered BA composition regulates the target genes involved in BA synthesis and transport in the gut–liver axis, ultimately causing liver damage [13]. The incidence of CLABSI in children receiving PN reaches 1.5 episodes of infection per patient–year [14], and patients with SBS are more likely to have CLABSI than other diseases, which may be because of bacterial translocation [15].

The aim of our study was to explore alteration of gut microbiota in infants with SBS, with and without complications, and its relationship to fecal metabolites, calprotectin and IgA.

1. Methods

1.1. Patients and controls

Eighteen Chinese infants with SBS were enrolled for intestinal rehabilitation at the Department of Pediatric Gastroenterology and Nutrition, Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine from March 2014 to June 2015. These infants all had malabsorption resulting from extensive surgical resection or congenital short bowel, and had received PN since the day of admission to the Department of Pediatric Gastroenterology and Nutrition after evaluation by dieticians. Patients' demographic parameters and medical/surgery history records were collected. The age-adjusted predicted small bowel length (ASL) was also calculated as described previously [16]. Serum biochemical indexes were examined weekly on the same day of the week. Anthropometric data (weight and height), nutritional intake (both enteral nutrition and PN) and ostomy/stool output were recorded daily.

PN duration was defined as the period between the start of PN and the day of weaning off PN, death of the patient, or reaching the study deadline (June 2015). During PN, if the patients had any one of the following conditions, samples were collected and labeled accordingly. (1) Asymptomatic: patients showed good tolerance to PN administration (stool output maintained at 10–20 mL/kg/day or ostomy output maintained at 2–3 mL/kg/h [17]) and no complications occurred for at least 1 week. (2) PNALD: patients showed elevated serum direct bilirubin level (>34 μmol/L) on two separate examinations conducted at least 1 week apart [18]. (3) CLABSI: patients had fever, increased neutrophils, and documented positive catheter blood culture, but were not subject to other sources of infection [19]. Finally, there were 26 SBS samples included in this study, seven in the Asymptomatic Group, 14 in the PNALD Group, and five in the CLABSI Group, and there were no replicated samples from one patient in each group (Table 1).

The antibiotic use of each SBS patient at the time of sample collection during PN (except one sample without a record) was classified as follows: (1) no use: no antibiotics received before sample collection ($n = 2$); (2) re-use: patient had taken antibiotics before and after a pause and was re-using at the time of sampling ($n = 7$); (3) using: patient was continually using antibiotics until the time of sampling ($n = 5$); and (4) used: patient had received antibiotics previously but was not taking them at the time of sampling ($n = 11$). For samples labeled as re-use or using, days of continuous antibiotic exposure when the sample was collected were also recorded (Table 1).

Seven healthy infants (aged 4–10 months) were randomly recruited from the community in Yangpu District, Shanghai, by contacting their parents or guardians during the period when we collected the samples from infants with SBS. The inclusion criteria included: (1) no gastrointestinal tract disorders or congenital defects; and (2) no treatment with antibiotics, probiotics or steroids from birth to the time of sampling. All healthy infants were transitioning from milk/formula to table food during sample collection. Fecal samples were collected immediately into a sterile tube after defecation, and then stored at −20 °C at home for healthy infants or in the hospital for infants with SBS. They were then shipped in ice to the laboratory and stored at −80 °C for <1 month before processing.

This study was approved by the Ethics Committee of Xin Hua Hospital. Written informed consent for sample collection was obtained from the patients' parents or guardians. This study was also registered in ClinicalTrials.gov (Identifier: NCT02699320).

1.2. DNA extraction and polymerase chain reaction (PCR) amplification

Microbial DNA was extracted from each sample using the QIAampFast DNA Stool Mini Kit (Qiagen, Hilden, Germany). The extracted DNA was quantitated by the Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY, USA) and the V4–V5 region of the bacterial 16S

Table 1
Information of eligible samples collected from each patient.

Patient number	RSL (cm)	ASL (cm)	Asymptomatic			PNALD			CLABSI		
			Sample	Antibiotics [a] (day)	PN [b] (%)	Sample	Antibiotics [a] (day)	PN [b] (%)	Sample	Antibiotics [a] (day)	PN [b] (%)
SB1	120	186.2	None	--	--	1	re-use (1)	100	None	--	--
SB2	50	167.8	None	--	--	None	--	--	1	using (6)	54.3
SB3	23	196	1	used	28.0	1	used	45.7	None	--	--
SB4	38	200.7	None	--	--	1	using (15)	65.5	1	using (8)	90.3
SB5	80	162.1	1	no use	100.0	None	--	--	None	--	--
SB6	46	263.7	1	used	23.6	None	--	--	1	used	28.3
SB7	35	186.2	1	used	36.8	1	re-use (12)	47.9	None	--	--
SB8	100	297.7	1	re-use (2)	12.3	1	used	16.8	None	--	--
SB9	60	181.1	None	--	--	1	no use	27.4	None	--	--
SB10	40	203	None	--	--	1	NA	NA	None	--	--
SB11	98	162.1	None	--	--	1	using (4)	28.9	None	--	--
SB12	61	248.9	None	--	--	1	used	31.5	None	--	--
SB13	30	227.1	1	used	11.5	1	using (6)	100.0	None	--	--
SB14	31	186.2	1	used	22.9	None	--	--	None	--	--
SB15	100	178.5	None	--	--	1	re-use (3)	50.1	1	re-use (6)	100.0
SB16	46	198.4	None	--	--	1	used	86.0	1	re-use (7)	75.0
SB17	70	165	None	--	--	1	using (31)	49.5	None	--	--
SB18	94	250.8	None	--	--	1	used	29.2	None	--	--

–, no data. [a] antibiotics use condition were labeled for each sample and for samples labeled re-use and using, continuous antibiotic exposure time (day) when the sample was collected were also recorded. [b] calorie percentage provided by PN on the day of sample collection.
ASL, age-adjusted predicted small bowel length; CLABSI, central-line-associated bloodstream infection; NA, not available; PNALD, parenteral nutrition–associated liver disease; RSL, remained small bowel length.