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Original Article

Ultrasonographic study of intestinal Doppler blood flow in infantile non-IgE-mediated gastrointestinal food allergy

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AGE, acute viral gastroenteritis;
ALPT, antigen-specific lymphocyte proliferation test; ANOVA, analysis of variance; AUC, area under the curve; Eos, blood eosinophil counts; ES, effect size; FEIA, fluorescence enzyme immunoassay; FPE, food protein-induced enteropathy syndrome; FPIES, food protein-induced enterocolitis syndrome; FPIP, food protein-induced proctocolitis syndrome; IgE, immunoglobulin E; OFCT, oral food challenge test; ROC, receiver operating characteristic; US, ultrasonography; VD, vessel density

ABSTRACT

Background: Although non-IgE-mediated gastrointestinal food allergy has increased rapidly in Japan, a small number of reports has evaluated B-mode and Doppler ultrasonographic findings in the acute phase of infantile gastrointestinal milk allergy. The aim of the present study was to compare the diagnostic utility of ultrasonographic findings and laboratory allergic data in non-IgE-mediated infantile gastrointestinal milk allergy.

Methods: Sixteen cases of active non-IgE-mediated infantile gastrointestinal milk allergy, diagnosed by food elimination tests and oral food challenge tests (OFCTs) (group A), 15 cases of acute viral gastroenteritis (AGE) (group B), and 15 controls (group C) were enrolled. 1) B-mode abdominal ultrasound findings, 2) laboratory allergic data including eosinophil counts (Eos), serum IgE, and the antigen-specific lymphocyte proliferation test (ALPT) against milk protein, and 3) vessel density (VD) indirectly quantified by gastrointestinal Doppler flow at jejunum, ileum, and sigmoid colonic mucosae were evaluated and compared among the groups.

Results: In the small intestine, wall thickening, dilation, mesenteric thickening, and poor peristalsis were found in 100%, 62.5%, 93.7%, and 100%, respectively, in group A. Eos, IgE, ALPT, and VD were positive in 25.0%, 0%, 87.5%, and 100%, respectively, in group A. Small intestinal VD was significantly greater in group A than in groups B (jejunum $p < .001$; ileum $p < .001$) and C (jejunum $p < .001$; ileum $p < .001$), with no significant differences between groups B and C (jejunum: $p = .74$; ileum: $p = .73$).

Conclusions: Abdominal Doppler ultrasonography and small intestinal VD at symptomatic state can support the diagnosis and evaluation of non-IgE-mediated infantile gastrointestinal milk allergy with symptoms of vomiting, diarrhea, and failure to thrive.

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Introduction

Cow's milk allergy, which is a food allergy induced by cow's milk proteins, is a common disease in pediatric patients in Western countries.^{1,2} However, the frequency of gastrointestinal food allergy

in infants has been a matter of discussion, and accurate estimates are difficult to obtain.³ Early exposure of the infantile gastrointestinal tract to cow's milk protein is considered one of the causes of immunologic responses and intestinal inflammation of variable intensity, both in IgE-mediated and non-IgE-mediated cow's milk allergy.^{4,5} Non-IgE-mediated gastrointestinal food allergy including cow's milk allergy has also been increasing rapidly since the end of the 1990s in Japan. Non-IgE-mediated gastrointestinal food allergy develops after starting breast milk or formula in the neonatal or infancy period and includes food protein-induced enterocolitis syndrome (FPIES), food protein-induced proctocolitis syndrome

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(FPIP), and food protein-induced enteropathy syndrome (FPE), which are already established in Europe and the United States.^{4,6–10} For diagnosis, it is important to confirm the improvement of symptoms by elimination of the causative foods and the evocation of symptoms after oral food challenge test (OFCT).¹¹ However, not all cases have undergone OFCT due to medical and social circumstances, and comprehensive diagnosis by other examinations is required.¹² Several other factors also make diagnosis of gastrointestinal food allergy difficult. Most symptoms of gastrointestinal food allergy are non-specific, such as failure to thrive, irritability, and intense crying, and no disease-specific findings of laboratory data and pathological findings that directly lead to the diagnosis of gastrointestinal food allergy have been reported.^{13,14}

In recent years, pediatric ultrasonography has been disseminated worldwide, since ultrasonography is noninvasive and has no radiation exposure. Gray-scale ultrasonography, which is synonymous with B-mode ultrasonography, is effective in detecting thickening of the gastrointestinal wall, which is triggered by infectious and inflammatory gastrointestinal diseases.^{15,16} Color Doppler ultrasonography is also effective in detecting active inflammation with increased blood flow signals.^{17,18} In the acute phase of infantile gastrointestinal milk allergy, a very small number of reports has evaluated B-mode and color Doppler ultrasonography.^{19,20} The aim of this study was to compare the diagnostic accuracies of ultrasonographic findings and laboratory data, including the antigen-specific lymphocyte proliferation test (ALPT), in 16 infants diagnosed with non-IgE-mediated infantile gastrointestinal milk allergy.

Methods

Patient population

During the two-year period between 2014 and 2015 at our institute, three groups (groups A, B, and C) of Japanese children were enrolled in the present study. All patients were admitted to our institute from outpatient through the direct consultation or referral from another hospital. Group A included 16 cases of active non-IgE-mediated infantile gastrointestinal milk allergy associated with formula milk. The criteria for enrolment to group A included all of the following: 1) having gastrointestinal symptoms such as vomiting, bloody stool, diarrhea, and failure to thrive; 2) having no history of anaphylaxis such as respiratory failure or shock in the acute phase; 3) disappearance of gastrointestinal symptoms by a food elimination test; 4) no reproduction of the same gastrointestinal symptoms by drinking hydrolyzed formulas; and 5) reproduction of gastrointestinal symptoms by an OFCT with formula milk. Group B included 15 cases of acute viral gastroenteritis (AGE) who had not been immunized with oral rotavirus vaccines. The inclusion criteria were: positive findings of both fecal viral antigen tests, such as rotavirus and adenovirus, and B-mode ultrasonographic findings, such as multiple mesenteric lymphadenopathy and poor intestinal peristalsis. Lymph node enlargement was defined as a lymph node whose maximum diameter was at least 5 mm. Group C included 15 controls, who showed neither gastrointestinal symptoms nor significant B-mode abdominal ultrasonographic findings, but had pneumonia or urinary tract infections. Additionally, low-birth weight infants whose birth weight was lower than 2000 g and preterm infants whose gestational age was less than 37 weeks were excluded. Infants with a prior history of abdominal surgery and genetic, hematological, and metabolic diseases were also excluded. All study protocols were approved by the Institutional Ethics Committee, and informed consent for participation was obtained from the parents of all children prior to their enrolment in the study.

Study design

This study consisted of three phases. The first phase was observation of group A children. The B-mode abdominal ultrasonographic findings, such as wall thickening of the small bowel and colon, small bowel dilation, mesenteric thickening, and peristalsis of the small bowel, of group A patients in the symptomatic phase were observed. Groups A and B with gastrointestinal symptoms underwent ultrasonographic examination before the diagnosis of non-IgE-mediated infantile gastrointestinal milk allergy and AGE and group C underwent ultrasonographic examination immediately after the diagnosis. Wall thicknesses of the small bowel and colon were defined as normal if the thicknesses were less than 3.0 mm and 4.0 mm, respectively. Thickness was measured by placing the pointer between the mucosal surface and the serosa in the same bowel loop. The small bowel was considered dilated if the diameter was more than 22 mm.²¹ Mesenteric thickening was confirmed when the mesentery showed high echogenicity with internal vessels and small lymph nodes on ultrasonography, since these changes are not detected in the normal state (Fig. 1).

The second phase of this study was a comparison of various laboratory examinations, including blood eosinophil counts (Eos), serum IgE, fluorescence enzyme immunoassay (FEIA) against milk components (β -lactoglobulin and casein), ALPT against milk components (β -lactoglobulin, lactoferrin, and κ -casein), and vessel density (VD).²⁰ The cut-off values of Eos, IgE, ALPT- β -lactoglobulin, ALPT-lactoferrin, and ALPT- κ -casein were defined as 700/ μ L, 20 IU/mL, 1.78 SI, 2.7 SI, and 1.59 SI, respectively.

The third phase of this study was to compare the fasting VD in the jejunum, ileum, and sigmoid colon in groups A, B, and C. In addition, VD was again measured one week after the disappearance of clinical symptoms with treatment, and VD was compared among the post-treatment A, B, and C groups (group A', B', and C') and between before and after treatment of groups A, B, and C, respectively. In addition, the sensitivity and the specificity of each Eos, ALPT against β -lactoglobulin, lactoferrin, and κ -casein, and jejunal, ileal, and sigmoidal VD were calculated in order to compare the accuracy of each examination in differentiating acute non-IgE-mediated gastrointestinal food allergy and others. The primary outcome of this study was to examine whether VD was more useful than other examinations in the differentiation of non-IgE-mediated gastrointestinal food allergy from AGE and controls.

Vessel density (VD)

Measurement of VD, measurement was limited to the intestinal tract and mesentery (interstitial), and the site where blood flow was delineated most strongly with power Doppler imaging, which measures the scalar quantity of blood flow better than color Doppler, was selected. The gain of the power Doppler imaging was adjusted to eliminate signal artifacts but retain vessel signals. The ultrasonographic equipment was adjusted to detect slow-velocity blood flow at a velocity of 8 cm/s with a low wall filter in this study. In addition, ultrasonographic studies were performed when the patients were either sleeping or not moving to avoid signal artifacts. The measurement range, in which large blood vessels, muscle, and other solid organs were excluded, was adjusted to 4 cm² in the jejunum and ileum and 2 cm² in the sigmoid colon, because the space between the abdominal wall and the iliopsoas muscle was limited. These ultrasonographic images were converted to two colors, in which the power Doppler flow-signal was white, and the others were red, with free software (ImageJ, National Institutes of Health, Bethesda, MD), and the percentage of the white colored area in the 2 or 4 cm² area was calculated. The average VD value was calculated from four Doppler images without the effects of body

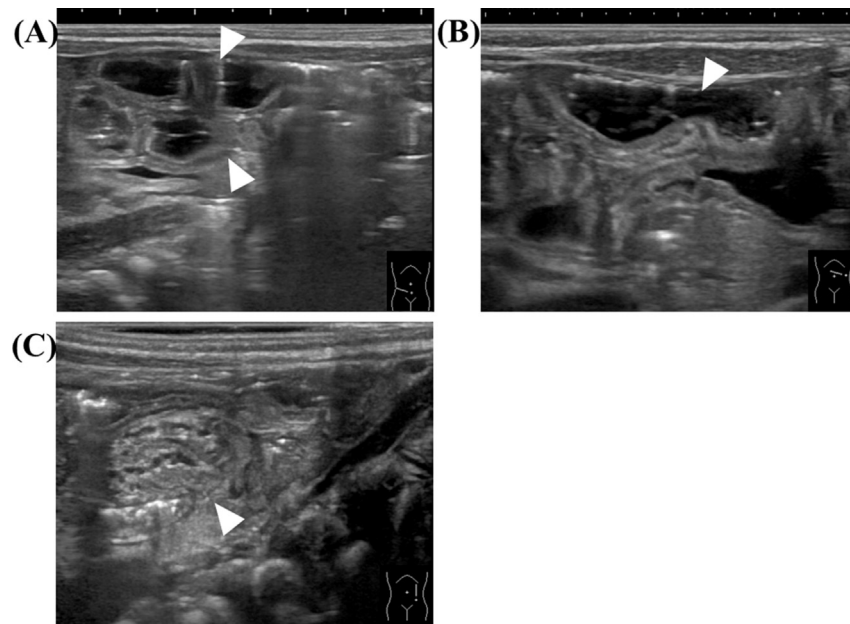


Fig. 1. B-mode abdominal ultrasound findings of group A. (A) Wall thickening of the ileum (arrow head) with edema. (B) Bowel dilation of the jejunum (arrow head). (C) The jejunal mesenteric thickening shows a high echogenic mass-like lesion (arrow head) including internal vessels and small lymph nodes.

movement to reduce selection bias (Fig. 2). As in a previous report,²⁰ a VD value of more than 18.7% was regarded as a positive finding.

Ultrasonographic findings

All ultrasonographic images in this study were visualized with a HI VISION Preirus® (Hitachi-Aloka Medical, Tokyo, Japan) and an 8- to 12-MHz high-frequency linear transducer EUP-L54MA® (Hitachi-

Aloka Medical). Ultrasonographic findings were confirmed by two physicians (Jimbo K and Kono T) and a technician (Asai N) familiar with pediatric ultrasonographic diagnosis. In addition, ultrasonographic examinations were performed by the same examiner (Jimbo K) with 14 years of experience in pediatric sonography, and ultrasonographic findings were accepted when the interpretations of the two physicians (Jimbo K and Kono T) and technician (Asai N) matched.

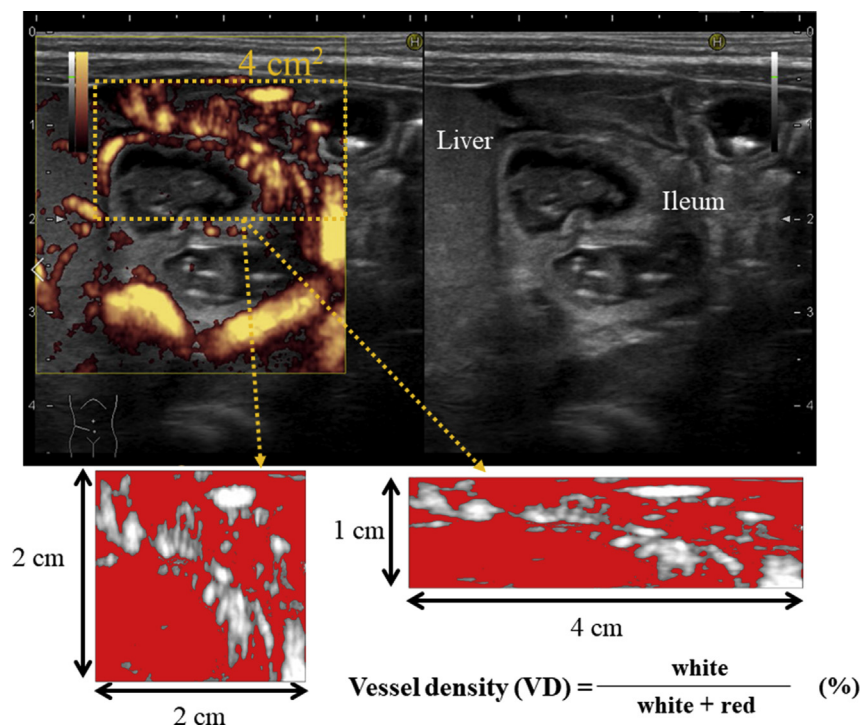


Fig. 2. Method for calculating vessel density (VD). The measurement range is adjusted to 4 cm² in the jejunum without large blood vessels, muscle, and other solid organs. Power Doppler flow signals and the other signals are converted to red and white, respectively, with ImageJ, and the percentage of the red in the 4 cm² area is calculated.

Statistics

The VD values of groups A, B, and C were analyzed for each measurement site by the Mann–Whitney U test, and a p -value less than .05 was considered significant. In addition, receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic performance of each VD with regards to Eos, ALPT- β -lactoglobulin, ALPT-lactoferrin, and ALPT- κ -casein. The areas under the ROC curve (AUC) were compared for significant differences between parameters, and the cut-off values with the point on the ROC curve closest to (0, 1), as well as sensitivity and specificity, were calculated. A p -value < .05 was considered significant. The statistical analyses were performed with BellCurve for Excel (Social Survey Research Information, Tokyo, Japan).

Results

Clinical characteristics

Sixteen children (2.9 ± 3.6 months old) with non-IgE-mediated infantile gastrointestinal milk allergy (group A), 15 children (10.4 ± 2.4 months old) with AGE (group B), and 15 controls (7.3 ± 3.5 months old) (group C) were enrolled in this study. Group A children were diagnosed as having non-IgE-mediated gastrointestinal food allergy between 1 week and 12 months after birth. There was no apparent sex disparity in group A patients. Chief complaints included vomiting in 75.0% (12/16), bloody stool in 12.5% (2/16), watery diarrhea in 37.5% (6/16), and failure to thrive in 37.5% (6/16). Based on these gastrointestinal symptoms, group A children were classified into three categories (clusters 1, 2, and 3), as reported previously¹²; 1 patient, 7 patients, and 3 patients were categorized in clusters 1, 2, and 3, respectively. However, four children could not be clearly distinguished between clusters 2 and 3, and one child could not be distinguished between clusters 3 and 4 based on the clinical manifestations (Table 1).

B-mode ultrasonographic findings of group A

On B-mode ultrasonographic analysis, small bowel wall thickening, small bowel dilation, mesenteric thickening, and poor peristalsis of the small bowel were found in 100% (16/16), 62.5% (10/16), 93.7% (15/16), and 100% (16/16), respectively, of group A patients. In addition, colonic wall thickening was also found in one case in cluster 1 and another case in cluster 3 + 4 (12.5%) (Table 2). These gastrointestinal ultrasonographic findings disappeared a few days after the milk elimination tests in all group A patients. In addition, the reproduction of similar ultrasonographic findings by an oral milk challenge test was also confirmed within several days in all group A patients.

Comparison of various data among the three groups

Eos was elevated in 25.0% (4/16), 20.0% (3/15), and 13.3% (2/15) of group A, B, and C patients, respectively. In 87.5% (14/16), 20.0% (3/15), and 20.0% (3/15) of group A, B, and C patients, ALPT was positive to one of β -lactoglobulin, lactoferrin, or κ -casein. The VD values were higher than 18.7% in all group A cases, including 2 ALPT-negative cases, and one group B case in either the jejunum or ileum. In contrast, no VD value was increased in the sigmoid colon in any cases, although slight elevation of VD was observed in a group A case with colonic wall thickening (Table 3).

Comparison of vessel density among the three groups

In the acute phase, VD was significantly higher in group A than in groups B ($p < .001$, effect size (ES) 3.7) and C ($p < .001$, ES 4.6) in the jejunum. In the ileum, VD was significantly higher in group A than in groups B ($p < .001$, ES 3.0) and C ($p < .001$, ES 3.5). In contrast, no significant differences were found in jejunal and ileal VD between groups B and C (jejunum $p = .74$; ileum $p = .73$). In addition, there were no significant differences in sigmoidal VD among the 3 groups (A vs B, $p = .31$; A vs C, $p = .54$) (Fig. 3). In the remission phase, there were no significant differences in jejunal, ileal, and sigmoidal VD among the 3 groups (jejunal A' vs B', $p = .17$; A' vs C', $p = .18$; B' vs C', $p = .77$; ileal A' vs B', $p = .06$; A' vs C', $p = .52$; B' vs C', $p = .06$; sigmoidal A' vs B', $p = .76$; A' vs C', $p = .27$; B' vs C', $p = .32$) (Fig. 3). In the comparison between group A and A', significant differences were found in jejunal and ileal VD (jejunum $p < .001$, ES 4.1; ileum $p < .001$, ES 3.2; sigmoid $p = .27$). On the other hand, there were no significant differences in the comparisons of jejunal, ileal, and sigmoidal VD between groups B and B' (jejunum $p = .90$; ileum $p = .29$; sigmoid $p = .74$) and groups C and C' (jejunum $p = .96$, ileum $p = .96$, sigmoid $p = .18$) (Fig. 3).

Diagnostic performance of vessel density

The diagnostic performance of VD in differentiating non-IgE-mediated gastrointestinal food allergy (group A) and others (group B + C) is summarized in Table 4. Among Eos, ALPT- β -lactoglobulin, ALPT-lactoferrin, ALPT- κ -casein, jejunal VD, ileal VD, and sigmoidal VD, jejunal and ileal VD had significantly higher AUCs for differentiation of non-IgE-mediated gastrointestinal food allergy from AGE and controls than other parameters (AUC = 0.995 and 0.987, respectively). ALPT- β -lactoglobulin and ALPT-lactoferrin also had significantly higher AUCs (AUC = 0.768 and 0.831, respectively). Despite this, there were significant differences between jejunal VD and ALPT- β -lactoglobulin and ALPT-lactoferrin ($p = .005$ and $.013$, respectively; Fig. 4) and between ileal VD and ALPT- β -lactoglobulin and ALPT-lactoferrin ($p = .009$ and $.025$, respectively; Fig. 4).

Discussion

In the present study, the usefulness of B-mode and power Doppler ultrasonography as a diagnostic tool for non-IgE-mediated gastrointestinal food allergy in infancy was evaluated. Color Doppler was the function that was standard on almost all abdominal ultrasonographic equipment. B-mode ultrasound (US) findings of active non-IgE-mediated gastrointestinal food allergy were evaluated first. Then, an ultrasonographic diagnostic method such as VD was compared with other clinical exams such as Eos, IgE, FEIA, and ALPT. Finally, the VD values of gastrointestinal food allergy cases were compared with those of normal controls and AGE patients. VD, which was first reported by Epifanio, changed the concept of gastrointestinal Doppler flow from merely “visual findings” to a “quantifying image analysis tool”.²⁰ In a previous report, they evaluated the quantity of peripheral mesenteric vessels in a 4 cm² area with color Doppler imaging. Saved ultrasonographic images were analyzed with ImageJ, and they calculated the percentage of colored vessel areas in the 4 cm² tissue sample area. They calculated the VD cut-off value with receiver operating characteristic (ROC) curve analysis and showed that, when VD was 18.7% or more, children with cow's milk allergy and healthy children could be clearly distinguished with sensitivity of 81.8%, specificity of 94.1%, positive predictive value of 90.0%, and negative predictive value of 88.9%. However, there were no data about VD in other diseases. In the present study, VD was examined

quantitatively not only in non-IgE-mediated gastrointestinal food allergy, but also in AGE, and it was found that non-IgE-mediated gastrointestinal food allergy and AGE or normal controls were clearly distinguished. Statistical tests of the variance of jejunal VD values measured by a single examiner (Jimbo K) at the first and second examinations of 16 patients with non-IgE-mediated gastrointestinal food allergy showed no significant difference between the measurements (one-way analysis of variance [ANOVA], $p = .8129$). Moreover, there was no significant difference (one-way ANOVA, $p = .8632$) in the variance of jejunal VD values measured in another 5 patients with non-IgE-mediated gastrointestinal food allergy by two examiners (Jimbo K and Arai N with three years of experience in pediatric sonography).

In the present study, the diagnosis of non-IgE-mediated gastrointestinal food allergy was made by confirming both the disappearance of clinical symptoms by the milk elimination test and the reproduction of symptoms by the milk challenge test, which met the diagnostic gold standard for gastrointestinal allergy. Among the cases, there was 1 patient in cluster 1, 8 in cluster 2, 2 in cluster 3, 3 in cluster 2&3, and one in cluster 3&4. Except for 4

Table 2

B-mode ultrasound findings of group A.

Cluster	Classification	Small bowel wall thickening	Small bowel dilation	Mesenteric thickening	Poor peristalsis of the small bowel	Colonic wall thickening
1	1	P		P	P	P
2	2	P		P	P	
3	2	P	P	P	P	
4	2	P	P	P	P	
5	2	P	P	P	P	
6	2	P	P	P	P	
7	2	P		P	P	
8	2	P	P	P	P	
9	2	P	P	P	P	
10	2 (+3)	P	P	P	P	
11	2 (+3)	P	P	P	P	
12	2 (+3)	P	P	P	P	
13	3	P			P	
14	3	P	P	P	P	
15	3	P		P	P	
16	3 (+4)	P		P	P	P

P, positive.

Table 1

Details of the three groups.

		Age (months)	Weight (g)	Male/female	Gestational age	Birth weight (g)	Vomiting	Bloody stool	Diarrhea	Failure to thrive	Cluster classification
Group A	1	0.3	2230	F	38w6d	2230	P	P			1
	2	3	5210	M	38w3d	3000	P				2
	3	0.5	3862	F	39w6d	3248	P				2
	4	1	4590	F	38w5d	3240	P				2
	5	0.25	2890	F	40w1d	2992	P				2
	6	0.25	3010	M	39w2d	3028	P				2
	7	0.25	2780	F	36w5d	2130	P				2
	8	0.25	3220	F	40w2d	2996	P				2
	9	7	7530	F	37w1d	2340	P		P		2 (+3)
	10	0.5	3300	M	37w3d	3060	P			P	2 (+3)
	11	4	6250	M	38w2d	3204	P			P	2 (+3)
	12	9	7900	M	41w6d	3420	P		P	P	2 (+3)
	13	12	6985	M	38w0d	2212			P	P	3
	14	4	5115	F	37w3d	3344			P	P	3
	15	7	7585	M	37w3d	2465			P	P	3
	16	1	3650	M	40w0d	3028		P	P		3 (+4)
Group B	1	8	7.330	M	39w0d	3141	P		P		
	2	6	7.800	F	40w2d	4183	P		P		
	3	12	9.312	M	40w0d	3561	P		P		
	4	6	6.903	M	38w4d	3012	P		P		
	5	8	7.012	F	39w3d	3297	P		P		
	6	12	10.39	M	40w4d	4465	P		P		
	7	12	10.59	F	37w1d	2711	P				
	8	8	7.128	F	39w0d	2918	P				
	9	12	9.101	M	39w2d	3510	P				
	10	12	9.900	M	41w1d	3986	P				
	11	12	10.18	M	40w5d	3020			P		
	12	12	10.05	F	39w1d	4003			P		
	13	12	9.102	F	39w6d	3649			P		
	14	12	9.109	M	38w6d	3222			P		
	15	12	10.39	M	41w0d	4110			P		
Group C	1	11	7.810	F	39w1d	3610					
	2	4	6.740	F	38w0d	2981					
	3	1	5.013	M	41w3d	3892					
	4	5	6.700	M	39w2d	2934					
	5	5	8.595	M	40w3d	3387					
	6	8	10.53	M	39w6d	4119					
	7	8	6.305	F	37w4d	2654					
	8	8	6.285	F	37w1d	2734					
	9	0.5	3.402	F	39w1d	2662					
	10	11	9.308	F	39w1d	3116					
	11	10	9.628	M	38w6d	3652					
	12	11	9.361	F	39w0d	3619					
	13	10	8.602	M	38w1d	3212					
	14	10	7.519	F	37w3d	2985					
	15	8	7.821	F	38w3d	3001					

F, female; M, male; P, positive.

Table 3
Comparison of various data among the three groups.

		Eos (/μL)	IgE (IU/mL)	FEIA (class)		ALPT (SI)			VD (%)		
				βLG	Ca	βLG	Lf	κ-Ca	Jejunum	Ileum	Sigmoid
Group A	1	408	5	0	0	4.35 P	10.9 P	1.53	35.6 P	33.7 P	14.5
	2	515	10.2	0	0	1.20	3.79 P	0.79	41.7 P	19.6 P	4.69
	3	586	8.9	0	0	8.03 P	33.2 P	1.22	28.1 P	28.4 P	1.73
	4	50	<5.0	0	0	2.21 P	5.37 P	0.32	35.3 P	34.3 P	1.75
	5	770 P	<5.0	0	0	1.16	16.9 P	1.61 P	33.8 P	27.3 P	13.5
	6	890 P	13	0	0	6.43 P	6.33 P	7.75 P	36.4 P	33.6 P	5.02
	7	71	<5.0	0	0	5.53 P	9.75 P	0.71	28.4 P	24.3 P	7.80
	8	1150 P	<5.0	0	0	5.66 P	20.6 P	1.33	30.4 P	31.1 P	6.55
	9	50	11.9	0	0	0.57	5.97 P	0.86	38.6 P	22.3 P	3.19
	10	350	<5.0	0	0	2.60 P	4.02 P	1.57	33.8 P	20.1 P	3.99
	11	469	19.2	0	0	1.23	1.73	0.12	27.8 P	22.3 P	13.2
	12	113	18.6	0	0	0.65	5.38 P	1.58	20.3 P	23.3 P	2.71
	13	137	8.7	0	0	2.51 P	13.6 P	0.37	25.5 P	16.8 P	0.88
	14	65	<5.0	0	0	1.29	13.1 P	0.12	32.1 P	7.85	3.06
	15	504	6.6	0	0	1.07	1.05	0.20	19.9 P	19.3 P	2.81
	16	1150 P	<5.0	0	0	1.20 P	3.45 P	6.75 P	26.1 P	19.3 P	13.3
Group B	1	380	11.5	0	0	1.11	2.33	1.31	9.76	4.67	3.98
	2	90	14.8	0	0	1.32	4.02 P	1.71 P	16.7	8.43	9.33
	3	350	18.1	0	0	0.95	1.78	0.89	10.6	3.66	2.82
	4	200	10.4	0	0	1.19	1.88	1.27	14.2	6.89	4.55
	5	680	16.9	0	0	0.68	1.62	1.03	9.83	6.52	2.36
	6	793 P	36.7 P	1	0	0.88	6.32 P	0.73	2.74	3.24	3.23
	7	181	17.1	0	0	0.71	1.92	0.97	2.34	5.16	0.48
	8	321	11.0	0	0	1.02	1.20	0.91	8.96	2.26	0.78
	9	950 P	15.1	0	0	1.08	2.31	1.00	3.36	2.83	1.50
	10	290	18.9	0	0	0.64	2.01	0.83	24.7 P	4.05	4.43
	11	78	5.6	0	0	0.93	1.97	0.91	6.33	12.3	11.3
	12	592	13.2	0	0	0.81	0.91	0.71	9.22	15.2	11.6
	13	1428 P	19.8	0	0	1.68	2.11	0.85	4.44	15.5	8.87
	14	183	7.1	0	0	1.27	2.09	1.38	3.09	1.55	0.24
	15	649	33.4 P	1	1	1.54	5.81 P	1.25	5.83	4.95	3.31
Group C	1	210	6.8	0	0	1.33	4.56 P	0.88	2.82	4.32	5.19
	2	85	3.5	0	0	0.70	0.83	0.81	5.43	4.08	8.37
	3	40	19.1	0	0	0.45	0.91	0.47	13.9	7.60	5.50
	4	130	8.2	0	0	1.19	1.36	0.60	10.9	6.33	8.07
	5	164	9.3	0	0	0.90	1.29	1.11	7.75	2.35	4.96
	6	121	9.7	0	0	0.89	0.92	0.78	12.2	3.49	3.71
	7	503	13.8	0	0	0.71	1.92	0.97	5.96	6.67	2.88
	8	370	17.1	0	0	1.37	0.81	1.03	10.3	5.89	4.36
	9	46	4.8	0	0	0.83	0.99	1.02	12.6	3.85	3.82
	10	1210 P	18.4	0	0	2.11 P	5.95 P	0.43	7.47	8.56	6.32
	11	143	18.0	0	0	1.20	4.91	0.91	5.78	7.38	3.98
	12	493	6.1	0	0	1.13	3.31	1.03	7.44	4.93	6.43
	13	728 P	13.1	0	0	0.23	6.98 P	0.32	3.96	8.65	9.55
	14	421	9.7	0	0	1.24	3.77	0.46	11.399	5.02	5.78
	15	435	43.1 P	0	0	0.94	1.38	1.13	8.65	6.7	3.233

ALPT, allergen-specific lymphocyte proliferation test; Ca, casein; FEIA, fluorescence enzyme immunoassay; Lf, lactoferrin; LG, lactoglobulin; Eos, blood eosinophil count; P, positive; VD, vessel density.

cases, most of the cases were clearly distinguished by cluster classification, as reported in past Japanese data, with 75.1% in clusters 1 + 2 and 17.4% in cluster 3.¹² However, no specific ultrasonographic findings were observed in each cluster in this study.

On B-mode US, 100% (16/16) of non-IgE-mediated gastrointestinal food allergy cases, including clusters 1, 2, and 3, showed small intestinal wall thickening, 100% (16/16) showed poor peristalsis, and 93.7% (15/16) showed mesenteric thickening. In addition, gastric wall thickening was found in most cases of each cluster. Although it is difficult to differentiate whether these lesions were caused by gastrointestinal allergy itself or other reasons, such as disease-related stress, it was very interesting to see gastric involvement in this particular disease.

On the other hand, 16 non-IgE-mediated gastrointestinal food allergy cases of clusters 1, 2, and 3 had abnormal ultrasonographic findings of the small intestine that coincided substantially with the cluster classification.¹² However, in addition to the small intestine, one cluster 3 case had ultrasonographic colonic wall thickening

that was not expected in cluster 3 and 4 cases. Therefore, the involved site visualized by ultrasonography helped identify the unexpected lesion with cluster classification, confirming the cluster-overlapped cases in the present study. Cluster classification based on clinical symptoms has been suggested as a convenient method for roughly estimating the involved gastrointestinal tract, but ultrasonographic examination seemed to detect involved sites with greater accuracy.

In addition to small intestinal wall thickening, poor peristalsis, and mesenteric thickening, fluid collection, high echogenic mesenteric thickening, increased Doppler flow signal, and abnormal bowel peristalsis were observed in non-IgE-mediated gastrointestinal food allergy, which are frequently observed in patients with emergent gastrointestinal disorders.²² These results suggest that the gastrointestinal tract in non-IgE-mediated gastrointestinal food allergy was relatively severely affected, as in acute abdomen, as previously reported.^{12,21,23} In particular, intestinal mesenteric thickening, which includes mesenteric adipose

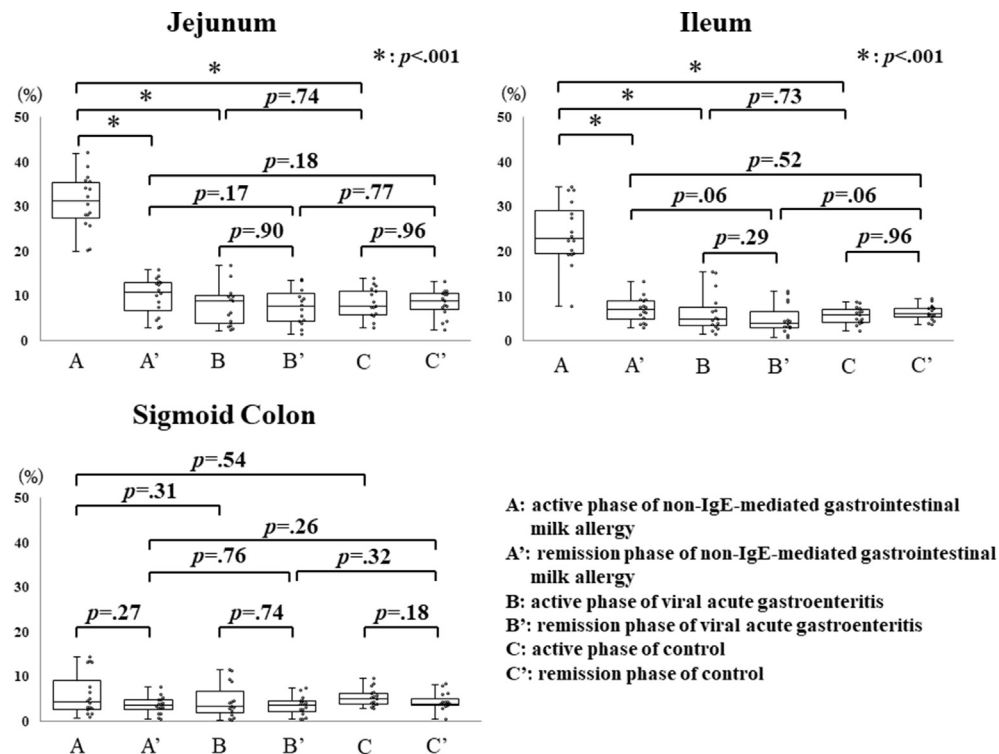


Fig. 3. Comparison of VD among the 3 groups in the active and remission phases. In the jejunum, VD is significantly higher in A than in B, C, and A'. In the ileum, the same results as for the jejunum are obtained. In the sigmoid, no significant differences are observed among the 3 groups in the active and remission phases.

tissue, is found in emergent diseases such as perforation of the gastrointestinal tract and Crohn's disease,^{21,22,24,25} but it is not found in mild gastrointestinal diseases such as viral gastroenteritis.^{21,26} These findings clearly suggest that ultrasonographic analysis is a very useful and important tool for the diagnosis and evaluation of non-IgE-mediated gastrointestinal food allergy.

In comparisons of various data in group A, ALPT was positive to either β -lactoglobulin, lactoferrin, or κ -casein in 87.5% (14/16). However, the single-positive rates of β -lactoglobulin, lactoferrin, and κ -casein were 50.0%, 87.5%, and 18.7%, respectively. In a previous study, sensitivities of ALPT for β -lactoglobulin, lactoferrin, and κ -casein were reported to be 81.0%, 77.4%, and 83.3–90.0%, respectively.²⁷ On the other hand, another study reported that ALPT had a false-positive rate of about 40% and recommended care in using ALPT alone in making the diagnosis of non-IgE-mediated infantile gastrointestinal milk allergy, because OFCT is needed.²⁸

Table 4
Result of ROC analysis.

	AUC (95% confidence intervals)	Cut off	Sensitivity (%)	Specificity (%)
Non-IgE-mediated gastrointestinal milk allergy (group A) vs. others (group B + C)				
Eos	0.517 (0.325–0.710)	408	56.2	60.0
ALPT- β LG	0.768 (0.605–0.932)	1.20	75.0	70.0
ALPT-Lf	0.831 (0.699–0.963)	3.45	87.5	73.3
ALPT- κ -Ca	0.531 (0.306–0.755)	1.22	50.0	83.3
Jejunal VD	0.995 (0.985–1.005)	19.9	100	96.6
Ileal VD	0.987 (0.961–1.013)	16.8	93.7	100
Sigmoidal VD	0.520 (0.324–0.716)	4.69	50.0	56.6

AUC, area under ROC curve; ALPT, allergen-specific lymphocyte proliferation test; Ca, casein; Lf, lactoferrin; LG, lactoglobulin; Eos, blood eosinophil count; VD, vessel density.

Meanwhile, B-mode US findings reflect the findings of food elimination tests and OFCT more precisely, which proved the usefulness of US, because there are cases in which it is difficult to identify a positive reaction on OFCT and ALPT. Compared to the results of ALPT, all VD values of group A, which included two ALPT-negative cases, showed positive results in either the jejunum or ileum. In groups B and C, the average VD of the jejunum exceeded 18.7% in only one case of group B. Therefore, the sensitivity and specificity of jejunal and ileal VD in the present study were 100%, 93.7%, 96.6%, and 100%, respectively according to the ROC curve

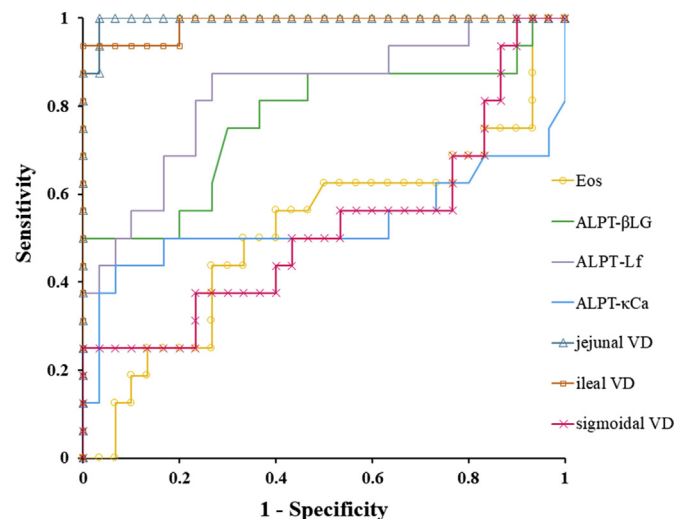


Fig. 4. ROC curves of Eos, ALPT- β -lactoglobulin, ALPT-lactoferrin, ALPT- κ -casein, jejunal VD, ileal VD, and sigmoidal VD for differentiation of non-IgE-mediated gastrointestinal food allergy from others.

results. VD was considered to be a more useful diagnostic tool than ALPT, because VD could provide more timely evaluation of the intestinal condition during the acute phase and OFCT in non-IgE-mediated gastrointestinal food allergy than ALPT.

In comparisons of VD among the three groups, group A had significantly higher VD in the jejunum and ileum than groups B and C. In contrast, no significant difference was found between groups B and C. Empirically it has been shown that no elevation of ultrasonographic power Doppler flow is observed in controls and viral AGE, but there have been no reports to objectively confirm this. In the present study, jejunal and ileal VD was not increased in viral AGE, which suggests that small intestinal VD is useful to differentiate viral AGE from IgE-mediated gastrointestinal food allergy. However, the present study has a limitation due to the small number of cases. There were significant age differences among the three groups (group A vs B, $p < .01$; A vs C, $p < .01$; B vs C, $p < .01$). In addition, there were significant negative correlations between age and jejunal VD ($r = -0.69$, $p < .01$) and ileal VD ($r = -0.62$, $p < .01$), which suggests the necessity for further studies with a large number of cases in the future. In conclusion, abdominal Doppler ultrasonography and measurements of small intestinal VD at symptomatic state can support the diagnosis and evaluation of infantile non-IgE-mediated gastrointestinal food allergy with symptoms of vomiting, diarrhea, and failure to thrive.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

KJ and YO conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. TKo and NAs supervised data collection and reviewed and revised the manuscript. NAr, RK, KH, YA, and TKu collected data and performed the initial analyses. TS critically reviewed the manuscript.

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