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Increased Anti-Flagellin and Anti-Lipopolysaccharide Immunoglobulins in Pediatric Intestinal Failure: Associations With Fever and Central Line–Associated Bloodstream Infections

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Abstract

Background—Central line–associated bloodstream infections (CLABSIs) pose a significant challenge in the lives of patients with intestinal failure (IF). We hypothesized that plasma immunoglobulins against flagellin (FLiC) and lipopolysaccharide (LPS) would be able to differentiate CLABSIs from nonbacterial febrile episodes and that levels would increase with infection and decline following appropriate antibiotic treatment.

Materials and Methods—Patients with IF, due to short bowel syndrome, between the ages of 3 months and 4 years of age, were recruited at Cincinnati Children’s Hospital Medical Center. Anti-FLiC and anti-LPS plasma antibody levels were measured in 13 children with IF at baseline, during febrile events, and also following treatment with antibiotics. These were also measured in 11 healthy children without IF who were recruited as controls.

Results—Plasma anti-FLiC IgA levels increased during febrile episodes in all patients with IF (baseline mean of 1.10 vs febrile episode mean of 1.32 optical density units, respectively; $P = .046$). Neither plasma anti-FLiC nor anti-LPS IgA or IgG levels distinguished CLABSI from nonbacterial febrile episodes compared with baseline levels. Compared with controls, patients with IF had significantly higher plasma levels of anti-FLiC and anti-LPS IgA at baseline.

Conclusion—Plasma anti-FLiC IgA antibody levels rise during febrile episodes but do not differentiate between nonbacterial febrile illnesses and CLABSIs in pediatric IF. However, the upregulation of these antibodies in IF suggests the baseline systemic presence of Gram-negative bacterial products.
Keywords
short bowel syndrome; parenteral nutrition; biomarker; fever; bacterial infection

Introduction
Short bowel syndrome (SBS) is a disorder characterized by diarrhea and malabsorption associated with complications, including malnutrition secondary to insufficient functional bowel length. Small bowel bacterial overgrowth (SBBO), bacterial translocation, and excessive intestinal permeability are likely to occur with greater frequency in this population due to gut barrier dysfunction. These conditions may contribute to higher rates of central line–associated bloodstream infections (CLABSIs). Identifying biomarkers to differentiate CLABSIs from nonbacterial causes of fever in children with SBS and other causes of intestinal failure (IF) would be valuable.

Lipopolysaccharide-binding protein (LBP) has been identified as a potential diagnostic marker for Gram-negative bacteremia in patients with neutropenic cancer as well as Gram-positive and fungal sepsis. Kevan et al recently reported that soluble triggering receptor expressed on myeloid cells-1 (STREM-1) and LBP both increase with fever and decline after treatment in children with IF. However, neither biomarker was able to distinguish CLABSIs from nonbacterial febrile episodes. Lipopolysaccharide (LPS)–specific and flagellin (FLiC)–specific antibodies have previously been studied in adult and pediatric patients with SBS. Adults with parenteral nutrition (PN)–dependent SBS had detectable serum anti-FLiC and anti-LPS and upregulated serum anti–FLiC-specific IgM and IgA and anti–LPS-specific IgA compared with normal controls. In infants with SBS, serum anti-FLiC and anti-LPS IgG levels were significantly lower than in healthy controls but significantly rose over time in patients with SBS. Anti-FLiC and anti-LPS IgA levels were comparable at baseline among healthy controls and patients with SBS. The potential role of systemic levels of immunoglobulins against either LPS or FLiC as biomarkers in bacterial infection in children with IF has not been previously evaluated.

The primary objective of this study was to evaluate changes in the plasma levels of anti-LPS and anti-FLiC immunoglobulins in children with IF during an acute episode of CLABSI compared with a non-CLABSI febrile episode. We also evaluated the trend in these levels following antibiotic treatment for a CLABSI and in comparison to children without IF.

Methods
Patients with IF as identified in our institutional database were recruited for this study following informed parental consent. Patients with IF were defined as children with a primary gastrointestinal (GI) disease requiring at least partial PN to maintain adequate nutrition, hydration, electrolyte balance, and growth for at least 90 days. All patients with PN dependence received standard PN with dextrose, amino acids, and lipids. No alternative lipid strategies such as fish oil–based emulsions were used among study participants. Inclusion criteria for study patients were children with IF between ages 3 months and 4 years, at least partial dependence on PN for 90 consecutive days, and the
presence of a central venous catheter. Patients were excluded from enrollment if they had undergone liver, small bowel, liver/small bowel, or multivisceral transplantation; were diagnosed with an immune disorder; had a current infection other than CLABSI; or had been taking systemic antibiotics or immunosuppressing medication for more than 24 hours prior to enrollment. Patients receiving antibiotic regimens as prophylaxis for SBBO were not excluded. None of the patients with IF were on ethanol locks at the time of enrollment or throughout the study. Patients were enrolled prospectively from September 2008 through October 2009 in the Cincinnati Children’s Hospital Medical Center Comprehensive Nutrition Clinic and during inpatient hospital admissions. This report is a subsequent analysis of available plasma samples (13 of 22) obtained from patients enrolled in a previous study evaluating potential biomarkers for CLABSI.9 For the control population, age-matched participants were selected from the general gastroenterology population and recruited at the time of routine endoscopy for the common indications of abdominal pain and vomiting. After patients were recruited, blood was collected and categorized by patient type/condition into 4 separate groups: IF at baseline (at least 60–90 days prior to or following a CLABSI), IF with blood culture obtained for fever and suspected CLABSI, IF posttreatment (within 10 days of completing therapy for a CLABSI), and non-IF controls. All blood draws from patients with IF were obtained using preestablished central access. In the IF group, a laboratory-confirmed CLABSI was defined according to the criteria established by the National Nosocomial Infections Surveillance System.11 Infections with fungal organisms were excluded since this study was designed to capture bacterial CLABSI episodes. At our institution, patients with fever in the presence of a central line are treated empirically with vancomycin and piperacillin-tazobactam. However, this regimen is modified depending on factors, including drug allergy status, prior infections, organism sensitivity, and clinical symptoms. Duration of therapy is usually 10 days from the first negative culture, although this period can be prolonged accordingly to the clinical status and organism resistance patterns. Multiple distinct CLABSIs could be captured from the same individual patient, but such episodes were at least 30 days apart.

Analysis for plasma FLiC-specific and LPS-specific IgA and IgG antibodies was by enzyme-linked immunosorbent (ELISA) assay, and optical density (OD) was read at 650 nm with an ELISA plate reader, as previously described.2,12

Data Analysis

Statistical analysis was performed using SPSS software (version 19; SPSS, Inc, an IBM Company, Chicago, IL) and GraphPad Prism 5 (version 5.03; GraphPad Software, La Jolla, CA). Student t tests were used to compare anti–FLiC-specific and anti–LPS-specific immunoglobulin values in control plasma samples compared with patients with IF at baseline. Baseline values in these patients were compared with levels obtained at the onset of fever and after completing antibiotics. Paired t test analysis was used to compare anti–FLiC-specific and anti–LPS-specific IgA and IgG levels from baseline, at onset of fever, and after completing antibiotics in patients diagnosed with CLABSI. FLiC- and LPS-specific IgA and IgG plasma levels were also analyzed at baseline among patients with IF and controls. FLiC- and LPS-specific IgA and IgG levels measured in patients with IF during febrile episodes were evaluated using the Student t test to assess if levels differed when
obtained during febrile episodes without CLABSI and CLABSI febrile episodes. Analysis of variance (ANOVA) and Student t test were used to evaluate differences between anti-LPS and anti-FLiC antibodies with type of bacterial organism cultured during a CLABSI. In addition, the ANOVA model adjusted for age was employed to compare levels of anti-FLiC and anti-LPS antibodies at baseline among patients and controls. Statistical significance was based on a 5% significance level.

Post hoc power analysis was performed using Simple Interactive Statistical Analysis software online (“SISA-Binomial,” 1997; http://www.quantitativeskills.como/sisa/distributions/binomial.htm).

Results

There were 13 children (10 boys and 3 girls) with IF with a median age of 11 months (range, 4–55 months). The primary diagnosis associated with IF included necrotizing enterocolitis (NEC) (6), gastrochisis with atresia (4), midgut volvulus (1), malrotation with gastric perforation (1), and spontaneous ileal perforation (1). None of these children had biochemical evidence of IF-associated liver disease when enrolled in this study. The percentage of calories from PN ranged from 13%–100% (median, 56%).

The control group comprised 11 children (5 boys and 6 girls) with normal small bowel length and function. The median age was 19 months (age range, 8–42 months). The clinical diagnoses of these controls were gastroesophageal reflux disease (2), food allergy (1), inadequate intake from oral aversion and dysphagia (4), functional constipation (2), and Chiari I malformation (2).

Prevalence of CLABSI

There were 17 confirmed CLABSI events among 10 of the 13 (77%) children with IF (consistent with our earlier report in pediatric SBS).6 A regimen as prophylaxis for SBBO was being administered during 6 (35%) of these CLABSI events. As patients were followed over time, multiple CLABSI events were captured and observed in 4 of the 10 (40%) who had CLABSI. The number of infections in these patients ranged from 2–4 each. Organisms cultured included Klebsiella pneumoniae, Enterobacter, Escherichia coli, Serratia, and Bifidobacterium. Gram-negative organisms were responsible for 53% (9 of 17) of CLABSI events, with K pneumoniae being the most common Gram-negative organism (5 of 9 events). A smaller proportion (18%) of CLABSI events was due to Gram-positive organisms (3 of 17). Finally, CLABSI events whose cultures grew multiple organisms (polymicrobial) encompassed 29% (5 of 17) of all infectious events. These infections comprised various combinations of Gram-negative and Gram-positive organisms and a fungal species (Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus, Pseudomonas, Citrobacter and a Candida species).

Anti-FLiC and LPS Antibody Response

Anti-FLiC and anti-LPS antibody (IgA and IgG) levels were obtained at baseline in all patients with IF and controls (Figure 1). Timing of baseline sample collection among patients with IF varied. Although most patients (9 of 13) with IF had baseline laboratory
values drawn prior to a febrile episode, there were 4 patients in whom baseline laboratory values were drawn after their initial febrile episodes. The time interval between laboratory draws among this group of patients was 2–4 months. Anti-FLiC IgA and IgG and anti-LPS IgA and IgG were detected in all patients and controls. Patients with IF had significantly higher levels of anti-FLiC IgA compared with controls (mean ± SD; 1.01 ± 0.56 vs 0.52 ± 0.29 OD units, respectively; \( P = .02 \)) and higher plasma levels of anti-LPS IgA compared with controls (1.43 ± 0.10 vs 0.45 ± 0.09, respectively; \( P = .004 \)). There was no difference in plasma levels of anti-FLiC IgG (1.29 vs 1.64; \( P = .40 \)) or anti-LPS IgG (0.85 vs 0.62; \( P = .12 \)) in patients with IF compared with controls at baseline. The ANOVA model, adjusted for age to evaluate baseline antibody levels, found similar results at baseline: levels of anti-FLiC IgA and anti-LPS IgA were significantly elevated in patients with IF compared with controls (mean value, 1.54 vs. 1.11; \( P = .21 \)) or anti-LPS IgG (mean value, 0.92 vs. 1.16; \( P = .45 \)), respectively.

A comparison evaluation of all biomarkers at baseline was also performed considering the variable of prophylaxis for SBBO among patients with IF. Anti-FLiC IgA (\( P = .02 \)), IgG (\( P = .01 \)) and anti-LPS IgA (\( P = .02 \)), IgG (\( P = .01 \)) were all significantly higher in plasma at baseline in patients with IF receiving prophylaxis compared with patients with IF not receiving prophylaxis.

Anti-FLiC and anti-LPS IgA and IgG antibody levels were obtained from the study patients during each febrile episode. Among the 13 patients with IF, there were a total of 27 febrile events. Anti-FLiC IgA was found to significantly increase in all patients during febrile episodes (Figure 2) from a mean value of 1.10–1.32 (\( P = .046 \)). There was no significant difference in anti-FLiC IgG, LPS IgA, and LPS IgG levels during febrile episodes compared with levels obtained in the baseline or prefebrile state.

Further evaluation to assess whether plasma anti-FLiC or anti-LPS antibody levels could distinguish nonbacterial febrile events from CLABSIs among patients with IF was done. There was no difference found in anti-FLiC IgA (mean value, 1.54 vs 1.11; \( P = .21 \)) or anti-FLiC IgG antibody levels (mean value, 1.88 vs 1.50; \( P = .06 \)) comparing non-CLABSI febrile events with CLABSI febrile events. Similarly, there was no difference between these conditions in patients with IF in plasma anti-LPS IgA (1.50 vs 1.66; \( P = .70 \)) or anti-LPS IgG antibody levels (0.92 vs. 1.16; \( P = .45 \)), respectively.

Anti-FLiC and anti-LPS IgA and IgG plasma antibody levels were obtained from patients with IF following treatment for culture-positive CLABSI (within 10 days of completing antibiotics). These results were compared with levels at baseline and at the time of a CLABSI in patients with IF to ascertain significant trends. There was no significant difference following treatment in anti-FLiC IgA (mean value, 1.37 vs 0.87; \( P = .57 \)), anti-FLiC IgG (mean value, 1.17 vs. 0.98; \( P = .47 \)), anti-LPS IgA (mean value, 0.98 vs. 1.59; \( P = .51 \)), or anti-LPS IgG (mean value, 0.79 vs. 1.12; \( P = .25 \)) antibody levels compared with baseline levels (Figure 3).

Additional analysis on anti-FLiC and anti-LPS IgA antibody levels was performed to distinguish a possible correlation between change (increase or decrease) of antibody
response and type of infection (Gram positive, Gram negative, polymicrobial). There was no association between anti-LPS or anti-FLiC antibody levels in plasma and type of infection.

A 2-sample, double-sided post hoc power analysis revealed that a sample size of 11 in the control and subject groups would attain a power of 0.71 (n = 11; α = 5%) to detect a statistical difference between anti-FLiC and anti-LPS IgA and IgG at baseline and with fever.

A comparison analysis was performed with the STREM-1 and LBP data obtained from these patients and previously published to determine possible relationships among these biomarkers. This was possible given that all the data were obtained simultaneously in the same patients. For this analysis, we specifically looked at anti-FLiC IgA only in comparison with STREM-1 and LBP given that anti-FLiC IgA was the only antibody that increased in febrile episodes. Our analysis revealed that the change in levels of anti-FLiC IgA from baseline to a febrile episode is marginally correlated to the degree of change observed in levels of STREM-1 (r = 0.36, P = .06). There was no relationship between changes in anti-FLiC IgA and LBP.

Discussion

CLABSIs pose a frequent and real challenge in the lives of patients with IF. Although various preventive measures have been established, reducing the incidence of CLABSI in this population remains a challenge. Due to the morbidity and mortality associated with a CLABSI in IF, these children are routinely hospitalized at the time of the febrile episode pending blood culture results. Identification of biomarkers that can reliably differentiate bacterial infections from other causes of fever could make a significant impact in clinical care for these children and their providers.

The primary objective of this study was to evaluate changes in the plasma levels of anti-FLiC and anti-LPS antibodies in children with IF during an acute episode of CLABSI. Our data revealed that anti-FLiC IgA and anti-LPS IgA levels were present in higher concentrations at baseline in patients with IF compared with age-matched non-IF controls. This is likely due to increased mucosal permeability and Gram-negative bacterial translocation, as previously described in this population. Also, these antibody levels have been shown to increase with age, and the infants enrolled in this study were older than those in previous reports. Levels of anti-FLiC and anti-LPS IgG antibodies did not differ between controls and patients with IF at baseline. One possible explanation for this observation may be the nutrition status of both controls and patients with IF. Most controls did have chronic conditions (vomiting, oral aversion, and dysphagia) with poor weight gain, which may have rendered their protein status substandard and thus approximating nutrition conditions in children with IF. Another reason may be timing of samples. In a separate study, levels of anti-FLiC and anti-LPS IgG were lower than controls at baseline but increased over a 4-month period to levels above control subjects. This study further confirms that levels of anti-FLiC and anti-LPS IgG rise over a period of months following bacterial translocation and overgrowth events.
In addition, our results provide unique information regarding the production of anti-FLiC IgA and anti-LPS IgA in response to fever. Anti-FLiC IgA levels increase with fever, but this marker does not differentiate between fever due to bacterial CLABSI and other causes of fever (eg, mucosal inflammation, nonsystemic infections). Anti-FLiC IgA and anti-LPS IgG levels in plasma at baseline and during febrile levels were similar. A possible reason for these findings is the half-life for these antibodies. The known half-life of IgA antibodies is approximately 5 days, while the half-life of IgG is approximately 20 days. IgA levels rise in the acute response phase to an infection while the increase in IgG is delayed. With this understanding, the timing of blood sample collections may not have provided a sufficient interval between the onset of fever and antibody formation in order for us to detect an appreciable increase in IgG levels. Furthermore, 4 of 13 (31%) patients with IF had baseline anti-FLiC and anti-LPS antibody levels obtained following an infection or between infections and not in a traditional longitudinal fashion (ie, prior to first infection). The time interval among these 4 patients between laboratory draw and last infection was 60–90 days. When these 4 patients were removed from the analysis, our results did not differ significantly (data not shown).

The limitations to our study include the study size of 13 patients and 11 controls with a calculated power of 71%. Selection bias should also be considered since the blood samples used (n = 13) were the remaining samples used in a previous study where potential biomarkers were analyzed in 22 patients with IF. Almost half of our patients with IF (6 of 13 [46%]) received cycled antibiotics enterally as prophylaxis for SBBO. However, there was no uniformity with regard to the prophylaxis regimen used or timing in regard to duration between the start of such a regimen and the development of a CLABSI. Among patients with IF receiving a prophylaxis regimen, 4 of 6 (66.7%) were taking oral metronidazole, 1 received rifaximin, and another had a combination of colistin, tobramycin, and nystatin. Antibodies against FLiC and LPS were elevated statistically at baseline in patients with IF who received a regimen as prophylaxis compared with patients with IF who were not on any similar regimen. A possible explanation for this is the distinction among genera of bacteria that are able to translocate vs those that have the propensity to cause SBBO. Grampositive anaerobes are frequently responsible for malabsorption in SBBO by deconjugating bile acids, while Gram-negative aerobes have a greater ability to translocate across the mucosal barrier into the portal circulation. Theoretically, if the genera of bacteria that cause SBBO are being suppressed with prophylaxis (ie, metronidazole), the likelihood for the remaining flora (ie, Gram-negative aerobes) to proliferate and subsequently translocate is greater. This increased rate of translocation could therefore lead to further activation of inflammatory pathways as well as a greater number of CLABSI events. Since the diagnosis of SBBO was not part of the study interventions, the relationship between the use of prophylaxis for bacterial overgrowth and biomarker levels could not be interpreted. More specifically, the degree to which an antibacterial regimen for SBBO could alter measurable antibody formation to FLiC or LPS immunoglobulins needs to be evaluated.

A second objective in our study was to determine if anti-FLiC and anti-LPS antibodies decline following therapy for CLABSI. Although our study revealed that anti-FLiC IgA
levels increase with febrile episodes, levels of anti-FLiC or anti-LPS antibodies did not differ significantly between CLABSI and non-CLASBSI febrile episodes. There was not a significant fall in antibody levels following treatment. This may be explained partially by the half-life of IgA and IgG antibodies. The posttreatment measurements done 10 days after completion of therapy may not have provided sufficient time for antibody levels to reach their peak and appropriately reflect a true upward or downward trend. Several studies have shown that peak levels of anti-LPS and anti-LBP antibodies occur 2–3 days after onset of infection. Therefore, detectable differences that are statistically significant following treatment for a CLABSI would be more difficult to obtain in these patients.

A subsequent evaluation was directed to determine if the degree of change in biomarker levels with CLABSI was associated with the type of organism responsible for the CLABSI. Although the largest degree of increase in biomarker levels occurred with anti-LPS IgA and Gram-negative infections compared with both Gram-positive and polymicrobial infections, no statistically significant relationship was identified. Antibodies directed toward LPS have been found in the serum of different types of infections, including fungal and Gram-positive organisms, and are more specific to Gram-negative infections. The degree to which this occurs may be substantial enough to contribute to the level of detectable antibody in our patients.

In summary, both anti-FLiC and anti-LPS antibodies are present at higher levels in the plasma of patients with IF at baseline compared with patients without IF. Anti-FLiC IgA antibody levels rise with the onset of fever but do not differentiate between nonbacterial febrile illnesses and CLABSIs. Future considerations for these and other potential biomarkers should include a larger sample size powered to determine potential relationships and longitudinal blood collections. In addition, the role of antibiotics as prophylaxis for SBSO should be considered a potential confounder for the incidence of CLABSIs and the development of anti-FLiC and anti-LPS antibodies in the plasma of children with IF due to SBS and other causes.

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References

Biomarkers directed at identifying central line–associated bloodstream infections in children with intestinal failure are a clinical necessity. This work evaluates the potential use of antiflagellin (FLiC) and anti-lipopolysaccharide (LPS) immunoglobulins in the diagnosis of bacterial-derived infections in pediatric patients with intestinal failure. Among patients with intestinal failure, anti-FLiC IgA antibody levels increased with the onset of fever. Furthermore, we reveal potential interactions between small bowel bacterial overgrowth prophylaxis and baseline levels of both anti-FLiC and anti-LPS antibodies and the incidence of central line–associated bloodstream infections.
Figure 1.
Comparison of baseline anti-flagellin (FLiC) IgA and anti-lipopolysaccharide (LPS) IgA antibody levels among controls and patients with intestinal failure (IF). Patients with IF had higher levels of anti-FLiC IgA (1.01 vs 0.52; \( P = .02 \)) and higher levels of anti-LPS IgA (1.43 vs 0.45; \( P = .004 \)) compared with controls.
Figure 2.
Levels of anti-flagellin (FLiC) IgA antibody at baseline compared with time of febrile episodes in patients with intestinal failure (IF). Anti-FLiC IgA increased in all patients with IF during febrile episodes (mean value of 1.10–1.32; \( P = .046 \)). There was no statistical change in anti-lipopolysaccharide (LPS) IgA.
Figure 3.
Trends in anti-flagellin (FLiC) IgA, IgG and anti-lipopolysaccharide (LPS) IgA, IgG antibody levels from baseline to time of infection to posttreatment in patients with intestinal failure (IF). There was no statistical difference in mean antibody levels following treatment in anti-FLiC IgA (1.37 vs. 0.87; \( P = .57 \)), anti-FLiC IgG (1.17 vs. 0.98; \( P = .47 \)), anti-LPS IgA (0.98 vs. 1.59; \( P = .51 \)), or anti-LPS IgG (0.79 vs. 1.12; \( P = .25 \)) compared with baseline levels.