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Usefulness of thymus and activation-regulated chemokine (TARC) for FPIES diagnosis

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Running title:

Usefulness of TARC for FPIES diagnosis

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To the Editor:

Food protein-induced enterocolitis syndrome (FPIES) is diagnosed based on clinical symptoms or positive results of an oral food challenge (OFC) test.^{1,2} The lack of disease-specific biomarkers renders early diagnosis difficult. Solid food FPIES often develops 5–12 months after birth and may be misdiagnosed as various infectious diseases that are common during that period. Therefore, it is necessary to establish new biomarkers for FPIES diagnosis. In this study, we measured serum thymus and activation-regulated chemokine (TARC)³ and general biomarkers in the acute phase of FPIES, infectious gastroenteritis, and sepsis to evaluate the usefulness of each biomarker for FPIES diagnosis.

This case-control study evaluated 20 episodes (9 emergency outpatient visits and 11 positive oral food challenges) in 13 pediatric patients with FPIES (8 egg yolk, 1 egg white, 1 whole egg, 1 wheat, 1 rice, and 1 short-neck clam) (FPIES group) and 40 age-matched patients with infectious gastroenteritis or sepsis (control group) between April 2019 and March 2021 (Figure S1, Table S1_Supporting Information). This study was approved by the Ethics Review Committee of the Saitama Medical Center, Jichi Medical University (approval number S19-077). Written consent was obtained from the patient's parents.

Patient background was evaluated. In patients with FPIES, the causative antigen, age of onset, age at event, symptoms, and severity (SuppInfo) of each episode were evaluated.

Serum TARC levels were quantified using a chemiluminescent enzyme immunoassay via a HISCL® TARC Assay Kit (Sysmex, Hyogo, Japan). For patients who visited the emergency

department, serum TARC values were measured at the time of outpatient visit. For OFC cases, serum TARC values were measured before antigen ingestion and at 6 h, 24 h, and 1 week after ingestion. Peak serum TARC values were evaluated. Since normal TARC values differ by age, modified TARC values (serum TARC value – normal mean for each age⁴) also were evaluated (SuppInfo). Concurrently, complete blood count (CBC) including white blood cell, neutrophil, eosinophil, and platelet, C-reactive protein (CRP), lactate dehydrogenase, venous blood pH, HCO₃⁻, and methemoglobin (%) were measured. OFC was conducted via the open, single-ingestion method (SuppInfo).

The patients with FPIES were five boys and eight girls. The age at onset ranged 0.5–3.0 (median 0.7) years. In the FPIES group, the age for 20 episodes analyzed was 0.5–6.2 (median 1.1) years. No patient had eczema at the time of episode. Of these, 7 were severe, 8 were moderate, and 5 were mild in severity (Table 1). The control group, aged 0.1–6.0 (median 1.5) years, had 20 patients each with infectious gastroenteritis and sepsis. There were no cases of eczema (Table S1_SuppInfo).

Table 2 shows the blood test results of the FPIES and control groups. The serum TARC (pg/mL) and modified TARC (pg/mL) levels were significantly higher in the FPIES group than in the control group ($p < 0.001$; Figure 1A). The eosinophil count was also significantly higher in the FPIES group. There were no significant differences regarding other parameters between the groups.

Table S2_SuppInfo shows the results when the control group was divided into infectious gastroenteritis and sepsis patients and compared to FPIES patients.

The results of the receiver operating characteristic analysis for each parameter for FPIES diagnosis are shown in Figure 1B and Table S3_SuppInfo. The area under the curve (AUC) of serum TARC was 0.946 (95% confidence interval [CI], 0.871–1.000), with 95% sensitivity, 95% specificity, and a cutoff value of 1062 pg/mL (Youden Index). The AUC of serum TARC and modified TARC values were higher than those of other parameters.

In univariate analysis, serum TARC level was a significant factor for FPIES diagnosis, with an odds ratio (OR) of 29.5 (95% CI 4.3–200.0, $p < 0.001$) per 1000 pg/mL increase. The age-adjusted TARC OR per 1000 pg/mL increase was 23.4 (95% CI 3.3–162.0, $p = 0.001$).

The median (range) serum TARC levels for patients with severe ($n = 7$), moderate ($n = 8$), and mild ($n = 5$) FPIES were 6320 (2911–8147), 2020 (1084–4097), and 1087 (410–1678) pg/mL, respectively. Significant differences were found between the severe, moderate, and mild groups ($p = 0.007$, $p = 0.037$) (Figure 3C). The correlation coefficients between the serum TARC value and number of vomiting episodes and vomiting time were $R = 0.570$ ($p = 0.008$) and $R = 0.699$ ($p < 0.001$), respectively, showing a moderate correlation (Figure S2).

Figure 1D shows the transition of the 11 OFC-positive TARC values. The median (range) serum TARC (pg/mL) level was 555 (227–1702) before ingestion, 1283 (410–3821) after 6 h, and 3748 (886–7816) after 24 h, increasing significantly over time. After 1 week, the median (range) TARC level decreased to 961 (468–3359).

This study revealed that serum TARC is more useful than other biomarkers, such as CBC, CRP, and methemoglobin for FPIES diagnosis. We also found a correlation between the severity of FPIES and serum TARC.

In a previous study comparing acute phase biomarkers in patients with FPIES, infectious gastroenteritis, and sepsis, patients with FPIES had higher white blood cell, eosinophil, and platelet counts and lower CRP levels. However, the usefulness of these biomarkers for FPIES diagnosis is unclear.⁵ We reported that neonates with FPIES had significantly higher methemoglobin levels than those with other diseases with vomiting. Those results were limited to the neonatal period with less overlap of methemoglobin levels in both groups.⁶ There are reports measuring various cytokines, although specific recommendations have not been determined.¹

TARC is a Th2 type chemokine produced by dendritic cells, endothelial cells, keratinocytes, and fibroblasts.³ Although TARC is used in diagnosing and assessing atopic dermatitis, some reports associate it with food allergies.^{4,7,8} We recently reported that compared to infectious gastroenteritis cases, FPIES cases are more likely to have elevated post-emetic serum TARC levels.⁹ Here, we included more cases of FPIES and added patients with sepsis as a control group. Hence, the usefulness of serum TARC in FPIES diagnosis and an appropriate cutoff value were clarified.

A longitudinal evaluation of TARC levels showed an increase at 6 h post-ingestion, with a further increase 24 h later. After 1 week, serum TARC levels had decreased, although they had not normalized. Therefore, TARC measurement after 24 h is recommended for the diagnosis of FPIES. Furthermore, serum TARC levels in patients with FPIES were positively correlated with symptom severity, vomiting frequency, and vomiting duration. Since there were no test items that

reflect FPIES severity, we expect that the TARC value after vomiting may be used as an objective evaluation item for FPIES severity.

Regarding the mechanism of TARC induction, TARC was expressed in the intestine when mice with allergic diarrhea ingested an antigen;¹⁰ the same reaction may occur in patients with FPIES.

This study had some limitations. First, all participants had acute solid food FPIES. Patients with cow's milk FPIES or chronic FPIES were excluded. Second, our sample size was small. Nonetheless, there was a significant difference in the TARC levels between patients with FPIES and the controls, indicating that TARC level elevation is specific to FPIES.

In conclusion, post-emetic serum TARC levels are useful for FPIES diagnosis. Early diagnosis may be possible by measuring TARC after vomiting. It may also be useful for objective severity assessment. Further larger-scale research is needed.

Keywords: biomarkers; food allergy; food protein–induced enterocolitis syndrome; infectious gastroenteritis; pediatrics; sepsis

Signature:

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Key message

Solid food protein-induced enterocolitis syndrome (FPIES) often develops 5–12 months after birth and may be misdiagnosed as various infectious diseases that are common during that period. So far, there have been no biomarkers for FPIES, although this study shows a clear increase in certain serologic markers, particularly thymus and activation-regulated chemokine (TARC) levels, especially 24 h post-ingestion of antigen. This indicates that TARC levels may be of use for the diagnosis of FPIES, although further research is still needed.

References

1. Nowak-Węgrzyn A, Berin MC, Mehr S. Food protein-induced enterocolitis syndrome. *J Allergy Clin Immunol Pract* 2020;8:24-35.
2. Nowak-Węgrzyn A, Chehade M, Groetch ME, et al. International consensus guidelines for the diagnosis and management of food protein-induced enterocolitis syndrome: Executive summary-Workgroup Report of the Adverse Reactions to Foods Committee, American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol* 2017;139:1111-1126.e4.
3. Knipping K, Knippels LM, Dupont C, Garssen J. Serum biomarkers for allergy in children. *Pediatr Allergy Immunol* 2017;28:114-123.
4. Fujisawa T, Nagao M, Hiraguchi Y, et al. Serum measurement of thymus and activation-regulated chemokine/CCL17 in children with atopic dermatitis: elevated normal levels in infancy and age-specific analysis in atopic dermatitis. *Pediatr Allergy Immunol* 2009;20:633-641.
5. Lee E, Barnes EH, Mehr S, Campbell DE. Differentiating acute food protein-induced enterocolitis syndrome from its mimics: a comparison of clinical features and routine laboratory biomarkers. *J Allergy Clin Immunol Pract* 2019;7:471-478.e3.
6. Makita E, Kuroda S, Sato H, et al. Comparison of methemoglobin levels in food protein-induced enterocolitis syndrome and other gastrointestinal diseases in neonates. *Allergol Immunopathol* 2020;48:490-495.

7. Furue M, Matsumoto T, Yamamoto T, et al. Correlation between serum thymus and activation-regulated chemokine levels and stratum corneum barrier function in healthy individuals and patients with mild atopic dermatitis. *J Dermatol Sci* 2012;66:60-63.
8. Hijnen D, De Bruin-Weller M, Oosting B, et al. Serum thymus and activation-regulated chemokine (TARC) and cutaneous T cell- attracting chemokine (CTACK) levels in allergic diseases: TARC and CTACK are disease-specific markers for atopic dermatitis. *J Allergy Clin Immunol* 2004;113:334-340
9. Makita E, Kuroda S, Itabashi K, Sugawara D, Ichihashi K. Evaluation of the diagnostic accuracy of thymus and activation-regulated chemokine to discriminate food protein-induced enterocolitis syndrome from infectious gastroenteritis. *Int Arch Allergy Immunol* 2020;182:229-233.
10. Knight AK, Blazquez AB, Zhang S, Mayer L, Sampson HA, Berin MC. CD4 T cells activated in the mesenteric lymph node mediate gastrointestinal food allergy in mice. *Am J Physiol Gastrointest Liver Physiol* 2007;293:1234-1243.

Table 1. Details of FPIES episodes

	All episodes (n = 20)
Median age, years (range)	1.1 (0.5–6.2)
Presentation (n, %)	
Emergency department visit	9 (45)
Oral food challenge test	11 (55)
Causative food (n, %)	
Egg yolk	13 (65)
Egg White	1 (5)
Whole egg	1 (5)
Wheat	1 (5)
Rice	3 (15)
Short-neck clam	1 (5)
Median time to onset of symptoms, hours (range)	2.5 (1.5–4)
Symptom	
Vomiting, n (%)	20 (100)
Median number of vomiting episodes (range)	5 (1–10)
Median vomiting duration, hours (range)	3 (0.5–10)
Diarrhea, n (%)	5 (25)
Bloody stool, n (%)	0 (0)

Lethargy, n (%)	15 (75)
Pallor, n (%)	8 (40)
Severity, n (%)	
Severe	7 (35)
Moderate	8 (40)
Mild	5 (25)
Treatments, n (%)	
Intravenous fluid	16 (80)
Intravenous hydrocortisone	9 (45)

FPIES, food protein-induced enterocolitis syndrome

Table 2. Comparison of laboratory findings between the FPIES and control groups

	FPIES group (n = 20)	Control group (n = 40)	<i>p</i> -value
TARC (pg/mL)	2053 (410–8147)	502 (104–2034)	<0.001
Modified TARC (pg/mL)	1346 (0–7440)	0 (0–1327)	<0.001
WBC (/μL)	14085 (5190–35050)	11205 (3360–24060)	0.095
Neutrophils (/μL)	8578 (934–24114)	7046 (1584–17684)	0.176
Eosinophils (/μL)	203 (0–832)	0 (0–992)	0.006
Platelets (×10 ⁴ /mm ³)	36.1 (20.7–53.4)	31.7 (11.9–58.4)	0.567
CRP (mg/dL)	0.26 (0.01–5.92)	0.87 (0.01–11.08)	0.083
LDH (U/L)	308 (232–383)	298 (193–543)	0.270
pH	7.380 (7.281–7.415)	7.384 (7.262–7.510)	0.426
HCO ₃ ⁻ (mEq/L)	19.7 (15.4–23.5)	20.9 (12.8–26.4)	0.225
Methemoglobin (%)	0.4 (0.1–0.6)	0.3 (0–1.1)	0.075

Data are presented as medians (ranges). Differences were evaluated using the Mann–Whitney *U* test.

FPIES, food protein-induced enterocolitis syndrome; TARC, thymus and activation-regulated chemokine; WBC, white blood cell count; CRP, C-reactive protein; LDH, lactate dehydrogenase.

Figure legends

Figure 1.

(A) Comparison of the serum and modified TARC levels between the FPIES and control groups.

The medians and interquartile ranges are indicated by horizontal lines.

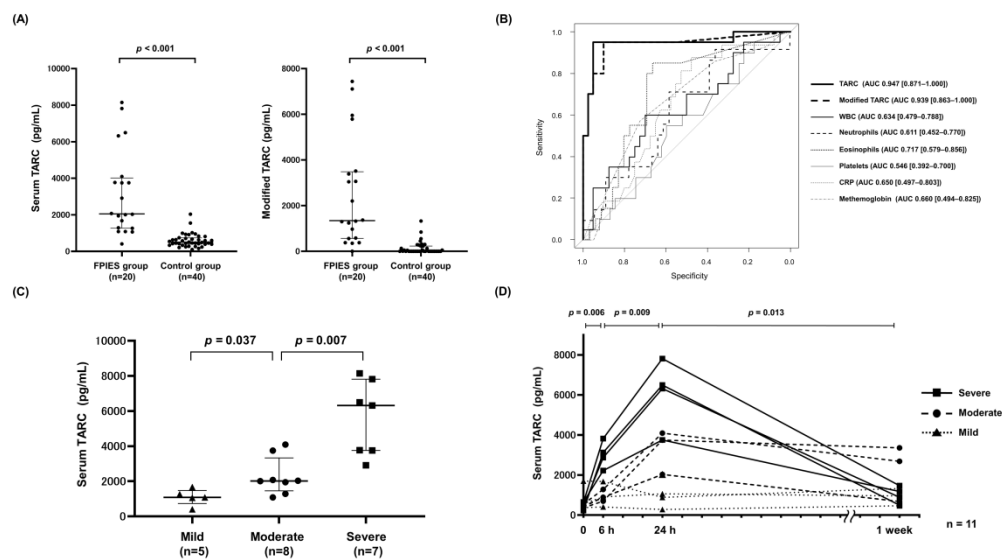
(B) Receiver operating characteristic curve for the diagnosis of FPIES.

(C) Comparison of serum TARC and modified TARC levels between mild, moderate, and severe episodes.

(D) The temporal changes in serum TARC values.

CRP, C-reactive protein; FPIES, food protein-induced enterocolitis syndrome; TARC, thymus and activation-regulated chemokine; WBC, white blood cell count

Figure 1



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Usefulness of thymus and activation-regulated chemokine (TARC) for FPIES diagnosis

Supporting Information

■ Methods

Definition of the study groups

FPIES group:

FPIES diagnosis was based on the diagnostic criteria of the International Consensus Guideline 2017.¹ Subjects with solid food FPIES visited the emergency outpatient department due to vomiting after ingesting causative food, or underwent OFC for confirmation of diagnosis or tolerance. OFC-negative cases were excluded from the study.

Control group:

Patients who visited the emergency department and were hospitalized with infectious gastroenteritis or sepsis, important differential diseases of FPIES, were included as the control group.

Infectious gastroenteritis was diagnosed as follows:

- 1) Cases wherein pathogenic bacteria were detected using stool culture.
- 2) Positivity on the norovirus or rotavirus stool rapid test.
- 3) Patients with watery diarrhea after vomiting, diagnosed as having viral gastroenteritis.

Patients who were hospitalized for infectious diseases other than gastroenteritis and met the pediatric systemic inflammatory response syndrome criteria were diagnosed with sepsis. Based on the definition of childhood sepsis, it does not matter whether the blood culture is positive or not.²

We matched the control group (patients with infectious gastroenteritis and sepsis) with the FPIES group by age. Case allocation was FPIES: infectious gastroenteritis: sepsis = 1: 1: 1 (FPIES group: control group = 1: 2).

Severity of FPIES

Regarding the severity of FPIES symptoms, the severity criteria of symptoms described in the International Consensus Guideline¹ was used (mild if there were between one and two vomiting episodes without lethargy, moderate if there were three or more vomiting episodes with mild lethargy, and severe if there were three or more vomiting episodes with severe lethargy, hypotonia, ashen, or cyanotic appearance).

Modifier TARC

Normal TARC values differ by age. The previously reported age-specific mean TARC values in healthy children aged 0–1, 2–5, and ≥ 5 years were 707, 365, and 273 pg/mL, respectively.³ A value obtained by subtracting the age-appropriate mean TARC value in healthy children from the measured serum TARC value was defined as the modified TARC value. If the modified TARC was less than 0, it was set to 0.

Oral food challenge test

OFC was conducted via the open, single-ingestion method using boiled egg yolk (2 or 7 g), boiled egg white (20 g), boiled wheat noodles (15 g), rice (3 g), or cooked short-neck clam (10 g). The patient was allowed to rest for 24 h after ingestion. Following the onset of vomiting and associated symptoms, intravenous fluid and/or intravenous hydrocortisone therapy was initiated at the discretion of the attending physician. Positive OFC results were ascertained based on the International Consensus Guideline.¹

Statistical analyses

Blood test data were compared between the FPIES and control groups. The Mann-Whitney U test was used to compare two groups of continuous variables. For TARC values, modified TARC values, and items reported previously to differ between FPIES and other diseases, a receiver operating characteristic analysis was performed as an index for FPIES diagnosis. Univariate and age-adjusted multivariate analyses were performed for FPIES diagnosis and TARC values. In patients with FPIES, the Mann-Whitney U test with Bonferroni correction was used to compare TARC values between the three severity groups. The correlation between TARC and the number of vomiting episodes and duration of vomiting was evaluated using Spearman's rank correlation coefficient. The Wilcoxon signed-rank sum test was performed to evaluate the TARC

value change over time of OFC cases. Statistical significance was set at $p < 0.05$.

All statistical analyses were performed using EZR 1.33 software (Saitama Medical Center, Jichi Medical University, Saitama, Japan).⁴

References

1. Nowak-Węgrzyn A, Chehade M, Groetch ME, et al. International consensus guidelines for the diagnosis and management of food protein-induced enterocolitis syndrome: Executive summary-Workgroup Report of the Adverse Reactions to Foods Committee, American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol* 2017;139:1111-1126.e4.
2. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Crit Care Med* 2013;41:580-637.
3. Fujisawa T, Nagao M, Hiraguchi Y, et al. Serum measurement of thymus and activation-regulated chemokine/CCL17 in children with atopic dermatitis: elevated normal levels in infancy and age-specific analysis in atopic dermatitis. *Pediatr Allergy Immunol* 2009;20:633-641.
4. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013;48:452-458.

Table S1. Patient (episode) characteristics

	FPIES group (n = 20)	Control group (n = 40)	
		Infectious gastroenteritis (n = 20)	Sepsis (n = 20)
Sex, male, (n, %)	7 (35)	11 (55)	8 (40)
Age (years)	1.1 (0.5–6.2)	1.7 (0.1–4.4)	1.3 (0.2–6.0)
History of IgE-dependent food allergy (n, %)	1 (5)	2 (10)	2 (10)
History of atopic dermatitis (n, %)	7 (35)	2 (10)	8 (40)
Current eczema (n, %)	0 (0)	0 (0)	0 (0)

Data are presented as medians (ranges) or n (%).

FPIES, food protein-induced enterocolitis syndrome; IgE, immunoglobulin E

Table S2. Comparison of laboratory findings in FPIES, infectious gastroenteritis, and sepsis patients

	FPIES (n = 20)	Infectious gastroenteritis (n = 20)	Sepsis (n = 20)
TARC (pg/mL)	2053 (410–8147) ^{†‡}	635 (277–2034) [†]	454 (104–998) [‡]
Modified TARC (pg/mL)	1346 (0–7440) ^{†‡}	91 (0–1327) ^{†§}	0 (0–303) ^{‡§}
WBC (/μL)	14085 (5190–35050)	10255 (5680–20320)	12280 (3360–24060)
Neutrophils (/μL)	8578 (934–24114)	7086 (2283–15240)	6929 (1584–17684)
Eosinophils (/μL)	203 (0–832) [‡]	0 (0–992)	57 (0–409) [‡]
Platelets (× 10 ⁴ /mm ³)	36.1 (20.7–53.4)	33.1 (22.5–52.9)	31.5 (11.9–58.4)
CRP (mg/dL)	0.26 (0.01–5.92)	0.17 (0.01–4.55) [§]	1.46 (0.05–11.08) [§]
LDH (U/L)	308 (232–383)	284 (193–370)	307 (226–543)
pH	7.380 (7.281–7.415) [‡]	7.383 (7.262–7.448)	7.384 (7.273–7.510) [‡]
HCO ₃ ⁻ (mEq/L)	19.7 (15.4–23.5) [§]	18.8 (12.8–24.4)	22.1 (17.8–26.4) [§]
Methemoglobin (%)	0.4 (0.1–0.6)	0.3 (0.2–0.6)	0.4 (0–1.1)

Data are presented as medians (ranges). Differences were evaluated using the Mann-Whitney U test with Bonferroni correction.

† p < 0.05 when FPIES and infectious gastroenteritis are compared

‡ p < 0.05 when FPIES and sepsis are compared

§ p < 0.05 when infectious gastroenteritis and sepsis are compared

FPIES, food protein-induced enterocolitis syndrome; TARC, thymus and activation-regulated chemokine; WBC, white blood cell count; CRP, C-reactive protein; LDH, lactate dehydrogenase.

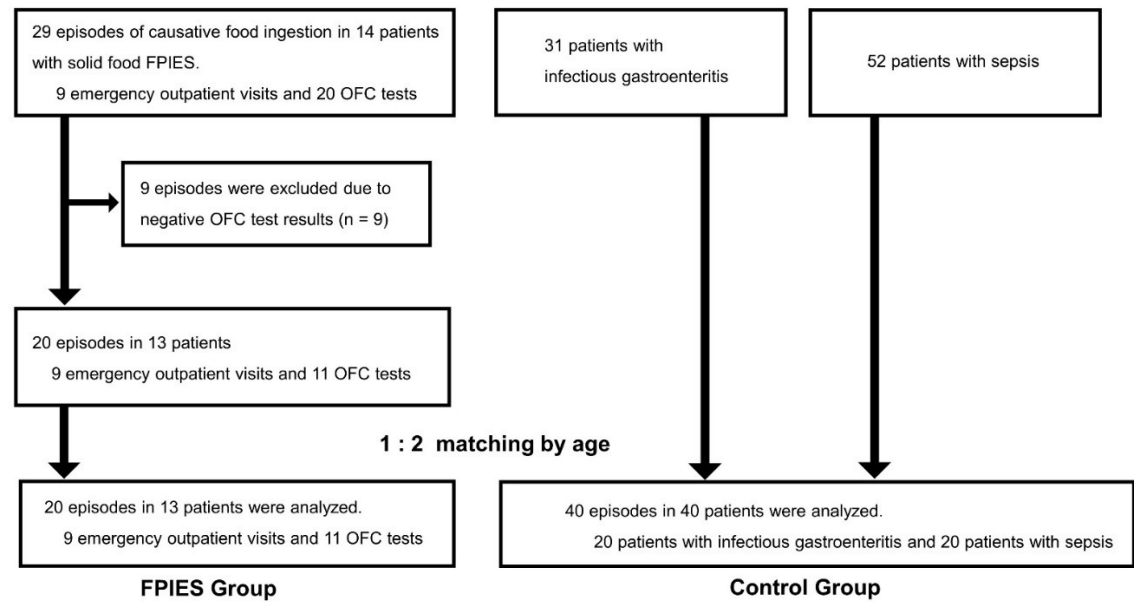
Table S3. Diagnostic performance for diagnosis of FPIES

	AUC	95% CI	Optimal cut-off point	Sensitivity, %	Specificity, %
TARC (pg/mL)	0.946	0.871–1.000	1062	95.0	95.0
Modified TARC (pg/mL)	0.939	0.863–1.000	355	95.0	90.0
WBC (/μL)	0.634	0.479–0.788	13870	60.0	70.0
Neutrophils (/μL)	0.611	0.452–0.770	7351	70.0	58.3
Eosinophils (/μL)	0.717	0.579–0.856	93	80.0	69.4
Platelets (× 10 ⁴ /mm ³)	0.546	0.392–0.700	36.0	55.0	60.0
CRP (mg/dL)	0.650	0.497–0.803	0.77	81.2	52.5
Methemoglobin (%)	0.660	0.494–0.825	0.4	57.1	72.2

FPIES, food protein-induced enterocolitis syndrome; AUC, area under the curve; CI, confidence interval; TARC, thymus and activation-regulated chemokine; WBC, white blood cell count; CRP, C-reactive protein

Figure S1. Study enrollment.

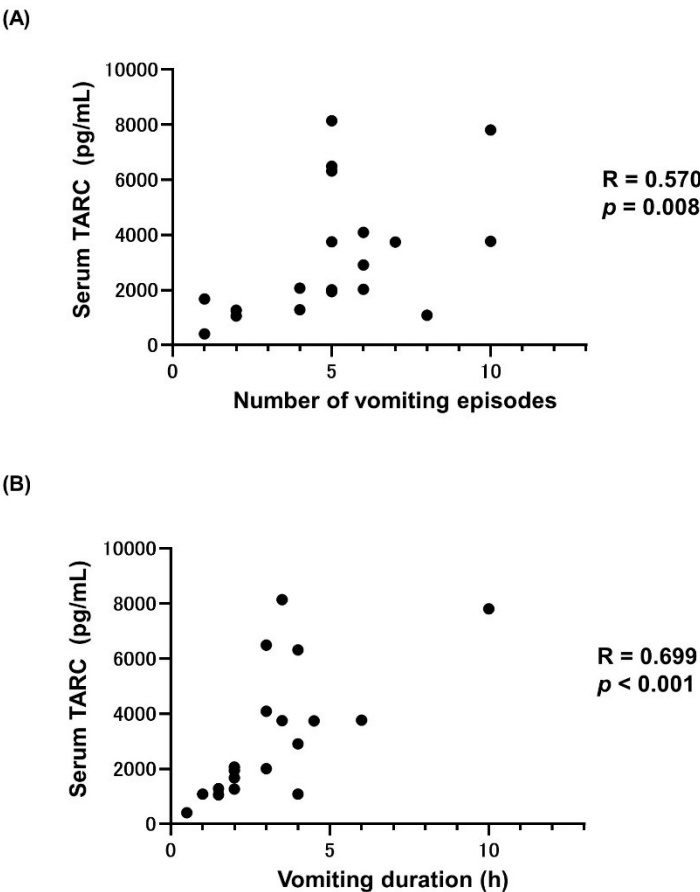
Figure E1



FPIES, food protein-induced enterocolitis syndrome; OFC, oral food challenge

Figure S2. Relationship of vomiting with serum TARC values.

Figure S2



(A) Relationship of the number of vomiting episodes with serum TARC values.

(B) Relationship of vomiting duration with serum TARC values.

TARC, thymus and activation-regulated chemokine