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Small bowel dilation in children with short bowel syndrome is associated with mucosal damage, bowel-derived bloodstream infections, and hepatic injury

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Background. *Liver disease occurs frequently in short bowel syndrome. Whether small bowel dilation in short bowel syndrome could influence the risk of liver injury through increased bacterial translocation remains unknown. Our aim was to analyze associations between small bowel dilation, mucosal damage, bloodstream infections, and liver injury in short bowel syndrome patients.*

Methods. *Among short bowel syndrome children (n = 50), maximal small bowel diameter was measured in contrast series and expressed as the ratio to the height of the fifth lumbar vertebra (small bowel diameter ratio), and correlated retrospectively to fecal calprotectin and plasma citrulline—respective markers of mucosal inflammation and mass—bloodstream infections, liver biochemistry, and liver histology.*

Results. *Patients with pathologic small bowel diameter ratio >2.17 had increased fecal calprotectin and decreased citrulline (P < .04 each). Of 33 bloodstream infections observed during treatment with parenteral nutrition, 16 were caused by intestinal bacteria, cultured 15 times more frequently when small bowel diameter ratio was >2.17 (P < .001). Intestinal bloodstream infections were predicted by small bowel diameter ratio (odds ratio 1.88, P = .017), and their frequency decreased after operative tapering procedures (P = .041). Plasma bilirubin concentration, gamma-glutamyl transferase activity, and histologic grade of cholestasis correlated with small bowel diameter ratio (0.356–0.534, P < .014 each), and were greater in the presence of intestinal bloodstream infections (P < .001 for all). Bloodstream infections associated with portal inflammation, cholestasis, and fibrosis grades (P < .031 for each). In linear regression, histologic cholestasis was predicted by intestinal bloodstream infections, small bowel diameter ratio, and parenteral nutrition (β = 0.36–1.29; P < .014 each), while portal inflammation by intestinal bloodstream infections only (β = 0.62; P = .033).*

Conclusion. *In children with short bowel syndrome, small bowel dilation correlates with mucosal damage, bloodstream infections of intestinal origin, and cholestatic liver injury. In addition to parenteral nutrition, small bowel dilation and intestinal bloodstream infections contribute to development of short bowel syndrome-associated liver disease. (Surgery 2017;162:670-9.)*

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Patients with short bowel syndrome (SBS) who are dependent on parenteral nutrition (PN) are at risk for several complications, of which bloodstream infections (BSI) and intestinal failure-associated liver disease (IFALD) carry the greatest morbidity and mortality and frequently coexist with pathologic small bowel (SB) dilation.[1-4] Excessive SB dilation is an independent predictor of prolonged PN,[5,6] predisposing to intestinal dysbiosis and malabsorption presumably due to impaired motility.[1,7-9] Although the pathophysiology of IFALD among

humans remains unresolved, results from experimental models suggest that in addition to the hepatotoxicity of PN solutions, translocation of altered intestinal microbiota and their endotoxins into bloodstream are among the key mechanisms promoting liver damage.[10-12]

Several factors can modify the intestinal microbiota in SBS.[13-15] Absence of the ileocecal region allows colonic bacteria to migrate into the SB, while excessive adaptive SB dilation may favor their proliferation and predisposes to symptomatic bacterial overgrowth.[5,9,16,17] During PN, intestinal mucosa undergoes atrophy, gram-negative species become overabundant, and simultaneously, the overall microbial diversity decreases.[13-15,18-20] Enteral nutrient deprivation and lipopolysaccharides (LPS) derived from gram-negative bacteria promote epithelial expression of proinflammatory cytokines which impairs epithelial barrier function and allows translocation of bacteria and their endotoxins into portal circulation.[15,21-24] More than half of bacteremia episodes among PN-dependent SBS patients are caused by gram-negative organisms, suggesting an intestinal origin.[25-27]

After being released into bloodstream, circulating bacterial constituents are cleared by the liver, where they activate Kupffer cells to produce proinflammatory cytokines.[11,28,29] Evidence mainly from animal models demonstrates this cytokine signaling promotes cholestasis through antagonism of liver nuclear farnesoid X receptor (FXR), downregulating expression of bile transporters.[29,30] Not only bacterial endotoxins but also plant sterols present in PN solutions are able to inhibit FXR and activate Kupffer cells, synergistically aggravating cholestasis and inflammation,[10-12,31] the key histopathologic findings in early-stage IFALD.[32,33] Frequent BSI, circulating endotoxins, and PN are known risk factors for cholestasis and IFALD.[1-3,34]

We hypothesized pathologic SB dilation would predispose to mucosal damage and bacterial translocation, and thereby promote liver disease in SBS. To study this, we analyzed fecal calprotectin (FC) and plasma citrulline, respective markers of mucosal inflammation and mass,[35,36] the frequency and origin of BSIs, as well as liver biochemistry and histology in relation to the degree of contrast-enhanced SB dilation among SBS children.

METHODS

Patients. All SBS patients with PN duration for >3 consecutive months or SB resection >50% of the age-adjusted reference value[1,37] having undergone SB contrast series during 2002–2016 (n = 50) were included. We excluded SBS patients without registered SB contrast series (n = 11) and patients with other underlying etiology of IF (n = 29). Medical records were reviewed for baseline patient characteristics, intestinal anatomy, PN duration, operative procedures, liver histology, laboratory test results, and BSI episodes.

Contrast SB series and SB diameter ratio. During the study period, 50 patients underwent altogether 179 contrast SB series at median 3.7-month intervals (interquartile range [IQR] 1.7–11.7) as part of their follow-up (median 3 investigations per patient, IQR 2–3). The width of the greatest SB segment perpendicular to the longitudinal axis of the bowel as well as the height of fifth lumbar vertebra were measured in each examination as described previously.[5] Mechanical intestinal occlusion was ruled out by confirming distribution of contrast material throughout the intestine. To obtain a comparable measure of the SB diameter controlled for age and size, the widest SB segment was expressed as the ratio to the height of the fifth lumbar vertebra (SB diameter ratio, SBDR).[5] Maximal SBDR was defined as the greatest value measured for each patient during follow-up.

Laboratory data. Levels of plasma alanine (ALT; n = 48) and aspartate aminotransferase activity (AST; n = 41), prealbumin (n = 45), bilirubin (n = 47), gamma-glutamyl transferase activity (GT; n = 41), and citrulline (n = 26) were included if analyzed within 6 months of the maximal measurement of the SBDR in contrast SB series. FC measurements were recorded if analyzed within 6 months of contrast series, and patients' greatest FC during follow-up was used for statistical analyses (n = 34). Plasma citrulline levels were analyzed with an automatic amino acid analyzer (Biochrom 30 Physiological and Midas Autosampler, Biochrom Limited, Cambridge, England),[38] while other tests were carried out using routine hospital laboratory methods. Blood samples were analyzed within a median of 6 (IQR 1–29) days of contrast series. FC levels were analyzed within 2 (0–29) days of contrast series with quantitative enzyme immunoassay (PhiCal Test, Calpro AS, Oslo, Norway).

Bloodstream infections. Altogether, 35 positive blood cultures were recorded within 6 months (median 3.13 [1.87–5.53] weeks) of the contrast SB series. Two positive cultures by *Staphylococcus epidermidis* in the absence of a central venous catheter were considered contaminated samples and were excluded from the analyses. Cultured

organisms were recorded and divided into subgroups for analytic purposes (skin flora: *Staphylococcus aureus* and coagulase negative *Staphylococci*; intestinal flora: *Proteobacteria* [*Citrobacter, Escherichia coli, Klebsiella*, and *Enterobacter cloacae*] and *Firmicutes* [*Lactobacillus, Bacillus cereus, Enterococci*, and *Streptococcus agalactiae*, cultured at the age >1 year]; and yeast: *Candida* species).

Liver biopsies. Altogether, 50 liver biopsies from 37 patients taken within 6 months (median 1.07 [0.14–5.14] weeks) of the contrast series were included. Nine patients had undergone 2 biopsies and 2 patients 3 biopsies. Biopsies were taken as ultrasonographic-guided core needle biopsies under general anesthesia by an experienced pediatric radiologist. Specimens were stained with conventional stains and evaluated by experienced pediatric pathologists without knowledge of clinical data, as described previously.[32] Histologic cholestasis and portal inflammation were graded as absent (=0), minimal (=1), marked (=2), or prominent (=3), and steatosis was assessed according to the proportion of hepatocytes affected (0 = absent, 1 ≤ 25%, 2 = 25–50%, 3 > 50% of hepatocytes). Grading of fibrosis was assessed according to Metavir stage as no fibrosis (=F0), portal fibrosis without septa (=F1), portal fibrosis and few septa (=F2), numerous septa without cirrhosis (=F3), or cirrhosis (=F4).[39]

Statistical analyses. Continuous data are expressed as median values and IQRs and categorical data as frequencies unless otherwise stated. Spearman rank correlation was used to examine associations between variables, Mann-Whitney *U* test to compare continuous variables, and Fisher exact test to compare frequencies between groups. Laboratory tests were analyzed by taking into account one measurement per patient for liver biochemistry and citrulline, while measurements taken within 6 months of maximal SBDR were included. The greatest FC value of each patient was correlated to SBDR measured within 6 months. Similarly, episodes of BSI and liver biopsies were correlated to SBDR measured within 6 months. When one liver biopsy per patient was analyzed using the first specimen during follow-up. Based on our previous finding that among SBS children, SBDR >2.17 was the best cutoff value for prediction of PN dependence, denoting pathologic dilation,[5] SBDR measurements were divided into 2 subgroups (≤2.17 and >2.17), across which clinical and histologic findings were compared. To evaluate predictors of BSIs and liver histology, simple logistic or linear regression models were conducted, from which statistically significant variables were chosen for multiple regression models.

Ethics. The hospital ethical committee approved the study protocol.

RESULTS

Baseline characteristics. Altogether, 50 children with SBS were included. Median length of remaining SB was 38 (25–60) cm, while that of the colon was 32 (24–40) cm, corresponding 25% (17–47) and 99% (71–100) percent of age-adjusted reference values, respectively.[37] The ileocecal valve was missing in 22 (44%), and no ileum was remaining in 21 (42%). Baseline diagnoses included necrotizing enterocolitis (n = 25), midgut volvulus (n = 11), gastroschisis (n = 4), and SB atresia with (n = 4) or without gastroschisis (n = 6). Median gestational age was 31 (26–36) weeks, and gestational weight was 1,410 (745–2,720) g. Altogether, 15 patients had undergone autologous intestinal reconstruction operation to alleviate dilation-associated intestinal dysfunction, including serial transverse enteroplasty (n = 10), longitudinal intestinal lengthening and tapering (n = 1), tapering enteroplasty (n = 3), or resection of dilated SB (n = 1), and 6 patients had required reoperations due to symptomatic redilation.

Contrast-enhanced small bowel dilation. Median age at contrast SB series was 1.04 (0.34–3.01) years, SB width 25 (19–32) mm, SBDR 2.14 (1.65–2.71), and patients' maximal SBDR 2.75 (2.10–3.46). Two-thirds (n = 122/179) of contrast SB series were performed on PN-dependent patients having received PN for 5.5 (2.5–12.6) months. Others had been weaned off PN for 36 (8.1–108) months prior the contrast study after 7.6 (4.7–11.1) PN months. PN-dependent patients were younger (0.55 vs 1.13 years, $P < .001$) and had greater SBDR (2.31 vs 1.67, $P < .001$) compared with those weaned off PN.

Fecal calprotectin and plasma citrulline in relation to SBDR. Median FC (greatest value for each patient) was 77 (20–272), with greater values observed in patients whose SBDR was >2.17 compared with ≤2.17 (Table I). The correlation between FC and SBDR, however, was not different (r = 0.272, P = .120). FC levels did not relate with remaining SB length or show difference across PN status (P = ns for both).

Plasma citrulline levels were less than normal in the whole sample (median 20, IQR 13–26) and lesser values were observed in patients whose maximal SBDR was >2.17 (Table I). The correlation between citrulline and maximal SBDR

Table I. Laboratory test results in relation to SB diameter ratio

	SB diameter ratio (n = *50*)		
	≤2.17 (n = *14*)	*>2.17* (n = *36*)	P *value*
Plasma bilirubin (μmol/L)	6.0 (5.0–8.0)	8.5 (5.0–94)	.072
Plasma GT (U/L)	11 (11–16)	61 (34–107)	<.001
Plasma ALT (U/L)	23 (22–38)	45 (22–67)	.092
Plasma AST (U/L)	39 (30–44)	53 (31–119)	.134
Plasma prealbumin	202 (176–256)	127 (88–182)	<.001
Plasma citrulline (μmol/L)	26 (24–34)	15 (12–25)	.033
Fecal calprotectin (μg/g)	24 (11–157)*	194 (76–400)†	.039

*n = 20.
†n = 14.
Laboratory test results measured within 6 months of contrast SB series according to SB diameter ratio. Liver biochemistry and citrulline are related to maximal SB diameter ratio (n = 50 measurements among 50 patients) while patients' maximal FC (n = 34) are related to SB diameter ratio measured within 6 months of FC. P values from the Mann-Whitney U test for the observed differences between subgroups are reported.

(r = −0.351, P = .079) or percentage of remaining SB (r = 318, P = .113) did not reach statistical significance. Plasma citrulline was less in patients receiving versus weaned off PN (15 vs 26, P = .001).

Bloodstream infections. Of the 33 BSIs observed in 13 patients, 39% (n = 13) were caused by skin flora, 12% (n = 4) by *Candida*, and 49% (n = 16) by intestinal organisms (*Proteobacteria*, n = 8 and *Firmicutes*, n = 8) among 12 patients. Notably, all BSIs occurred during PN in patients with a central venous catheter. When stratified according to causative organisms, all BSI subgroups correlated with an increased SBDR (Fig 1). BSIs by intestinal microbiota occurred more frequently among patients with SBDR >2.17 vs ≤2.17; although the difference did not reach statistical significance when only BSIs at maximal SBDR measurement were analyzed, all BSIs were observed in patients a maximal SBDR >2.17 (Fig 2). The remaining lengths of the small bowel, ileum, or colon, the presence of an ileoceal valve, and the duration of PN did not correlate with BSIs.

Predictors of bloodstream infections. In simple logistic regression, PN dependence (odds ratio, OR = 19.9, 95% confidence interval [CI], 2.65–149, P = .004), age (OR = 0.37, 95% CI, 0.20–0.71, P = .003), and SBDR (OR = 2.03, 95% CI, 1.32–3.13, P = .001) were predictors of all BSIs, but none of these variables remained statistically significant in a multiple regression model; importantly, SBDR was the only predictor of BSI caused by intestinal microbiota (OR = 1.88, 95% CI, 1.12–3.16, P = .017).

Liver biochemistry in relation to SB diameter ratio and bloodstream infections. Patients whose maximal SBDR was >2.17 had greater levels of GT, lesser levels of prealbumin, and tended to have

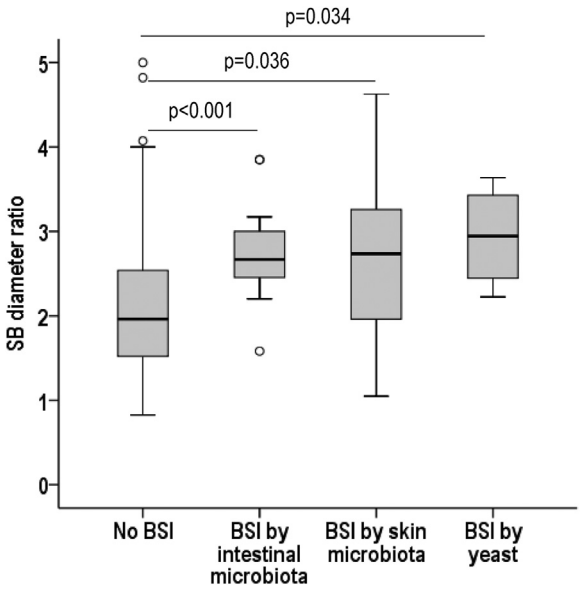

Fig 1. SB diameter ratio according to organisms cultured in blood samples within 6 months of contrast SB series (total n = 179). Statistically significant differences between subgroups are reported.

greater levels of serum bilirubin than patients with a maximal SBDR ≤2.17 (Table I). Maximal SBDR correlated positively with bilirubin (r = 0.356, P = .014) and GT (r = 0.504, P = .001), and negatively with prealbumin (r = −0.470, P = .001), while the correlation with ALT was 0.258 (P = .077). No correlations between maximal SBDR and laboratory test results were observed when analyzing patients on and off PN separately.

If measured within 6 months of BSIs by intestinal microbiota, plasma levels of bilirubin were greater (74 vs 7, P = .013), and if measured within 6 months of any BSIs, bilirubin (54 vs 6, P = .002), GT (66 vs 21, P = .008), and AST (77 vs 40,

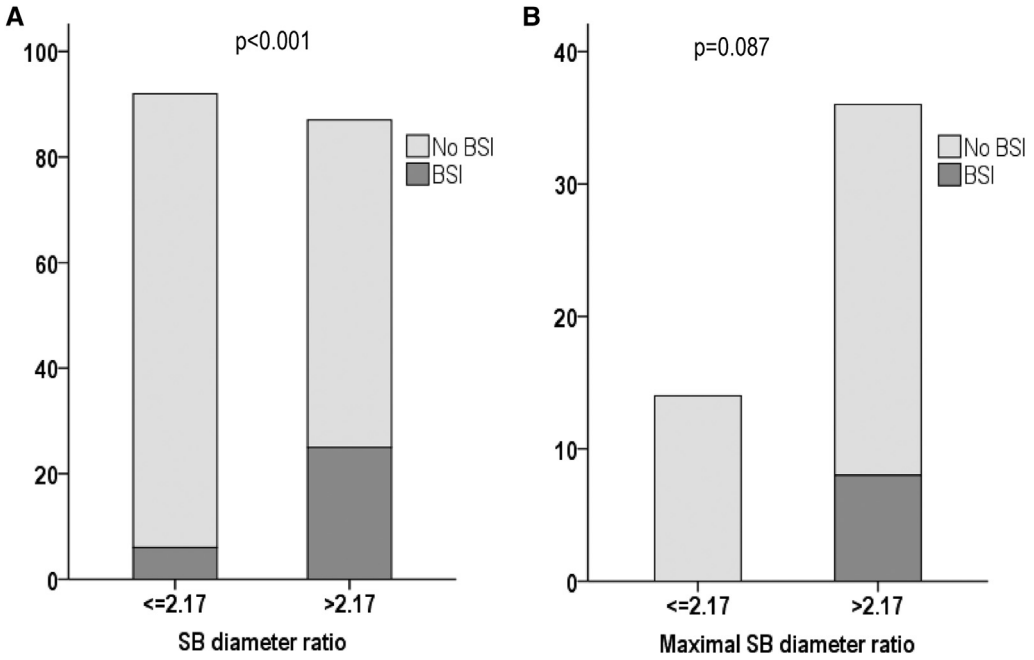

Fig 2. The occurrence of BSI by intestinal microbiota within 6 months of contrast SB series (*A*) according to all measurements of SBDR ($n = 179$) and (*B*) according to maximal SBDR ($n = 50$). *P* values for the differences between subgroups are reported.

$P = .015$) were greater and prealbumin less (94 vs 182, $P = .003$) compared with measurements performed in the absence of any BSIs.

Outcomes after intestinal tapering operations (autologous intestinal reconstruction). When all laboratory test results measured before and after autologous intestinal reconstruction ($n = 15$ patients) were analyzed, plasma levels of AST (51 [44–123] vs 39 [26–48], $P = .003$), GT (43 [24–76] vs 26 [15–37], $P = .034$), and bilirubin (7.0 [4.5–14] vs 3.0 [2.0–5.0], $P < .001$) decreased, while albumin levels increased (32 [29–36] vs 36 [31–38], $P = .047$) postoperatively. No differences were observed when the analysis was restricted to markers of liver function measured within 6 months of maximal SBDR. After autologous intestinal reconstruction, the frequency of all BSIs (mean 0.9 [range 3.0] vs 0.20 [1.0], $P = .040$) as well as those caused by intestinal organisms (0.60 [3.0] vs 0.00 (−), $P = .041$) decreased.

Liver histology. Cholestasis, portal inflammation, and steatosis were each present in 19 (38%), while any fibrosis (≥F1) was present in 32 (64%) of liver biopsies. Portal inflammation was more common in the presence versus in the absence of cholestasis (12/19 vs 7/31, $P = .005$). Cholestasis was more severe and occurred more frequently among patients with SBDR >2.17 vs ≤2.17, both when analyzing all liver biopsies or one biopsy per patient (Table II, Fig 3). SBDR correlated positively with grading of histologic cholestasis (r = 0.534, $P = .001$). Similarly, irrespective of the number of liver biopsies analyzed per patient, the grading of cholestasis, portal inflammation, and fibrosis was greater in biopsies taken within 6 months of BSIs of intestinal origin compared to other biopsies (Table III). The overall incidence of cholestasis, portal inflammation, and fibrosis (grades 1–3 versus absent) tended to be more frequent in the presence of intestinal BSIs when one biopsy per patient was analyzed ($P = .066$ for all), while significant differences in the incidence of cholestasis and portal inflammation were observed when analyzing all liver biopsies (Fig 4).

PN-dependent patients not only showed cholestasis and fibrosis (≥F1) more frequently than patients weaned off PN ($P \leq .001$–$.021$), but the grading for cholestasis and fibrosis was greater ($P \leq .001$–$.028$), both when one or multiple biopsies per patient were analyzed. The length of remaining SB, ileum, or colon and the presence of an ileocecal valve were unrelated with liver histology.

Predictors of cholestasis and portal inflammation. According to simple linear regression, predictors of the grade of cholestasis ($n = 37$ biopsies) were all BSIs and BSIs by intestinal organisms

Table II. Grading of steatosis, cholestasis, portal inflammation, and fibrosis according to small bowel diameter ratio measured in contrast series within 6 months of liver biopsy

	SB diameter ratio (n = *50 biopsies*)			*SB diameter ratio* (n = *37 biopsies*)		
	≤2.17 (n = *24*)	*>2.17* (n = *26*)	P *value*	*≤2.17* (n = *17*)	*>2.17* (n = *20*)	P *value*
Steatosis, (0–3)	0.63 (0.00–3.00)	0.58 (0.00–3.00)	.859	0.71 (0.00–3.00)	0.58 (0.00–3.00)	.950
Cholestasis, (0–3)	0.21 (0.00–2.0)	1.15 (0.00–3.00)	.001	0.23 (0.00–2.00)	1.20 (0.00–3.00)	.007
Portal inflammation, (0–3)	0.38 (0.00–1.00)	0.48 (0.00–2.00)	.699	0.41 (0.00–1.00)	0.55 (0.00–2.00)	.208
Fibrosis (Metavir 0–4)	1.08 (0.00–2.00)	1.16 (0.00–3.00)	.832	0.94 (0.00–2.00)	1.25 (0.00–3.00)	.407

Data are mean with range. Results based on all liver biopsies ($n = 50$) presented on the left and results based on one biopsy per patient (first specimen during follow-up, $n = 37$) presented on the right. *P* values from the Mann-Whitney *U* test for the observed differences between subgroups are reported.

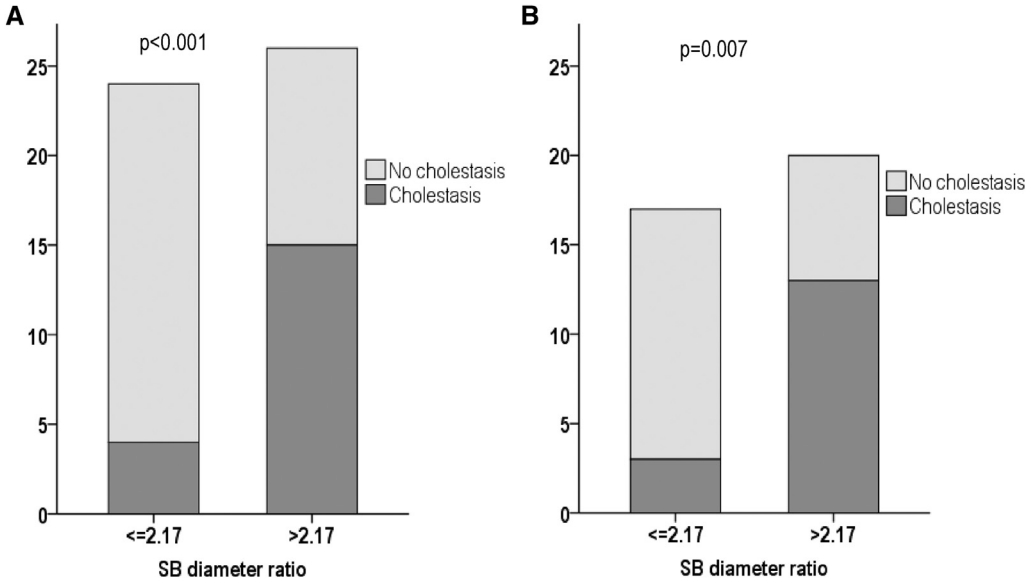

Fig 3. The presence of cholestasis by taking into account (*A*) all liver biopsies ($n = 50$) and (*B*) one liver biopsy per patient ($n = 37$) within 6 months of contrast SB series according to SB diameter ratio. *P* values for the differences between subgroups are reported.

within 6 months of liver biopsy (unstandardized coefficient, $\beta = 1.29$, 95% CI, 0.66–1.92, $P < .001$ and $\beta = 1.09$, 95% CI, 0.24–1.94, $P = .014$), PN-dependence ($\beta = 1.29$, 95% CI, 0.77–1.82, $P < .001$), SBDR ($\beta = 0.36$, 95% CI, 0.08–0.64, $P = .012$), and age ($\beta = -0.08$, 95% CI, −0.14 to −0.03, $P = .004$). In a multiple regression model, all BSIs ($\beta = 0.87$, 95% CI 0.23–1.51, $P = .009$) and PN-dependence ($\beta = 0.72$ 95% CI, 0.01–1.42, $P = .047$) remained significant predictors of cholestasis. PN duration and SB or ileum length were unrelated with the grade of cholestasis.

The only predictor of the grade of portal inflammation was BSIs by intestinal organisms within 6 months of liver biopsy ($\beta = 0.61$, 95% CI, 0.10–1.13, $P = .021$) while the effects of PN-dependence, SBDR, and all BSIs did not reach statistical significance ($P = .052$–$.075$). In a multiple regression model adjusted also for variables with a borderline significance, BSIs by intestinal microbiota remained the only predictor of portal inflammation ($\beta = 0.62$, 95% CI, 0.06–1.19, $P = .033$).

DISCUSSION

This study is the first reporting clinical associations between excessive SB dilation and the most important clinical complications of SBS; BSIs and IFALD. The degree of bowel dilation associated with increased levels of FC and decreased plasma citrulline, reflecting inflammation and atrophy of the intestinal mucosa.[35,36] Bowel dilation was the only recognized risk factor for BSIs of intestinal origin, the frequency of which decreased after autologous intestinal reconstruction. Furthermore, SBDR was predictive of biochemical and histologic cholestasis, while BSIs of intestinal origin predicted both portal

Table III. Grading of steatosis, cholestasis, portal inflammation, and fibrosis according to the presence of bloodstream infections within 6 months of liver biopsy

	BSI of intestinal origin (n = *50 biopsies*)			*BSI of intestinal origin* (n = *37 biopsies*)		
	No (n = *43*)	*Yes* (n = *7*)	P *value*	*No* (n = *31*)	*Yes* (n = *6*)	P *value*
Steatosis, (0–3)	0.64 (0.00–3.00)	0.33 (0.00–1.00)	.725	0.70 (0.00–3.00)	0.33 (0.00–1.00)	.725
Cholestasis, (0–3)	0.52 (0.00–3.00)	1.86 (0.00–3.00)	.005	0.58 (0.00–3.00)	1.67 (0.00–3.00)	.031
Portal inflammation, (0–3)	0.35 (0.00–2.00)	1.00 (0.00–2.00)	.031	0.39 (0.00–2.00)	1.00 (0.00–2.00)	.048
Fibrosis (Metavir 0–4)	1.00 (0.00–3.00)	2.00 (1.00–3.00)	.022	0.94 (0.00–3.00)	2.00 (1.00–3.00)	.022

Data are mean with range. Results based on all liver biopsies ($n = 50$) presented on the left and results based on one biopsy per patient (first specimen during follow-up, $n = 37$) presented on the right. *P* values from the Mann-Whitney *U* test for the observed differences between subgroups are reported.

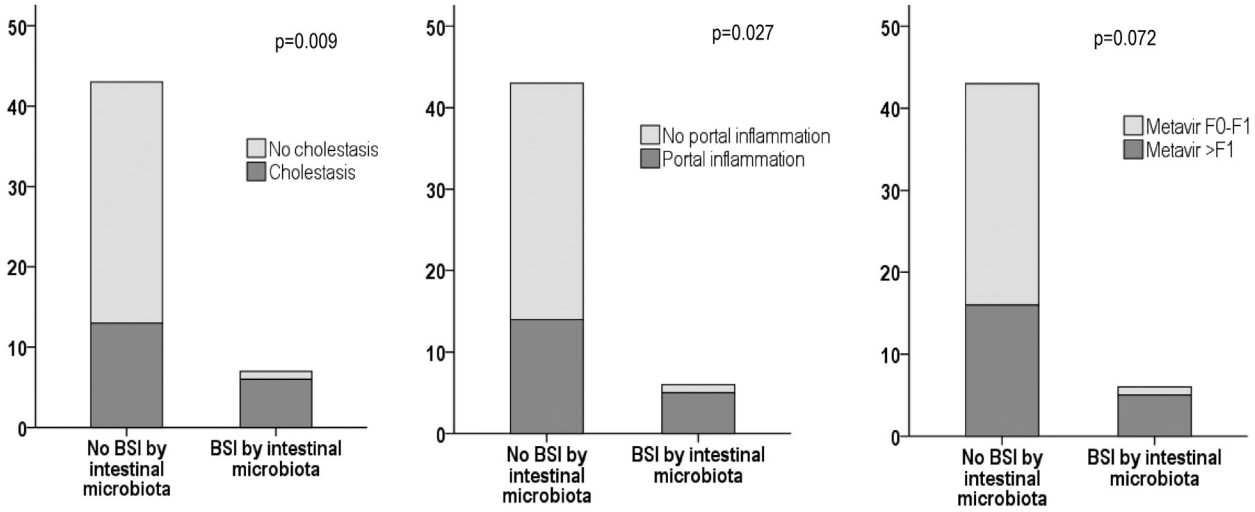

Fig 4. The presence of cholestasis, portal inflammation, and fibrosis (>F1) in liver biopsies ($n = 50$) taken within 6 months of BSI caused by intestinal microbiota ($n = 7$) versus other liver biopsies ($n = 43$). *P* values for the observed difference between subgroups are reported. No difference in the presence of steatosis between subgroups was observed ($P = .99$).

inflammation and cholestasis, the histologic hallmarks of early-stage IFALD.[32,33] Altogether, these findings suggest that excessive SB dilation predisposes to intestinal mucosal damage and BSIs, and reinforce the concept that translocated intestinal microbes play a crucial role in the pathophysiology of human IFALD by promoting inflammation and cholestasis, corresponding to observations in experimental models.[10-12]

Pathologic SB dilation occurs in a subset of SBS patients, predisposing to intestinal dysmotility, bacterial overgrowth, and prolonged PN duration.[1,5,7,8] Among SBS patients, the incidence of bacterial overgrowth is up to 70% and approaches 100% during PN.[19,26,40,41] Calprotectin is excreted in stools in abundance in the presence of mucosal inflammation.[36] FC levels have also been reported to be greater in SBS children with bacterial overgrowth both compared with other SBS patients or healthy controls.[26] Even though the presence of bacterial overgrowth was not measured objectively in our patients, the increased FC levels in patients with pathologic SBDR suggest mucosal inflammation becomes more prevalent along with bacterial overgrowth when the bowel undergoes dilation. Furthermore, decreased citrulline levels paralleled the increasing degree of SB dilation, suggesting atrophy of mucosal absorptive surface may accompany the above outlined changes in excessively dilated SB.[15,35] Plasma levels of itrulline are a reliable marker of decreased enterocyte mass and function in SBS.[35] However, possibly because clearly decreased citrulline levels were observed in all of our SBS patients, correlations between citrulline and SBDR as well as SB length were not statistically significant.

During PN, the intestinal microbiota of SBS patients is characterized by a loss of *Firmicutes* and

an overabundance of LPS-producing *Proteobacteria*, while mucosal inflammation becomes increasingly prevalent.[14,20,21,42] Simultaneously, deprivation of enteral nutrients creates a proinflammatory state within the intestine increasing epithelial permeability and translocation of bacterial products.[11,15,21] PN-dependent SBS patients show increased intestinal permeability and increased plasma antibodies against LPS, suggesting continuous exposure to bowel-derived bacterial antigens.[23,24,26] In our patients, more than half of BSIs were caused by intestinal microbiota, corresponding to the BSI origins and overabundant bacterial species in SBS patients reported previously.[14,24,26,27] Notably, all BSIs by intestinal microbiota occurred during PN, demonstrating that PN-associated factors are crucial for disruption of the epithelial barrier; however, because intestinal BSIs no longer occurred after autologous intestinal reconstruction and were predicted mainly by an increased SBDR, SB dilation favoring the overgrowth of abnormal microbiota and mucosal damage seems to promote bacterial translocation. Certain PN lipid compositions have been reported to modify intestinal microbiota adversely in experimental animals with an association to transcription of tight junction proteins and proinflammatory cytokines.[43,44] In addition, altered systemic immunity and low levels of antioxidant trace elements in PN-dependent SBS patients may contribute to the correlation between delivery of PN and BSIs.[45,46] Although we considered *Candida* infections to have originated from central venous catheters, *Candida* has been cultured in the SB of SBS patients,[19] and the increased SBDR related with such BSIs could also indicate an intestinal origin, further supporting our findings.

Experimental SBS models have demonstrated that both increased intestinal permeability and PN delivery are required for the activation of liver inflammation and development of cholestatic liver injury in mice.[11] Accordingly, in addition to the observed association between PN delivery and liver histopathology, we found BSIs of intestinal origin to be predictive of cholestasis and the only predictor of portal inflammation. BSIs of intestinal origin and histologic cholestasis were predicted by SB dilation, which was associated with markers of mucosal damage, suggesting that SB dilation may contribute to development of liver injury by favoring bacterial translocation and dysfunction of the epithelial barrier. In experimental animals, the downstream signaling pathways activated by bacterial LPS in the liver include release of inflammatory cytokines and suppression of the expression of bile acid transporter through antagonism of hepatocyte nuclear receptors, resulting particularly in portal inflammation and cholestasis.[11,28,29] Increased concentrations of inflammatory cytokines correlate with liver histopathology in SBS children.[47] Although LPS or cytokine levels were not measured in the present study, our findings suggest the direct hepatotoxic effects of bacterial constituents play a central role in IFALD pathophysiology.

In children, SB diameter standardized to the height of the fifth lumbar vertebra may be a more accurate measure of bowel dilation than absolute SB width as it is less affected by patient age and size.[5,6,48] We found previously that a SBDR >2.17 was predictive of PN-dependence,[5] while in this study, such a SBDR correlated with impaired liver function parameters, histologic cholestasis, and BSIs by intestinal microbiota. Improved liver biochemistry and decreased BSIs observed after autologous intestinal reconstructive operation support the previous findings that restoration of normal SB caliber prevents the harmful consequences of bacterial translocation,[49,50] and therefore cholestasis or frequent BSIs of intestinal origin should be considered as indications for intestinal tapering operations in SBS children with excessively dilated SB who are unable to wean from PN. Common practice involves controlling symptoms of bacterial overgrowth by antibiotics, which has also been reported to reverse cholestasis.[51,52] Antibiotics, however, cannot restore SB motility or SB caliber and the continuous use of antibiotics may favor the growth of resistant strains.[8] Our findings support the use of autologous intestinal reconstruction in SBS children unable to wean from PN with a SBDR >2.17.

The most important limitation of our work is the lack of direct evidence on causal relationships between SBDR, BSIs, and liver histopathology. We used retrospective data and had to deal with some missing laboratory test values. Furthermore, FC was analyzed instead of specific measures of bacterial overgrowth. Nevertheless, this clinical study which links bowel dilation with signs of IFALD and the incidence of BSIs as well as BSIs of intestinal origin with liver inflammation. Our results suggest excessive SB dilation in SBS predisposes to clinically relevant and severe systemic complications. Even though experimental studies are needed to unravel the molecular mechanisms behind IFALD, adaptive SB dilation is one of the clinical challenges which is difficult to take into account in rodent models but needs to be considered in the treatment of SBS patients.

In conclusion, increased SBDR among SBS children correlates with markers of mucosal damage, BSIs of intestinal origin, and both biochemical and histologic liver injury. SBDR was predictive of histologic cholestasis and BSIs of intestinal origin, while such BSIs were predictive of both cholestasis and portal inflammation. Further research on bacterial translocation and its hepatotoxicity among SBS patients is required to fully understand the role of SB dilation as a risk factor for IFALD.

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