International Archives of Allergy and Immunology

Clinical Allergy – Brief Report

Int Arch Allergy Immunol
DOI: 10.1159/000510723

Received: June 19, 2020
Accepted: August 5, 2020
Published online: October 6, 2020

Evaluation of the Diagnostic Accuracy of Thymus and Activation-Regulated Chemokine to Discriminate Food Protein-Induced Enterocolitis Syndrome from Infectious Gastroenteritis

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Keywords
Chemokine CCL17 · Food allergy · Food protein-induced enterocolitis syndrome · Thymus and activation-regulated chemokine · Infectious gastroenteritis

Abstract
Background: Post-emetic elevation in thymus and activation-regulated chemokine (TARC) levels has been reported in patients with food protein-induced enterocolitis syndrome (FPIES); however, no studies have investigated differences in TARC levels between FPIES and other diseases. ***Objectives:*** We evaluated the clinical usefulness of TARC measurement in differentiating between FPIES and infectious gastroenteritis. ***Methods:*** This study included 8 patients with solid-food FPIES (FPIES group; hen's egg [n = 6], rice [n = 1], and short-neck clam [n = 1]; a total of 11 episodes necessitating emergency department visit or positive result of oral food challenge test) and 17 patients with infectious gastroenteritis (control group), and all patients had no eczema. Post-emetic serum TARC levels and modified TARC levels (serum TARC value – normal mean for each age) were compared between the 2 groups. ***Results:*** The median (range) ages for the FPIES and control groups were 0.7 (0.5–6.2) and 1.8 (0.1–4.4) years, respectively ($p > 0.05$). In the FPIES and control groups, median (range) TARC levels were 2,911 (1,062–7,816) and 600 (277–2,034) pg/mL, and median (range) modified TARC levels were 2,204 (355–7,109) and 129 (0–1,314), respectively. The TARC and modified TARC levels were significantly higher in the FPIES group than in the control group ($p < 0.001$ for both). ***Conclusion:*** In the absence of eczema, post-emetic serum TARC levels might be a potential diagnostic biomarker for distinguishing FPIES from infectious gastroenteritis. © 2020 S. Karger AG, Basel

Introduction

Food protein-induced enterocolitis syndrome (FPIES) is an immunoglobulin E (IgE)-independent allergic disease that manifests with gastrointestinal symptoms, such as vomiting and diarrhea [1]. Therefore, diagnosing FPIES at the first episode is difficult in the absence of disease-specific biomarkers; in particular, solid-food FPIES often develops in infancy after the age of 5–6 months [1], which is a period when infants are more likely to have infectious gastroenteritis. Notably, thymus and activation-regulated chemokine (TARC), a Th2 chemokine, was found to be elevated in allergic diseases, such as atopic

Edited by: H.-U. Simon, Bern.

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Table 1. Comparison of patient characteristics and symptoms between FPIES and control groups

	FPIES group (n = 11)	Control group (n = 17)	p value
Sex, male, n (%)	4 (36.4)	8 (47.1)	0.705
Age, years	0.7 (0.5–6.2)	1.8 (0.1–4.4)	0.076
Vomiting, n (%)	11 (100)	17 (100)	>0.999
Diarrhea, n (%)	3 (27.3)	17 (100)	<0.001
Bloody stool, n (%)	0 (0)	1 (5.9)	>0.999
Lethargy, n (%)	11 (100)	17 (100)	>0.999
Pallor, n (%)	5 (45.5)	2 (11.8)	0.076
History of IgE-dependent food allergy, n (%)	1 (9.1)	2 (11.8)	>0.999
History of atopic dermatitis, n (%)	4 (36.4)	3 (17.6)	0.381
Current eczema, n (%)	0 (0)	0 (0)	>0.999

Data are presented as median (range) or n (%). Differences were evaluated using the Mann-Whitney U test for continuous variables or the Fisher's exact test for categorical variables. FPIES, food protein-induced enterocolitis syndrome; IgE, immunoglobulin E.

dermatitis [2], and a case report showed increased serum TARC levels after appearance of FPIES symptoms [3]. However, no studies have assessed TARC levels for the differential diagnosis of FPIES and other diseases, such as infectious gastroenteritis. Accordingly, this study compared post-emetic serum TARC levels between patients with solid-food FPIES and those with infectious gastroenteritis to evaluate the clinical usefulness of TARC measurements for differentiating between FPIES and infectious gastroenteritis.

Materials and Methods

This prospective study enrolled 8 patients aged 0–6 years (a total of 11 vomiting episodes: 7 emergency outpatient visits and 4 oral food challenge [OFC] tests) with acute solid-food FPIES who visited the Saitama Medical Center Jichi Medical University, Saitama, Japan, between April 2019 and April 2020. Negative results of the OFC tests and episodes within 1 week from a previous vomiting episode were excluded. Diagnosis and severity of FPIES were ascertained using the diagnostic criteria of the International Consensus Guideline published in the Journal of Allergy and Clinical Immunology [1]. The control group comprised 17 patients aged 0–6 years who were hospitalized due to viral gastroenteritis-induced vomiting (for definition of the group, see online suppl. material; see www.karger.com/doi/10.1159/000510723 for all online suppl. material). This study included only patients whose legal guardians provided consent to participate in this study (online suppl. Fig. 1). We collected data on age, sex, symptoms, medical history, and current eczema. Furthermore, in patients with FPIES, the causative food and age of onset were also evaluated.

Serum TARC was quantified by a chemiluminescence enzyme immunoassay using an HISCL® TARC Assay Kit (Sysmex, Hyogo, Japan). In patients who visited our hospital with complaints of vomiting, serum TARC levels were measured in the venous blood sample collected at the outpatient visit. In cases that were followed up, TARC levels were measured again several days later. In FPIES patients who underwent OFC, serum TARC levels were measured before ingestion and at 6 h, 24 h, and 1 week after ingestion. Peak serum TARC values were evaluated. Because normal TARC values differ by age [4], the "modified TARC value" (measured serum TARC value – age-appropriate mean TARC value in healthy children) was assessed (taken as 0 when the modified TARC level was less than 0). A time course of serum TARC levels was evaluated in patients who required repeated measurements.

WBC, neutrophil, eosinophil, and platelet counts were determined. The serum levels of C-reactive protein, lactate dehydrogenase, pH, HCO_3^-, and methemoglobin (%) were also measured. The OFC was conducted using an open, single-ingestion method with 2 g of boiled egg yolk or 10 g of cooked short-neck clam, and the patient was required to rest for 24 h after ingestion. Positive results of OFC were ascertained based on the International Consensus Guideline [1].

For statistical comparisons between the 2 groups, we used the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. $p < 0.05$ was considered statistically significant. For serum TARC and modified TARC values, an ROC analysis was conducted with the differentiation between FPIES and infectious gastroenteritis as the index (Methods of ROC analysis in the online suppl. material). All statistical analyses were conducted using the EZR 1.33 software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [5].

Results

The causative antigens in 8 patients with FPIES (11 episodes) included egg yolk (n = 5), whole egg (n = 1), rice (n = 1), and short-neck clam (n = 1). The FPIES group included 3 boys and 5 girls, and the age at onset ranged

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

	FPIES group (n = 11)	Control group (n = 17)	p value
TARC, pg/mL	2,911 (1,062–7,816)	600 (277–2,034)	<0.001
Modified TARC, pg/mL	2,204 (355–7,109)	129 (0–1,314)	<0.001
WBC, /μL	13,980 (4,920–35,050)	10,190 (5,680–18,500)	0.122
Neutrophils, /μL	9,902 (934–24,114)	6,975 (2,283–12,853)	0.202
Eosinophils, /μL	103 (0–832)	0 (0–992)	0.202
Platelets, $\times 10^4$/mm^3	37.2 (20.7–53.4)	34.7 (22.5–52.9)	0.510
CRP, mg/dL	0.22 (0.01–1.53)	0.19 (0.01–4.55)	0.521
LDH, U/L	299 (232–375)	284 (193–370)	0.771
pH	7.368 (7.281–7.498)	7.385 (7.262–7.448)	>0.999
HCO_3^-, mEq/L	20.6 (18.4–26.7)	18.6 (12.8–24.4)	0.106
Methemoglobin, %	0.4 (0.3–0.5)	0.3 (0.2–0.6)	0.093

Data are presented as median (range) or n (%). Differences were evaluated using the Mann-Whitney U test for continuous variables or the Fisher's exact test for categorical variables. FPIES, food protein-induced enterocolitis syndrome; TARC, thymus and activation-regulated chemokine; CRP, C-reactive protein; LDH, lactate dehydrogenase.

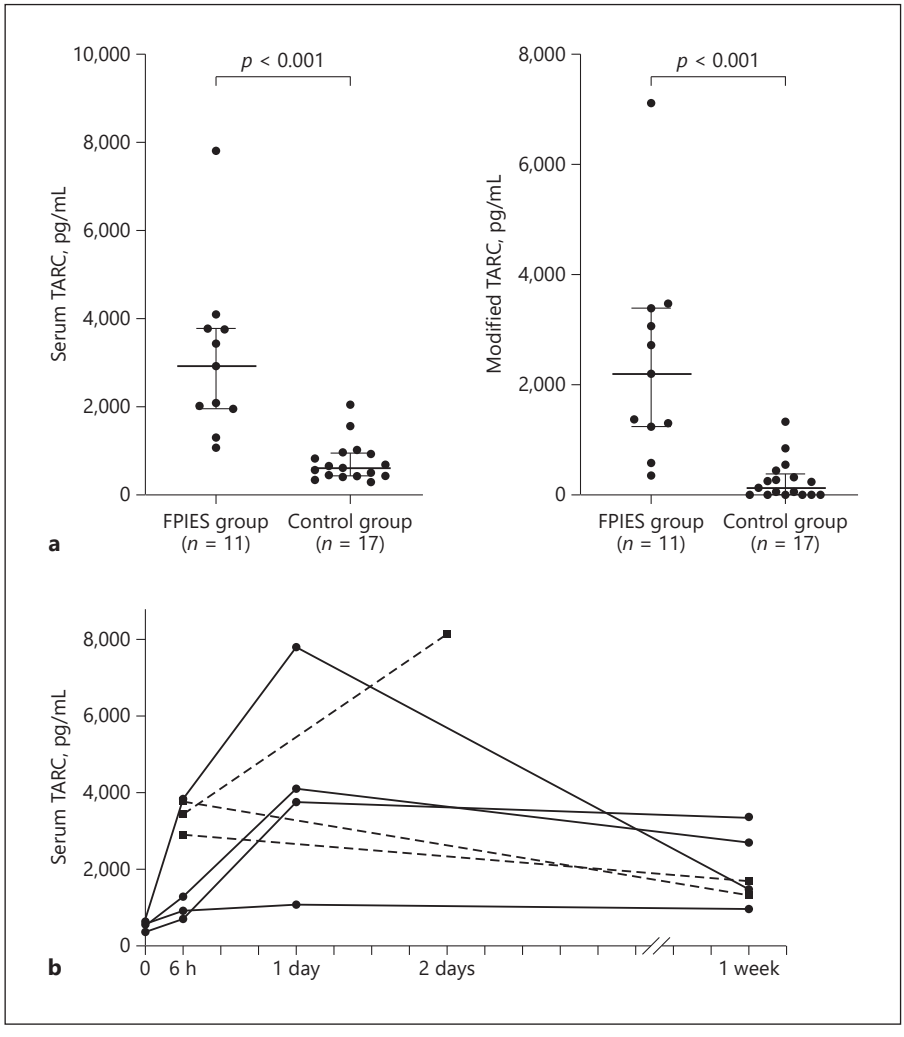

Fig. 1. Serum TARC levels. **a** Comparisons of serum and modified TARC levels between FPIES and control groups. The median and interquartile range are indicated by horizontal lines. **b** Temporal changes in serum TARC levels. The solid line represents the patients with positive OFC results (n = 4), while the dotted line represents patients who visited an emergency department (n = 3). TARC, thymus and activation-regulated chemokine; FPIES, food protein-induced enterocolitis syndrome; OFC, oral food challenge.

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from 0.5 to 3.0 (median, 0.7) years. The median age at the time of the episodes was 0.7 (range, 0.5–6.2) years. Of the 11 episodes, 4, 6, and 1 were severe, moderate, and mild in severity, respectively (Table 1; online suppl. Table 1). The control group included 17 patients with viral gastroenteritis (8 boys and 9 girls; median [range] age: 1.8 [0.1–4.4] years), and 7 patients had norovirus infections (Table 1). Patient characteristics and symptoms at onset in the FPIES and control groups are shown in Table 1. None of the participants in both groups had eczema.

As shown in Table 2, in the FPIES and control groups, the medians (range) of serum TARC levels were 2,911 (1,062–7,816) and 600 (277–2,034) pg/mL and those of modified TARC levels were 2,204 (355–7,109) and 129 (0–1,314) pg/mL, respectively; both TARC levels were significantly higher in the FPIES group than in the control group ($p < 0.001$ for both; Fig. 1a). There were no significant intergroup differences in other laboratory parameters (Table 2, online suppl. Table 1). In addition, an evaluation of the time course of TARC levels showed an increase at 6 h post ingestion and a further increase 24 h later. Despite a decrease in the levels 1 week later, TARC levels did not reach normal levels (Fig. 1b). The results of ROC analysis are shown in the online suppl. material (online suppl. Fig. 2).

Discussion

The FPIES is an IgE-independent allergic disease that manifests with gastrointestinal symptoms. It is diagnosed based on 1 major criterion and 3 or more minor criteria [1]. Solid-food FPIES occurs later than milk FPIES and often develops in infancy after the age of 5–6 months [1], which is a period when infants are more likely to have infectious gastroenteritis. Therefore, it is very difficult to diagnose solid-food FPIES and differentiate it from gastroenteritis in the first episode. To the best of our knowledge, this study is the first to investigate the clinical usefulness of post-emetic TARC levels in differentiating solid-food FPIES from infectious gastroenteritis.

Undiagnosed patients with FPIES who continue to ingest the causative food are at an increased risk of developing more severe symptoms. If FPIES is suspected and the symptoms and causative antigens are unclear, OFC is recommended [1]. Thus, for early diagnosis, biomarkers of FPIES are necessary. It has been reported that 2–20% of the FPIES patients are atypical (atypical-FPIES), and the IgE specific to the causative antigen can be positively detected during the long-term follow-up in these patients [1]. However, most FPIES patients are typically IgE negative, and the diagnosis based on specific IgE measurement is difficult. Although several studies have evaluated changes in neutrophil, C-reactive protein, methemoglobin, and cytokines in patients with FPIES, the results are inconsistent. Thus, no specific biomarkers for FPIES have yet been determined [6–9].

TARC is a Th2-type chemokine produced by dendritic cells, endothelial cells, keratinocytes, and fibroblasts [2]. Several studies have examined the association between allergic diseases and TARC. In particular, the usefulness of TARC in the diagnosis and evaluation of atopic dermatitis has been reported [4]. However, few studies have examined the relationship between food allergies and TARC [3]. In this study, we compared post-emetic TARC levels between patients with solid-food FPIES and those with infectious gastroenteritis and found significantly higher TARC levels in patients with FPIES. Considering that serum TARC levels are higher in younger children [4], we evaluated the modified TARC levels by subtracting the normal mean value for each age-group from TARC measurements. The modified TARC levels were also significantly higher in patients with FPIES than in those with infectious gastroenteritis. The findings suggest that increased post-emetic serum TARC levels might be a potential marker for the diagnosis of FPIES. In addition, some patients had a history of atopic dermatitis, but none had eczema at the time of examination because they were being treated with appropriate topical steroid therapy. Notably, patients with uncontrolled atopic dermatitis have high TARC levels. Thus, in patients with severe eczema, high TARC levels might not be indicative of FPIES. Therefore, it is important to evaluate the skin condition at the time of examination.

This study has some limitations. For instance, the sample size was small, and only patients with acute solid-food FPIES were included in this study. Further studies, including other FPIES patients, are needed to confirm our present findings.

In conclusion, we found that post-emetic TARC levels were significantly higher in patients with solid-food FPIES than in those with infectious gastroenteritis. The findings suggest that serum TARC levels might be a useful diagnostic biomarker for FPIES, and elevated TARC levels in the absence of eczema may help diagnose FPIES. Future studies with a large sample size are required to clarify the etiopathogenesis of FPIES.

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Acknowledgements

We thank all of the pediatricians and nurses at the Saitama Medical Center, Jichi Medical University, Saitama, Japan. We would like to thank Editage (www.editage.jp) for English language editing.

Statement of Ethics

In accordance with the tenets of the Declaration of Helsinki (1964), the study design and the risks were fully explained, both verbally and in writing, to the children's guardians. Written informed consent was obtained from the guardians prior to study participation. This study was approved by the Ethics Review Board of the Saitama Medical Center, Jichi Medical University (Approval No. S19-077).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study did not receive any funding.

Author Contributions

E.M. wrote the manuscript. E.M., S.K., K.I., and D.S. contributed to data collection and analysis. D.S. and K.I. contributed to study conception, study design, and drafting of the manuscript. All authors have read and approved the final version of this manuscript.

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Supplementary Materials

Definition of group

FPIES group

The diagnosis and severity of FPIES were ascertained using the diagnostic criteria of the International Consensus Guideline published in the Journal of Allergy and Clinical Immunology [1]. Negative results of the OFC tests and episodes within one week from a previous vomiting episode were excluded.

Control group

A case (diagnosed with viral gastroenteritis by a doctor) in which all of the following three items were satisfied was included in a control group.

- Watery diarrhea is shown after vomiting, and the symptoms improve after a few days with fluid therapy alone.
- A fecal culture test is performed and no bacteria that cause bacterial enteritis are detected.
- Blood and urine tests, ultrasonography, and X-ray rule out all other diseases.

A fecal norovirus/rotavirus antigen test was performed to determine norovirus/rotavirus infection.

ROC analysis

Methods

For serum TARC and modified TARC values, an ROC analysis was conducted with the differentiation between FPIES and infectious gastroenteritis as the index. The area under the curve (AUC) was evaluated, and the sensitivity and specificity of the cutoff values were determined. The diagnosis of FPIES and the post-emetic serum TARC levels were analyzed

using logistic regression analysis. All statistical analyses were conducted using the EZR 1.33 software (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

Results

The results of the ROC analysis of the serum and modified TARC levels are shown in Figure E2. The AUCs for serum and modified TARC levels were 0.968 (95% CI 0.914–1.000) and 0.957 (95% CI 0.893–1.000), with a specificity of 88.2% and 88.2% and sensitivity of 100% and 90.9% at cutoff values of 1062 and 584 pg/mL (Youden Index), respectively.

The univariate analysis showed that the post-emetic serum TARC level was a significant factor for the diagnosis of FPIES, and the odds ratio per 1000 pg/mL increase was 23.0 (95% CI 2.17–244.0, $p = 0.009$). In the multivariate analysis adjusted by age, which tended to be lower in the FPIES group than in the control group, the serum TARC level was also a significant factor, and the adjusted odds ratio per 1000 pg/mL increase was 17.6 (95% CI 2.02–153.0, $p = 0.009$).

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Supplementary figures

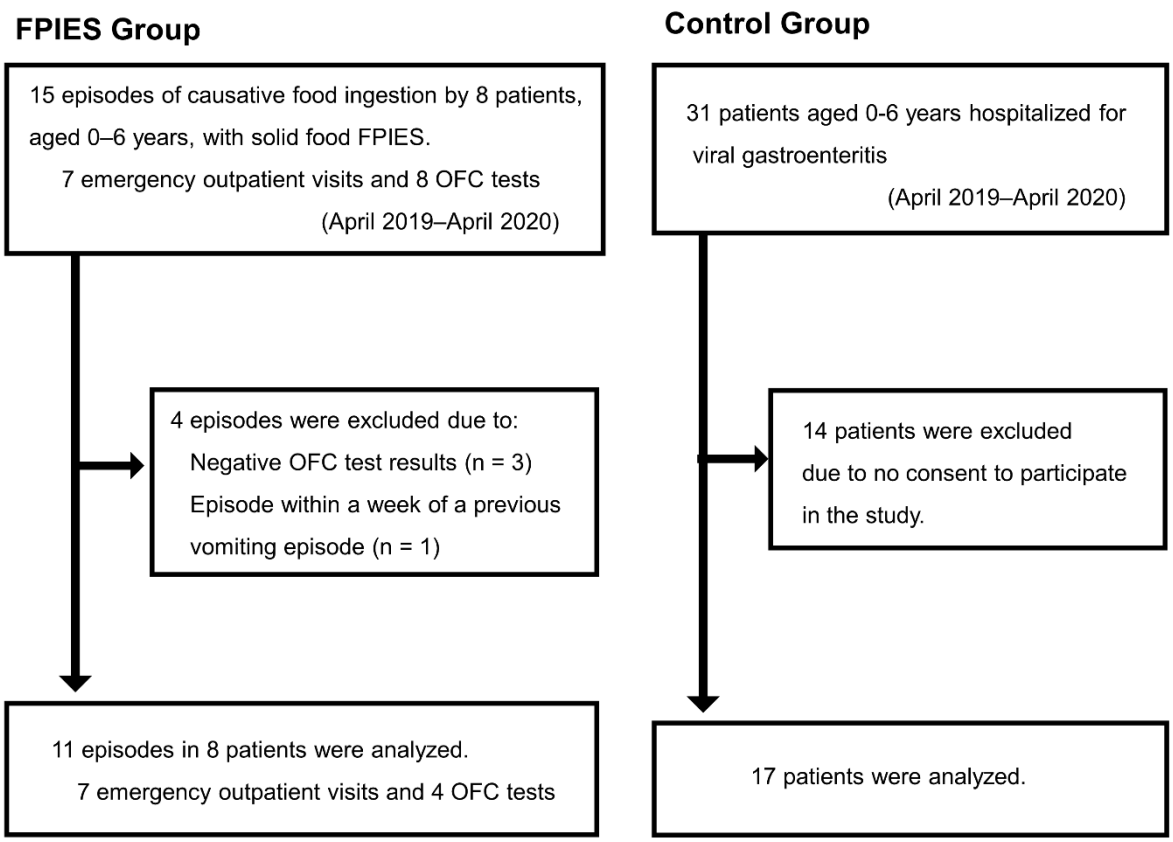

Fig. E1. Study enrolment.

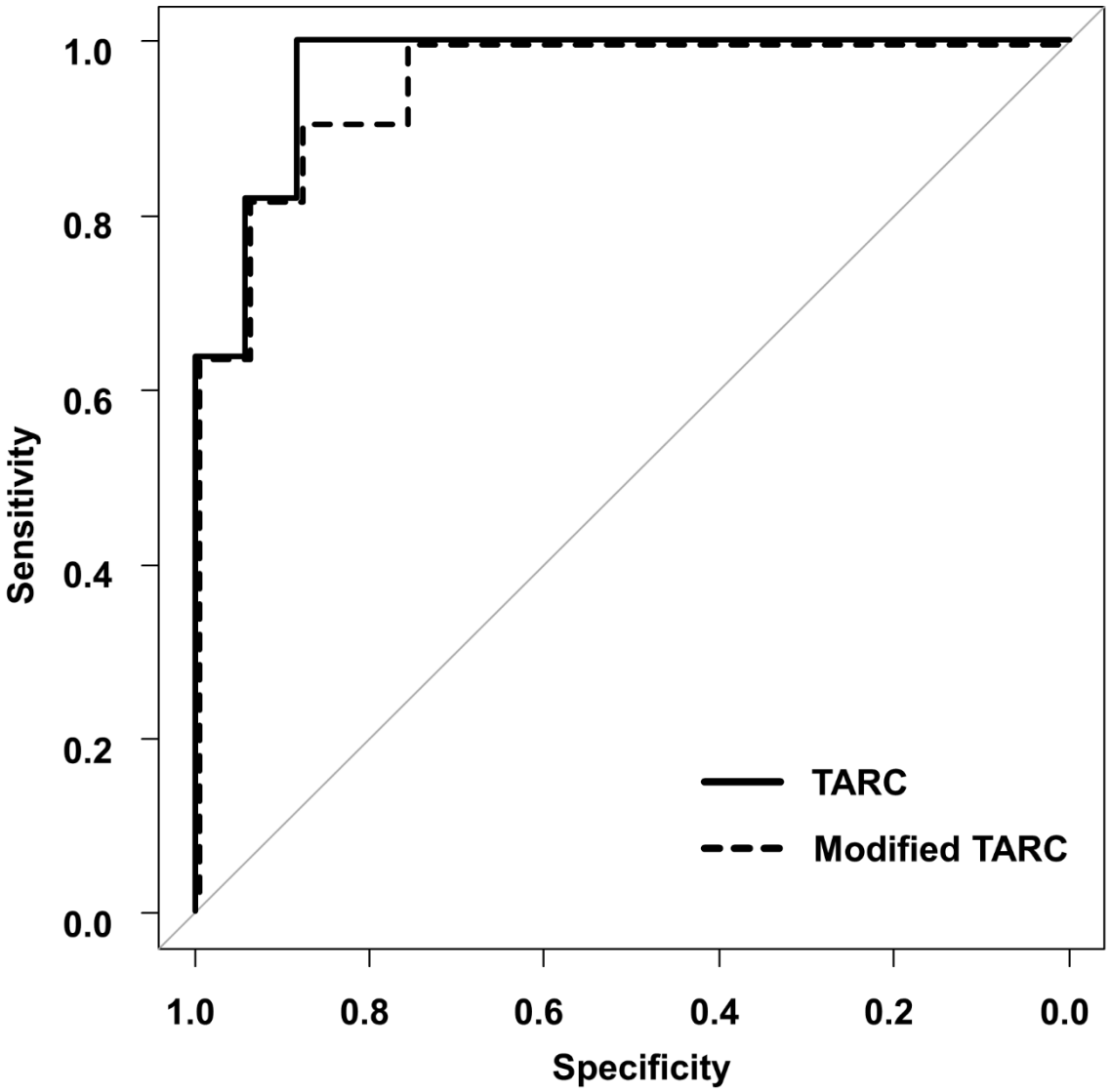

Fig. E2. Receiver operating characteristic curve for the diagnosis of FPIES.

Continuous and dashed lines indicate TARC and modified TARC levels, respectively.

FPIES, food protein-induced enterocolitis syndrome; IgE, immunoglobulin E; OFC, oral food challenge; TARC, thymus and activation-regulated chemokine.

Table E1

Clinical findings of all episodes of FPIES in the study population

Patient–episode	Age at onset, years	Causative food	Presentation	Ingestion dose (g)	Symptom	Vomiting episodes	Symptom severity	Treatment	Baseline TARC, (pg/mL)	TARC, (pg/mL)	Time from antigen ingestion to blood test (hours)
1-I	0.7	Egg yolk	ED	2	V, L	4	Moderate	None	630	2075	22
1-II	1.6	Egg yolk	OFC	2	V, L, P	>10	Severe	HC, IF	630	7816	24
2-I	0.7	Egg yolk	ED	7	V, D, L	5	Moderate	None	576	2009	6
2-II	1.0	Egg yolk	OFC	2	V, L	2	Mild	HC, IF	576	1062	24

3	1.9	Egg yolk	OFC	2	V, L	6	Moderate	HC, IF	555	4097	24
4	0.7	Egg yolk	ED	2	V, D, L, P, C	5	Severe	IF	NA	3428	6
5	0.7	Egg yolk	ED	7	V, L	5	Moderate	IF	NA	1948	4
6	0.8	Whole egg	ED	3	V, D, L, P	>10	Severe	IF	NA	3771	6
7-I	0.5	Rice	ED	3	V, L, P	4	Moderate	IF	886	1291	6
7-II	0.6	Rice	ED	5	V, L, P	6	Severe	IF	886	2911	6
8	6.2	Short-neck clam	OFC	10	V, L	7	Moderate	HC, IF	360	3748	24

FPIES, food protein-induced enterocolitis syndrome; TARC, thymus and activation-regulated chemokine; ED, emergency department visit; OFC, oral food challenge; V, vomiting; D, diarrhea; L, lethargy; P, pallor; C, cyanosis, HC, intravenous hydrocortisone; IF, intravenous fluid; NA, not available.