

Atopy patch test for the diagnosis of food protein-induced enterocolitis syndrome

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Food protein-induced enterocolitis syndrome (FPIES) is thought to be a non-IgE mediated food allergy syndrome. Affected infants typically demonstrate gastrointestinal symptoms after hours after ingestion of the offending food. Traditional allergy testing is not useful for this disorder because tests for food specific IgE are routinely negative. A diagnostic oral food challenge (OFC) is the only method to confirm the diagnosis of FPIES. This prospective study was undertaken to determine whether the atopy patch test (APT) is able to predict the results of the OFC. Nineteen infants with suspected FPIES by clinical history underwent APT to the suspected foods. After APT was performed, subjects underwent OFC to determine whether FPIES was present. The results of APT and OFC were compared and used to calculate sensitivity and specificity of the APT. APT predicted the results of oral food challenges in 28/33 instances. There were 16 cases of FPIES confirmed by oral food challenges. In all 16 cases of FPIES, the APT was positive to the suspected food. However, the APT was positive in five instances where the oral food challenge was negative. All 12 patients with a negative APT had a negative oral food challenge to the suspected food. APT appears to be a promising diagnostic tool for the diagnosis of FPIES.

Key words: food allergy; food protein-induced enterocolitis; atopy patch testing

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Food protein-induced enterocolitis syndrome (FPIES) is considered by many to be a non-IgE-mediated food allergy syndrome, which consists of severe vomiting and diarrhea 1–3 h after ingestion of the offending food. Patients usually present in early infancy with severe vomiting and diarrhea, which can cause dehydration, hypotension, lethargy, metabolic acidosis, and shock. In FPIES, patients with chronic exposure to the offending food may present with chronic diarrhea and/or vomiting. Milk and soy are the most common foods implicated in FPIES, but other foods have been implicated including chicken, turkey, peas, egg, wheat, fish, rice, oat, barley, lentil, sweet potato, squash, and string bean (1–3).

Little is known about the pathophysiology of FPIES. The absence of food-specific IgE as demonstrated by negative prick skin tests suggest

that the disease is not caused by an early onset IgE-mediated reaction. Van Sickle et al. showed *in vitro* proliferation to food antigen of peripheral blood mononuclear cells (PBMCs) in patients with FPIES as compared with non-allergic controls (4) suggesting a T-cell mediated response. TNF- α is thought to play a major role in FPIES. Heyman et al. showed that PBMCs of infants with gastrointestinal allergy to cow's milk secrete TNF- α , which alter intestinal permeability (5); also, fecal TNF- α levels are increased with patients with FPIES (6). Another study by Benlounes et al. revealed that lower doses of cow's milk protein are needed to stimulate TNF- α secretion from PBMCs of patients with active intestinal CMA as compared with patients whose allergy had resolved or those with skin rather than gastrointestinal sensitivity (7). Finally, increased fecal TNF- α levels were found in the

stool after positive cow's milk challenges in patients with enterocolitis (8). Although the precise mechanism of FPIES is still unclear, these results suggest that TNF- α plays a significant role in the pathophysiology of the disease.

Currently, a diagnostic food challenge is the only method to confirm the diagnosis of FPIES in children with suggestive symptoms as testing for allergen-specific IgE-foods is negative (3). This needs to be performed under the supervision of an experienced medical team and is routinely performed to establish the diagnosis in children with suspected FPIES. In addition, food challenges are performed to determine whether a child may have outgrown an already diagnosed FPIES. Consequently, any test that can predict the outcome of a food challenge with reasonable sensitivity and specificity would be useful in limiting the number of food challenges that need to be performed.

However, traditional allergy testing such as prick skin testing or *in vitro* testing for food-specific IgE are not useful for the diagnosis of FPIES as they are negative. Therefore, atopy patch testing (APT) is explored in this study. APT is a procedure that attempts to test for a delayed, non-IgE-mediated reaction to foods. Increasing evidence supports the use of APT for diagnosis of food hypersensitivity in atopic dermatitis (9, 10) and eosinophilic esophagitis (11). In addition, one small retrospective study showed that APT had a sensitivity of 79% and specificity of 91% for the detection of cow's milk allergy with digestive symptoms in 35 children (12). These results suggest that APT may be able to detect non-cutaneous delayed hypersensitivity reactions. The APT is performed by dissolving small amounts of suspected food proteins in saline and placing the mixture in aluminum Finn chambers.

This study was undertaken to see if APT is capable of predicting the presence or absence of FPIES. The results of the APT were compared with the results of the oral food challenge, the best diagnostic test.

Methods

Subjects

Nineteen patients aged 5–30 months participated in the study from the allergy clinic at The Children's Hospital of Philadelphia over 18-month period. The study was reviewed and approved by the Institutional Review Board of The Children's Hospital of Philadelphia. Informed consent was obtained from the parents

of the subjects in all cases. Patients were included in the study if they met clinical criteria for 'typical' FPIES as proposed by Sicherer (13).

Subjects were excluded from the study if they had an evidence of immediate hypersensitivity (IgE-mediated) reaction demonstrated by positive prick skin test to the suspected foods, thereby excluding the possibility of a co-existing IgE-mediated food allergy. In addition, patients were excluded if they had conditions that might affect interpretation of APT results for the following reasons: (i) presence of skin disorders other than atopic dermatitis which may affect interpretation of APT results; (ii) severe diffuse atopic dermatitis with no surface area available for APT; (iii) use of oral immunosuppressant medicines that may affect patch testing results; and (iv) use of oral medicines that may affect interpretation of oral food challenges such as antimotility agents, anti-inflammatory medicines and β -blockers.

Atopy patch tests were performed to suspected foods using previously published methods with 12-mm aluminum cups (Finn Chambers on Scanpore, Allerderm Laboratories, Inc., Petaluma, CA, USA) (11, 14). The patches were removed at 48 h and the results were read at 72 h. Reactions were classified as follows: +, erythema; ++, erythema and papules; ++++, erythema and vesicles.

Within 2 months after the APT, non-blinded oral food challenges were performed to ascertain whether the patient had FPIES. Patients were instructed to avoid all foods that were found positive on APT. Oral food challenges were performed by administering 0.05–0.15 g protein/kg body weight of the suspected protein in two increasing doses over a 30-min interval in the Allergy Clinic located at The Children's Hospital of Philadelphia. Patients were observed for 4 h and symptoms recorded by the physician (M.A.F. and J.M.S.). Patients tolerating the food challenge in hospital were sent home with a food reintroduction plan. On days 1–3, patients took 0.05–0.15 g/kg of protein per day. On days 4–6 patients took 0.1–0.3 g/kg of protein per day. On days 6–9 patients took 0.15–0.45 g/kg of protein per day. If no symptoms developed on the reintroduction, the food challenge was considered negative and the patient was allowed to ingest the food in any desired quantity. If gastrointestinal symptoms with no other cause developed during the reintroduction, the challenge was considered positive and the reintroduction was discontinued. If equivocal symptoms occurred, the reintroduction was suspended and the food was reintroduced under the same plan 2 wk later. Telephone follow up was performed

by a physician to ascertain the results of the reintroduction. For patients with multiple food challenges, the challenges were spaced 1 month apart for a washout period.

Results

The nineteen patients underwent atopy patch testing and subsequent food challenges. The mean age of these subjects was 15.6 months and a range of 5–30 months. Of the 19 subjects, nine were female and 10 were male. The median age for the onset of symptoms was 2 months, with a range from birth until 9 months. Only two patients (nos 6, 18) had atopic dermatitis.

The blank control patch tests were negative in all subjects who completed the study.

Atopy patch testing and subsequent food challenges

Atopy patch test results were predictive of oral food challenge results in 27/32 instances in the 19 patients who completed the study (Table 1). In this series, there were 10 patients with 13 episodes of FPIES confirmed by oral food challenges. One patient had FPIES to three foods (cow's milk, soy and rice) and one patient (no. 19) had FPIES to two foods (rice and oat). There were no cases of any cutaneous or respiratory reactions. In all 13 cases of FPIES confirmed by challenges (Table 2), the APT was positive to the suspected food. Three separate patients had accidental exposure to significant amounts of the suspected food after the APT but before the formal food challenge also had a positive APT. In all three cases, the exposure caused vomiting and diarrhea within 2 h of the ingestion per the family history. These are considered to be positive challenges to the suspected food for this study. Of these 16 cases of confirmed FPIES, 15 patients developed vomiting and/or diarrhea within 4 h of ingestion of the suspected food (Table 2). One of the 16 cases of confirmed FPIES developed diarrhea within 24 h of ingestion of the suspected food during the food reintroduction at home.

One patient in the study had positive challenges to milk, rice and soy. In each case, the patient developed severe vomiting within 2–3 h after ingestion of the challenge food. His reactions were clinically similar, although his reaction to soy involved diarrhea as well as vomiting. In addition, his reaction to soy was more protracted, lasting 12 h as compared with 2 h for milk and rice.

All 12 patients with negative APT had a negative office-based challenge and reintroduction at home. There were five additional cases of

Table 1. Demographics and results of atopy patch test (APT), oral food challenge (OFC)

Subject number	Age (months)	Gender	Age at onset of symptoms	Suspected food	APT	OFC
1	17	M	2 wk	Cow's milk	++	+
2	17	F	2 wk	Soy	+	+
3	15	F	1 wk	Soy	—	—
4	30	F	1 month	Cow's milk	—	—
5	15	F	1 wk	Soy	++	+
			2 months	Soy	—	—
			2 months	Cow's milk	—	—
			9 months	Egg	++	+
6	13	F†	4 months	Wheat	—	—
			2 months	Cow's milk	+	—
			2 months	Soy	+	—
7	13	M	6 months	Rice	—	—
			5 months	Oat	++	—
8	12	F	5 months	Soy	+++	—
			4 months	Cow's milk	+	+
9	8	M	4 months	Soy	++	+
			4 months	Cow's milk	—	—
			4 months	Cow's milk	+++	+
10	19	M	1 months	Cow's milk	+++	+
			9 months	Rice	++	+
			3 months	Soy	++	+
11	17	M	1 month	Cow's milk	++	+
12	16	F	4 months	Soy	—	—
13	5	F	1 month	Cow's milk	+	+
			1 month	Soy	—	—
14	18	M	4 months	Oat	—	—
15	16	M	2 months	Cow's milk	+	+
				Soy	—	—
16	15	M	3 months	Cow's milk	+	+
17	23	M	1 month	Cow's milk	—	—
18	11	F†	2 months	Cow's milk	++	+
				Soy	+	—
19	17	M	4 months	Rice	+++	+
				Oat	++	+

*Reactions at home.

†Patients with atopic dermatitis.

negative food challenges with the subjects having a false positive APT. Interestingly three out of the five cases had a weakly positive (+) APT. In this study, there were no cases of FPIES with a negative APT.

Statistical analysis

Based on the initial results from our first 19 patients, we can calculate an initial positive predictive value of 75%, negative predictive value of 100% with sensitivity of 100% and specificity of 71%.

Discussion

Currently, the only way to diagnose FPIES is by taking a careful history and performing an oral food challenge. Oral food challenges are the 'gold

Table 2. Challenge results

Patient	Food	Dose (gm/kg)	Time of reaction after ingestion (h)	Reaction (h)
1	Cow's milk	0.15	3	V, D × 3
2	Soy	*	2	V × 3
4	Soy	0.15	2	V, D × 12
5	Egg	0.15	3	V, D × 1
8	Cow's milk	0.15	4	V, D × 2
9	Soy	0.15	3	V × 2
10	Cow's milk	0.15	2	V × 2
	Rice	0.15	4	V × 2
	Soy	0.15	2	V, D × 12
11	Cow's milk	*	3	V, D × 2
13	Cow's milk	0.15	4	V × 2
15	Cow's milk	0.15	24	D × 6
16	Cow's milk	*	2	V, D × 2
18	Cow's milk	0.15	3	V, D × 1
19	Rice	0.15	4	V, D × 12
	Oat	0.15	2	V × 2

V, vomiting; D, diarrhea.

*Accidental reaction at home-dose is unknown.

standard' test for the diagnosis of any food allergy disorder. However, challenges are not without risks. In our cases, three of 30 challenges required intravenous fluid therapy and prolonged monitoring. In this series, 13 patients who had a restricted diet because of a clinical diagnosis of FPIES were able to liberalize their diet because of a negative food challenge. The purpose of this study was to determine how APT compared with the results of the oral food challenge for the diagnosis of FPIES.

The patients in the study had FPIES to commonly reported foods, especially milk and soy. Of 32 episodes that met the clinical criteria for a diagnosis of FPIES, only 16 of the episodes were confirmed by challenge. Because FPIES is usually outgrown before 36 months of age, it is likely that several of the patients lost reactivity to the suspected food in the interval between the onset of disease and the current study.

Importantly, there were no patients with a negative APT that still had FPIES upon challenge to the suspected food. There were five cases of false positive APT with a negative food challenge for FPIES. This suggests APT is similar to traditional allergy skin testing with patients developing tolerance to the food before percutaneous testing is negative.

However, there are several limitations to this study. One limitation is that the APT is not currently a standardized test for food allergens. This makes performance and interpretation of the test somewhat user dependent. Even after standardization, the APT will remain a qualit-

ative test subject to human errors in performance and interpretation.

Another potential critique of the study is the methodology of the food challenges. For the diagnosis of FPIES, protocols typically administer a range of 0.15–0.6 g of protein/kg of body weight for the in-hospital challenge (3). Although the initial dose of protein used for in-hospital challenges in this study was low (0.05–0.15 g of protein/kg body weight), the dose was sufficient to cause FPIES symptoms within 4 h in 15 of 16 cases of confirmed FPIES. In one of 16 cases, the subjects developed symptoms within 24 h. At the end of the 9-day food reintroduction period, there were no false negative challenges confirmed in all cases by telephone follow up.

Another valid critique of this study is that the oral food challenges were not blinded to the subject or the physicians. While double-blinded, placebo-controlled oral food challenges are ideal, open challenges are usually sufficient to demonstrate the presence or absence of FPIES as symptoms of FPIES are not subjective (15). Several studies have used open challenges for FPIES, where bias is less likely to occur (13, 16, 17).

It is interesting to note that APT appears to be useful in this disorder as well as other gastrointestinal reactions suggesting a delayed hypersensitivity reaction in several studies (10, 11) mediated by T cells. But, there are dramatic differences in skin T cells that are cutaneous leukocyte antigen (CLA) positive (18) and gastrointestinal T cells are primarily gamma-delta T cells with a higher proportion of CD45RO positive cells. In addition, gastrointestinal T cells have a different homing adhesion molecule (19, 20). These observations make it difficult to rationalize how T-cells reactions in the gastrointestinal tract could be detected in the skin. One possible explanation is the migration of T cells throughout the circulatory system or the interaction of T cells in regional lymph nodes.

Prior to this study, patients who met criteria for FPIES were instructed to completely avoid the offending protein for a year after the previous reaction. After a full year of complete avoidance, an oral food challenge was performed to ascertain whether the patient had outgrown their sensitivity to the food. Assuming the results of this study are confirmed by a larger study, our diagnostic algorithm for FPIES will change. We will recommend APT for patients who have a suggestive clinical history for FPIES. If the APT is negative to the suspected food, an oral food challenge will be performed immediately as there is a strong likelihood that the patient is either not

sensitive to the food or has outgrown their sensitivity. This strategy is supported by the negative predictive value of 100% found in the study. This will allow many patients to stop restricting their diet at an earlier age. If the APT is positive, the food challenge will be delayed until 1 year after the most recent reaction. An important question for the ongoing study is whether the APT reverts to negative when a patient outgrows FPIES.

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