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Severity scales of non-IgE-mediated gastrointestinal food allergies in neonates and infants

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non-IgE-mediated gastrointestinal food allergy, non-IgE-GI-FAs; gastrointestinal, GI; Japanese Pediatric Guideline for Food Allergy, JPGFA; food protein-induced enterocolitis syndrome, FPIES; food protein-induced allergic proctocolitis, FPIAP; food protein-induced enteropathy, FPE; oral food challenge, OFC; white blood cell, WBC; eosinophil, Eos; alanine aminotransferase, ALT; C-reactive protein, CRP; lymphocyte stimulation test, LST; 5-bromo-2′-deoxyuridine, BrdU; stimulation index, SI; Kruskal-Wallis test, K-W test; Jonckheere-Terpstra trend test, J-T trend test; Cochran-Armitage test, C-A test

ABSTRACT

Background: Non-IgE-mediated gastrointestinal food allergies (non-IgE-GI-FAs) are one type of food allergy found in neonates and infants. Few reports have defined the severity of non-IgE-GI-FAs in these populations.
Methods: Grading scales of the severity of non-IgE-GI-FAs according to extra-GI symptoms, such as poor weight gain, as well as systemic symptoms, including fever and shock, were developed and retrospectively applied to patients with non-IgE-GI-FAs. The relationship between the severity of non-IgE-GI-FAs and both clinical and laboratory findings were examined.
Results: Elevation of C-reactive protein levels and a decrease in total protein and albumin were observed in accordance with allergy severity. In an endoscopic examination, inflammatory findings were confirmed in large areas of the colonic mucosa in case of higher severity levels, and infiltration of inflammatory cells other than eosinophils was found in the severest grade. Extensively hydrolyzed milk or amino acid-based milk was required for all patients with the severest grade. In addition, the timing of acquiring tolerance tended to be late for this grade.
Conclusions: Classification and determination of the severity of non-IgE-GI-FAs in neonates and infants may not only contribute to elucidation of the pathogenesis but may also be useful in the clinical setting.

Introduction

The prevalence of non-IgE-mediated gastrointestinal (GI) food allergies (non-IgE-GI-FAs) has been increasing since first reported in 1967.[1] Non-IgE-GI-FAs include food protein-induced enterocolitis syndrome (FPIES), food protein-induced allergic proctocolitis (FPIAP), and food protein-induced enteropathy (FPE); FPIES mainly exhibits repetitive vomiting and diarrhea, FPIAP shows only bloody stools with poor weight gain, and FPE shows chronic, non-bloody diarrhea with malabsorption and a failure to thrive.[2–5] Diagnosis relies on the recognition of symptom patterns in FPIAP and FPIES, as well as biopsy in FPE. Although these conditions are separate clinical entities, they have many overlapping clinical features.

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The number of neonates and infants with non-IgE-GI-FAs has also been increasing rapidly in Japan since the late 1990s.[6–8] However, evidence has shown the diagnosis of most patients in Japan did not correspond to entities of non-IgE-GI-FAs that have been proposed in the United States because of variations in the clinical findings.[9–11] Non-IgE-GI-FAs in neonates and infants exhibit diverse symptoms, mainly through non-IgE-mediated mechanisms, and include a broad spectrum of symptoms from bloody stool alone to sepsis-like symptoms such as fever or shock.[12–14] Taking this information into account, the 2017 Japanese Pediatric Guideline for Food Allergy (JPGFA) tentatively named all clinical entities that show GI symptoms due to food allergens, especially during infancy, as "neonatal and infantile GI allergy"[15] or "non-IgE-GI-FAs in neonates and infants."

Since the urgency and intensity of treatment is dependent on the severity of non-IgE-GI-FAs, the classification based on severity is a prerequisite for the appropriate management of these types of diseases. FPIES subtypes with distinct severity are described in the 2017 International Consensus Guidelines,[16] in which a severe subtype represents repetitive projectile emesis, lethargy, dehydration, hypotension, and shock. The MAP (Milk Allergy in Primary Care) guideline categorizes non-IgE-mediated cow's milk allergy according to severity of GI symptoms, including mild-moderate and severe, rather than into distinct disease entities.[17,18] The main purpose of this guideline is to improve disease recognition in the primary care setting and for treatment of mild and moderate cases. At present, there have been no severity grading scales developed that encompass all typical and atypical symptoms of non-IgE-GI-FAs and can be widely used in clinical practice.

To develop such a severity grading scale, we constructed a new severity grading system for non-IgE-GI-FAs, which is based on extra-GI symptoms, such as poor weight gain and/or systemic symptoms, and retrospectively applied this system to patients who have been treated at our clinic. Severity in our grading scale was closely associated with laboratory and histopathological findings, treatment, and prognosis.

Methods

Patients

Consecutive neonatal or infant patients with non-IgE-GI-FAs, who were treated at Gunma University hospital between January 1, 2003 and August 31, 2016, were enrolled in this retrospective study. The causative food for allergies was cow's milk in all patients. Non-IgE-GI-FAs were diagnosed based on the following criteria proposed by the JPGFA,[15] which is modified from one proposed by Powell[19]: (i) development of GI symptoms after ingestion of causative foods; (ii) disappearance of symptoms after discontinuation of causative foods; (iii) exclusion of other disorders that could cause GI symptoms, such as infection or surgical problems; and (iv) recurrence of GI symptoms during oral food challenge (OFC) or re-administration. Patient medical records were reviewed to obtain detailed information, including patient background, blood test results, endoscopic findings, treatments, and prognoses. This study was conducted under the approval of the Ethics Committee of Gunma University.

Severity grading

All patients enrolled in this study were graded according to concomitant extra-GI symptoms: GI symptoms only (Grade 1); GI symptoms and poor weight gain (Grade 2); and systemic symptoms in addition to those in Grade 2 (Grade 3). GI symptoms included vomiting, diarrhea, bloody stool, and/or abdominal distension. Poor weight gain was defined as a decrease of −0.5 standard deviations of weight. Systemic symptoms included fever and shock. Fever was defined as a temperature of 38 °C or more. Shock was defined based on common diagnostic symptoms such as lethargy, fast breathing, cold skin, prolonged capillary refill, fast weak pulse, and low blood pressure as a late sign.[20] The standards of blood pressure for diagnosing hypotension were: 65 mmHg for ages 0–3 months, 70–90 mmHg for ages 3–6 months, and 80 mmHg for ages 6–12 months. The grading was based on the severest symptoms observed during the clinical course.

Laboratory tests

The results of the following laboratory tests at the first examination, as well as at their highest or lowest levels during the course, were collected: white blood cell (WBC), neutrophil, eosinophil (Eos), hemoglobin, platelet counts, and serum levels of total protein, albumin, alanine aminotransferase (ALT), and C-reactive protein (CRP). Serum levels of total IgE and milk-specific IgE, which were measured using the ImmunoCAP system (Thermo Fisher Scientific, Waltham, MA, USA), were also collected.

Lymphocyte stimulation test

The lymphocyte stimulation test (LST) was conducted with a few modifications to previously described implementations.[21–24] Briefly, PBMCs from each patient were incubated separately with cow's milk and 4 different milk protein components (α-lactalbumin, β-lactoglobulin, α-casein, and κ-casein) for 5 days. Then, proliferating cells were labeled by further incubation with 100 μM of 5-bromo-2′-deoxyuridine (BrdU) for 6 h, and the incorporated BrdU contents were determined by an enzyme-linked immunesolvent assay. The results were described as stimulation index (SI), which was calculated as the ratio of BrdU-contents in a stimulated to unstimulated sample. Cut-off levels were set based on results with receiver operating characteristic curve analysis of patients with non-IgE-GI-FAs, as well as 3 healthy controls and 5 with other diseases, such as pyloric stenosis, using the R package Epi for analysis.[25]

Colonoscopy

Colonoscopy was conducted without sedation. Pathologic findings were blindly judged by a specialized pediatric gastroenterologist who did not receive any information about a given patient's allergy severity.

Statistical analysis

Significant differences between patient groups were estimated using the Kruskal-Wallis (K-W) test for gestational age, birth weight, age of onset, weight of onset, and laboratory findings. The significance of positive LST rates, treatment, and prognosis were tested using the chi-squared test. The significance of patient trends was estimated using the Jonckheere-Terpstra (J-T) and Cochran-Armitage (C-A) trend tests for gestational age, birth weight, age of onset, weight of onset, laboratory findings, positive LST rate, treatment, and prognosis. K-W tests and chi-square tests were conducted using GraphPad Prism version 5 for Windows (GraphPad Inc., San Diego, CA, USA). The J-T and C-A trend tests were performed using the R packages Clinfun and Desc Tools.[25] The results were statistically significant if p values were less than 0.05.

Results

Patients

During the study period, 17 patients (9 boys, 8 girls) were diagnosed with non-IgE-GI-FAs and enrolled in this analysis. The median gestational age, birth weight, age, and body weight at onset were 39 weeks plus 1 day, 2718 g, 30 days, and 3610 g, respectively. Among the patients, 7, 5, and 5 were categorized as having Grade 1, Grade 2, and Grade 3 severity, respectively. GI symptoms and poor weight gain were found in all patients categorized as Grade 2 or 3. There was no significant difference among the 3 severity grades in terms of gestational age, birth weight, age, body weight at onset, the time between the onset and initiation of appropriate therapy, and nutrition at onset (Table 1). Patients showing simultaneous vomiting and bloody stool were present in all grades, while those without vomiting and bloody stool were only Grade 3 (Fig. 1).

Laboratory findings and LST

Serum CRP levels in Grade 3 patients were significantly higher than in those of other grades, both at the first medical examination and at the maximum CRP value, during the study period (Table 1). In addition, a significant decrease was observed in the lowest level of both total protein and albumin for Grade 3 patients. Furthermore, a trend test showed associations between grade and increasing neutrophils (maximum), increasing platelets (onset and maximum), and decreasing hemoglobin (minimum). There was no difference in the total IgE or milk-specific IgE antibody levels among the severity groups (Table 1).

All severity groups had positive results for LST with at least one of the milk components (Table 2). There was a tendency toward higher SI values in Grade 3 patients than the other severity groups associated with the milk formula and all components, except α-lactalbumin, though it was not significant. Notably, Grade 3 had higher SI values for α-casein ($p = 0.0504$, J-T trend-test). All Grade 3 patients tested positive for all antigens.

Colonoscopy

Colonoscopy was performed on 7 patients; 2 patients each with Grades 1 and 2, as well as 3 patients with Grade 3 severity. Regarding the endoscopic findings, erosion and hemorrhage of the colonic mucosa of patients were seen only in Grades 1 and 2, lymphoid follicles with erythema in Grade 2, and edema and vascular pattern loss in Grade 3. The histopathologic findings revealed Eos infiltration in most Grade 1 and 2 patients, while lymphocyte and neutrophil infiltration and edema were found in

Table 1
Demographic characteristics and laboratory findings of 3 grades.

	Grade 1	Grade 2	Grade 3	P^{*}	P^{**}
N	7	5	5		
Sex (males/females)	4/3	2/3	3/2		
Gestational age (weeks, days)	38, 5 (37, 5–39, 5)	40, 6 (37, 6–41, 2)	37,1 (36, 5–39, 1)	.103	.163
Birth weight (g)	2718 (2398–3286)	2628 (2400–3167)	2726 (2530–3595)	.740	.665
Age at onset (days)	30.0 (10.0–60.0)	18.0 (4.0–79.5)	6.0 (1.5–169.0)	.677	.401
Weight at onset (g)	4455 (2768–5424)	3218 (2251–5571)	3610 (2671–6430)	.719	.729
Time between onset and the initiation of appropriate therapy	24 (5–31)	23 (5.5–103.5)	30 (18.5–247.5)	.468	.256
Nutrition Breast/formula/mixed	0/3/4	1/0/4	1/2/2		
WBC (/μL)					
at onset	10,600 (9100–11,500)	12,700 (8855–16,550)	15,100 (10,950–23900)	.362	.160
max	10,600 (9500–20,700)	10,700 (10,350–15590)	18,200 (16,750–37050)	.079	.093
Neutrophil (/μL)					
at onset	3192 (2040–3508)	1590 (1273–7915)	7079 (3393–10,389)	.167	.160
max	3520 (3074–4917)	5910 (1707–8048)	8405 (5359–17,060)	.090	**.040**
Eos (/μL)					
at onset	373 (318, 1209)	540.0 (136, 1224)	486 (30–2050)	.824	.568
max	888 (343–2384)	1691 (408–1931)	883 (513–3098)	.932	.931
Hemoglobin (g/dL)					
at onset	11.2 (10.5–16.8)	11.7 (10.5–14.75)	11.2 (9.3–13.9)	.782	.596
min	10.8 (8.8–11.0)	8.7 (8.6–9.9)	7.2 (6.4–9.5)	.064	**.012**
Platelet count ($\times 10^4$/μL)					
at onset	44.0 (35.0–71.0)	48.5 (44.9–57.9)	71.3 (55.5–93.6)	.135	**.040**
max	57.9 (39.6–71.0)	51.7 (44.9–79.4)	89.7 (66.9–110.5)	.084	**.038**
Total protein (g/dL)					
at onset	5.8 (5.4–6.2)	5.0 (4.5–5.8)	5.7 (5.4–6.2)	.257	.847
min	5.4 (4.9–5.8)	4.8 (4.0–5.0)	4.1 (3.3–4.5)	**.007**†	**.002**
Albumin (g/dL)					
at onset	3.9 (3.5–4.2)	3.5 (2.9–4.1)	3.7 (3.7–3.9)	.666	.630
min	3.7 (3.5–4.1)	3.3 (2.3–3.6)	2.2 (1.7–2.7)	**.013**†	**.002**
ALT level (IU/mL)					
at onset	16 (11–28)	16 (7–27)	19 (13–40)	.639	.658
max	23 (20–32)	43 (27–91)	74 (23–308)	.170	.057
CRP (mg/dL)					
at onset	0.10 (0.00–0.18)	0.23 (0.06–0.24)	1.00 (0.27–1.75)	**.040**†	**.009**
max	0.10 (0.00–0.18)	0.40 (0.02–0.98)	3.88 (1.45–6.73)	**.007**†	**.002**
Total IgE at onset (IU/mL)	<3.0 (<3.0–10.0)	4.0 (1.9–23.5)	<3.0 (<3.0–105.2)	.152	.157
Milk specific IgE (U_A/mL) at onset	0.00 (0.00–0.10)	0.00 (0.00–0.98)	0.0 (0.0–0.0)	.307	.536

Data are expressed as medians (interquartile ranges).
p values less than 0.05 are indicated by bold letters.
* p values were calculated by the Kruskal-Wallis test.
** p values were calculated by the Jonckheere-Terpstra trend test.
† Post-hoc Dunn's multiple comparison test was used to compare Grade 1 and Grade 3 ($^a p < 0.05$).

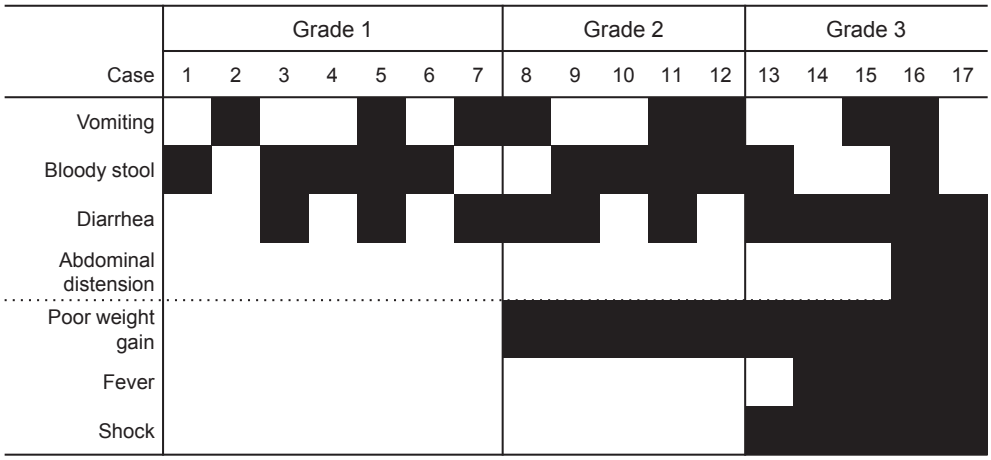

Fig. 1. Schematic representation of GI symptoms, poor weight gain, and systemic patient symptoms. Symptoms observed in each case are filled with black.

Table 2
Results of lymphocyte stimulation test.

		No.	Median (IQR)					Positive rate (%)				
			Grade 1	Grade 2	Grade 3	P^{*}	P^{**}	Grade 1	Grade 2	Grade 3	P^{***}	P^{****}
LST (SI)	formula	16	13.16 (3.887–24.15)	12.34 (7.585–25.87)	51.51 (17.44–86.13)	.092	.080	71.4	100	100	.230	.064
	α-casein	15	2.817 (1.960–3.970)	3.920 (1.880–5.675)	8.115 (7.043–12.96)	.092	.050	85.7	80	100	.719	.323
	κ-casein	13	3.370 (2.418–5.750)	3.060 (1.458–4.228)	12.79 (5.669–22.08)	.052	.169	100	80	100	.230	.618
	α-lactalbumin	16	3.690 (2.176–4.530)	2.880 (1.720–4.860)	3.260 (2.120–5.655)	.920	.961	85.7	100	100	.503	.149
	β-lactoglobulin	16	2.960 (2.620–7.620)	3.500 (1.895–6.035)	4.838 (3.505–11.00)	.400	.445	100	100	100	NA	NA

LST, lymphocyte stimulation test; IQR, interquartile range; SI, stimulation index.
* p values were calculated by the Kruskal-Wallis test.
** p values were calculated by the Jonckheere-Terpstra trend test.
*** p values were calculated by the chi-square test.
**** p values were calculated by the Cochran-Armitage test with an alternative hypothesis of an increasing trend with the covariate value and grade severity.

Grade 3 patients. These endoscopic and histopathologic findings of Grade 1 patients were mostly localized to the rectum, whereas those in Grade 2 and 3 patients were found in both the rectum and sigmoid colon (Fig. 2, Table 3).

Treatment and prognosis

All Grade 3 patients required extensively hydrolyzed or amino acid-based formula, compared to 42.9% of Grade 1 patients ($p = 0.087$, K-W; $p < 0.05$, C-A; Table 4). Exclusive breastfeeding with elimination of cow's milk from the diet of the mother or a combination of breastfeeding and moderately hydrolyzed milk were used for patients with non-IgE-GI-FAs caused by mixed breast and bottle feeding in Grades 1 or 2. Moderately hydrolyzed milk was used by 2 patients with non-IgE-GI-FAs caused by cow's milk in Grade 1. Regarding prognosis, a significantly smaller number of Grade 3 patients developed tolerance until 1.5-years of age ($p = 0.002$, K-W; $p < 0.001$, C-A; Table 4). In 2 Grade 3 patients, OFC induced GI symptoms and/or fever, even at the age of 4.

Discussion

The proposed grading system reflects disease intensity, aids in selecting the appropriate therapeutic approach, and predicts disease prognosis. Among the 3 severity grades proposed, Grade 3 showed distinct features in the clinical course, as well as laboratory and endoscopic findings. Grade 3 showed higher levels of CRP, and lower levels of total protein and albumin. Edema and infiltration of lymphocytes and neutrophils were also prominent. Additionally, all Grade 3 patients required either extensively hydrolyzed or amino acid-based formula. The acquisition of milk tolerance was observed for Grade 3 patients of older age, which was not found to be the case for the other groups. In addition to distinct Grade 3-based features, significant trends of elevated neutrophils and platelets, as well as decreased hemoglobin, according to severity grading, were also observed; this indicated that grading reflects some aspects of disease severity.

Regarding symptoms, some Grade 3 patients manifested both vomiting and bloody stool during the acute phase or showed sepsis-like symptoms that typically present in severe FPIES patients, while others presented FPE-like GI symptoms, such as chronic diarrhea with malabsorption and failure to thrive. Comorbidities of vomiting, bloody stool, and diarrhea were observed in patients of each grade, whereas a case with bloody stool-alone as a GI symptom, but presenting with poor weight gain, was found in the Grade 2 group, while 1 patient with bloody stool and diarrhea, but not vomiting, also presented with shock was Grade 3. It is difficult to classify these cases into canonical clinical entities such as FPIES, FPIAP, or FPE. Classification into clinical entities[18] or GI-symptoms-based clustering[12] provides insights into disease pathogenesis and aids in locating lesions within the GI tract. On the other hand, our grading system would be useful in the clinical setting since it incorporates clinically relevant extra-GI tract symptoms, which leads to differences in laboratory findings and histopathology in accordance with different severity grades.

Regarding laboratory findings, an increase in neutrophils has been found in patients with severe acute FPIES[26] and during OFC in patients with non-IgE-GI-FAs.[19] The latest 2017 FPIES guidelines indicate an increase in neutrophil count (>1500 cells/μL) as a minor diagnostic criterion for OFC interpretation[16]; this implies the

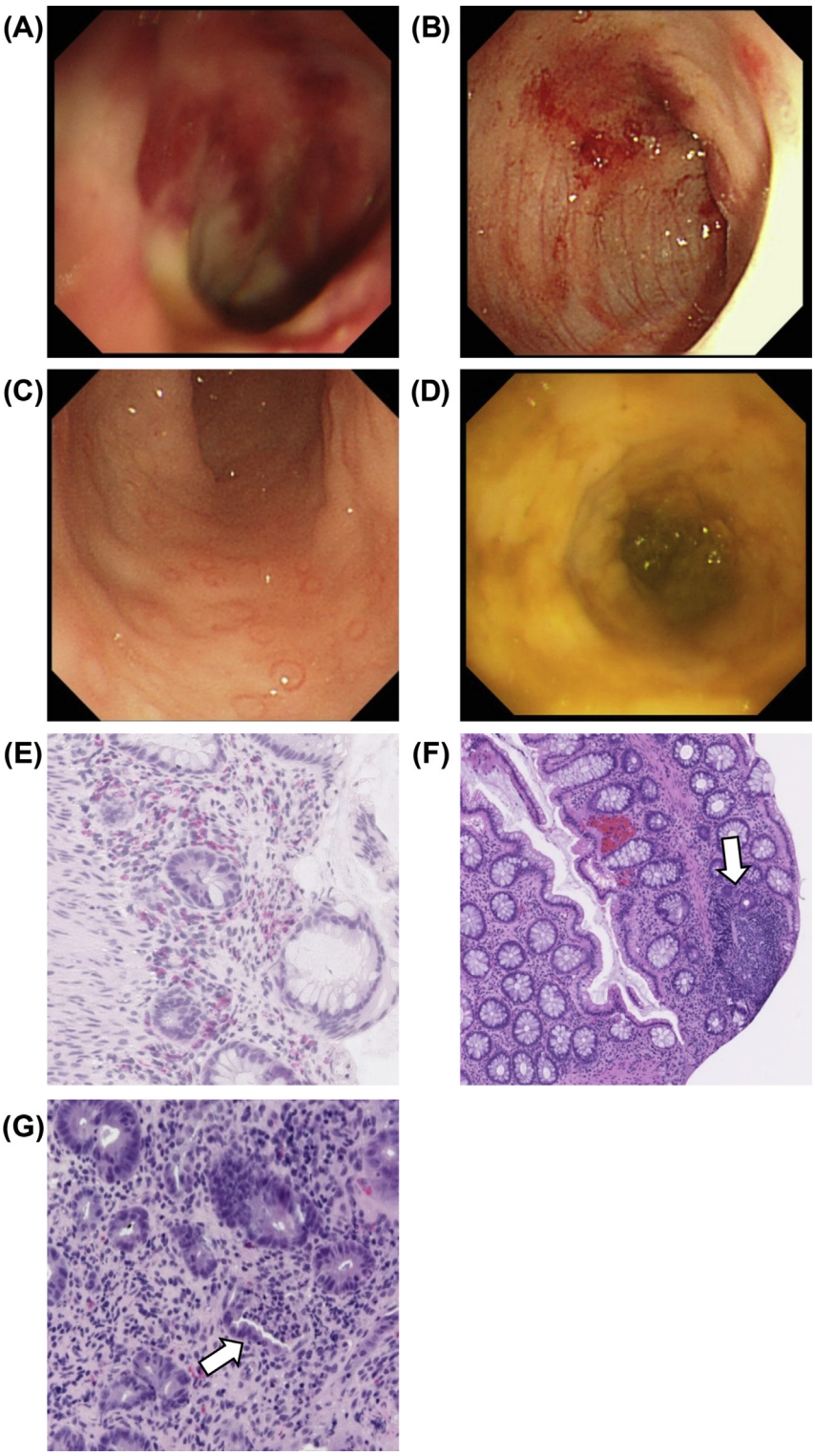

Fig. 2. Endoscopic and histopathologic findings (sigmoidoscopy). **(A–D)** Representative endoscopic images of the colon. Erosion and hemorrhage in the mucus of a Grade 1 patient **(A)**, erosion, hemorrhage, and lymphoid follicles with erythema of a Grade 2 patient **(B, C)**, edema and loss of the vascular pattern in a Grade 3 patient **(D)** were observed. **(E–G)** Representative histopathologic images of biopsy specimens of the colon after hematoxylin-eosin (HE) staining. Eosinophil infiltration in a Grade 1 patient **(E)**, eosinophil infiltration and the formation of follicles (arrow) in a Grade 2 patient **(F)**, lymphocyte and neutrophil infiltration, crypt abscess (arrow), and edema in a Grade 3 patient **(G)** were observed.

involvement of proinflammatory cytokines that induce neutrophilia. Indeed, interleukin (IL)-8 and IL-6 have been implicated in FPIES by *in vivo* and *in vitro* studies.[24,27,28] These observations suggest that proinflammatory cytokines produced from GI-tract lesions likely cause systemic inflammation in severe disease, or that non-specific stress caused by intense GI symptoms induce proinflammatory cytokine production, and hence inflammation in the patient.[29] In the current study, neutrophils increased

Table 3
Endoscopic findings.

	Grade	Rectum							Sigmoid colon						
		Grade 1		Grade 2		Grade 3			Grade 1		Grade 2		Grade 3		
	Case	4	6	9	10	13	14	17	4	6	9	10	13	14	17
Endoscopic findings	Erosion	+	+	+	+	−	−	−	−	−	+	−	−	−	−
	Hemorrhagic	+	+	+	+	−	−	−	−	−	+	−	−	−	−
	Lymphoid follicle with erythema	−	−	−	−	−	−	−	−	−	+	+	−	−	−
	Edema	−	−	−	−	+	+	+	−	+	−	+	+	+	+
	Loss of the vascular pattern	+	+	+	+	+	+	−	−	−	−	−	+	+	−
Histopathologic findings	Eosinophil infiltration	+	+	+	+	−	−	−	−	+	+	+	−	−	−
	Lymphocyte and neutrophil infiltration	+	−	+	+	+	+	+	+	−	+	−	+	+	+
	Nodular lymphoid hyperplasia	−	−	+	−	−	−	−	−	+	+	−	−	+	−
	Edema	−	−	−	−	−	+	+	−	−	−	−	−	+	+
	Crypt epithelial polymorphs	−	−	−	+	+	−	+	−	−	−	−	+	−	+
	Crypt irregularity	−	−	−	+	+	−	−	−	−	−	+	+	−	−

Table 4
Therapeutic milk and prognosis.

		No.	Grade 1	Grade 2	Grade 3	p^{*}	p^{**}
Therapeutic milk	Extensively hydrolyzed formula or amino acid-based formula	17	42.9%	80.0%	100%	0.087	**0.015**
Prognosis	Tolerance until 1.5 years old	16	100%	80.0%	0.0%	**0.002**	**<0.001**

p values less than 0.05 are indicated in bold letters.
* *p* values were calculated by the chi-square test.
** *p* values were calculated by the Cochran-Armitage test with an alternative hypothesis of an increasing trend with the covariate value and grade severity.

prominently in Grade 3 patients, although this result was not significant, suggesting that the current severity grading reflects disease intensity to some extent. Further detailed studies of cytokine profiles in non-IgE-GI-FA patients should be conducted in the future.

The LST has been reported to aid in diagnosing non-IgE-GI-FAs in neonates and infants.[23] In contrast, there has been controversy over the utility of the LST, and currently, no consensus has been reached.[30] In the present study, all patients with different grades showed LST-positive results with at least 1 milk component, and LST-positive rates and SI values were higher in patients with more severe non-IgE-GI-FAs. This supports the idea that the LST is useful in the supplementary diagnosis of non-IgE-GI-FAs.

Both endoscopic and histologic findings from colonoscopy were confined to the rectum for Grade 1 patients, while findings were discovered in the rectum and sigmoid colon for Grade 2 and 3 patients. These results suggest that the range of inflammation expands according to disease severity. Eos infiltration was found in most Grade 1 and 2 patients. In contrast, neutrophils and lymphocytes were mainly observed in Grade 3 patients. Interestingly, colonoscopy in acute phase patients with severe non-IgE-GI-FAs demonstrated that the presence of mast cells changed to that of eosinophils 3 weeks later.[13] This indicates that the infiltrating cell type can be altered between the acute phase to the chronic phase. Different infiltrating cell types in inflammation on the mucosa may be partly explained by different severity of local inflammation or different phases of the disease.

Non-IgE-GI-FAs generally have a favorable prognosis; the majority of patients have symptom resolution by 1 year of age in FPIAP, 1–3 years of age in FPE, and 1–5 years of age in FPIES.[2,5] In the case of FPIES, the timing of tolerance acquisition is diverse and may be dependent on different subgroups, such as mild and severe symptoms, or acute and chronic phase. Kimura *et al.* reported that serum CRP levels and the number of Eos in the peripheral blood at onset are correlated with the timing of tolerance acquisition in Japanese patients.[31] In this study, tolerance acquisition to milk proteins was late in Grade 3 patients. Therefore, this result implies that Grade 3 patients have different prognoses. Consistently, patients who presented with sepsis-like symptoms had poorer prognoses.

Limitations in this study include the small number of patients, and that LST and intestine endoscopy were not performed in all cases. Endoscopy was performed when consent was obtained from the patient's guardians. However, since atypical symptoms or poor response to initial treatment might have urged us to perform endoscopy for an accurate diagnosis, the selection of patients might be biased for this procedure, which might have affected endoscopic findings. In addition, this is a retrospective study. It is indispensable to increase the number of patients, investigate the relationship between severity and various tests, and determine the reproducibility of this study.

In summary, our severity grading system of non-IgE-GI-FAs in neonates and infants may be useful in the clinical setting to select the appropriateness of therapeutic milk and to predict prognosis. It remains unknown whether GI symptoms alone and those with systemic symptoms belong to the spectrum of a single disease or different diseases. However, since our grading reflects pathological findings, further studies using this grading system will also provide insights into basic pathology of non-IgE-GI-FAs in the future.

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Conflict of interest
The authors have no conflict of interest to declare.

Authors' contributions
HY performed the statistical analysis and interpretation of the results and wrote the manuscript. TT designed the study, reviewed data collection. TIs, YY and HA provided critique for the results and revision. HA supervised the study. All authors except for YY contributed to data collection. All authors read and approved the final manuscript.

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