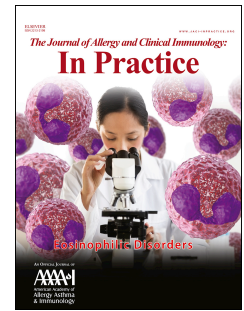


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**A prospective assessment of clinical characteristics and responses to dietary
elimination in food protein-induced allergic proctocolitis**

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Running title: Clinical characteristics of proctocolitis

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Abstract

Background

Some aspects of diagnostic elimination/challenge diets in food protein-induced allergic proctocolitis (FPIAP) are still poorly defined.

Objective

This study investigated the symptom spectrum, time required for resolution of each symptom, triggering foods, and risk factors for multiple food allergies (MFA) in FPIAP.

Methods

Infants referred with visible blood in stool were enrolled after etiologies other than FPIAP had been excluded. Laboratory evaluation, clinical features and elimination/challenge steps were performed prospectively during diagnostic management.

Results

Ninety-one out of 102 infants (53 boys) were diagnosed with FPIAP. Eleven children did not bleed during challenges. Visible blood in stool began before two months of age in 63.6% of the infants not diagnosed with FPIAP, compared to 18.9% of the patients with FPIAP ($p=0.003$). Offending foods were identified as cow's milk (94.5%), egg (37.4%), beef (10.9%), wheat (5.5%), and nuts (3.3%). MFA was determined in 42.9% of patients. Multivariate logistic regression analysis identified atopic dermatitis (AD) (OR 2.98, 95% CI 1.18-7.55, $p=0.021$) and an eosinophil count ≥ 300 cells/ μL (OR 2.72, 95% CI 1.09-6.80, $p=0.032$) as independent risk factors for MFA. Blood and mucus in stool disappeared in a median three days (IQR: 1-14.5 days) and 30 days (IQR: 8-75 days), respectively.

Conclusions

A tendency to transient bleeding occurs in infants who present with bloody stool before two months of age. A two-week duration of elimination for blood in stool is sufficient to reach a judgment of suspected foods for FPIAP. Mucus in stool is the last symptom to disappear. Concurrent AD suggests a high probability of MFA in FPIAP.

What is already known about this topic?

Diagnosis of food protein-induced allergic proctocolitis (FPIAP) is based on symptom responses to sequential elimination/challenge diets.

What does this article add to our knowledge?

Prospectively structured diagnostic dietary steps identify a tendency to transient bleeding before two months of age, prolonged presence of mucus in stool even if blood disappears, recovery time with elimination for each symptom, and atopic dermatitis as a risk factor for multiple food allergies.

How does this study impact current management guidelines?

A two-week duration of elimination for blood in stool is sufficient to form a judgment of suspected foods. Concurrent atopic dermatitis suggests a high probability of multiple allergies. Mucus in stool is the last symptom to disappear and should not affect diagnostic decisions alone.

Key words:

Allergic proctocolitis; elimination diet; food allergy; multiple food allergies; atopic dermatitis; transient proctocolitis

Abbreviations:

AD: Atopic dermatitis

109 FPIAP: Food protein-induced allergic proctocolitis
110 FPIES: Food protein-induced enterocolitis syndrome
111 IQR: Interquartile range
112 MFA: Multiple food allergies
113 OFC: Oral food challenge
114 OR: Odds ratio
115 SCORAD: Scoring atopic dermatitis
116 sIgE: Specific immunoglobulin E
117 SPT: Skin prick test
118 TC: Transient colitis

119
120

121 **Introduction**

122 Food protein-induced allergic proctocolitis (FPIAP) is one of the most common
123 manifestations of non-IgE-mediated food allergies in infants¹. This benign condition involves
124 bloody stools in a well-appearing infant. Onset is usually in the first few months of life, while
125 recovery generally occurs within one year. Diagnosis is based on sequential elimination and
126 challenge diets with the suspected triggering food²⁻⁴.

127 Despite these well-known clinical features of FPIAP, some aspects of dietary
128 management are still poorly described, especially in children with multiple food allergies (MFA).
129 In most infants, elimination of the offending protein, most frequently cow's milk, from the diet
130 leads to a clinical resolution of bleeding within 72 to 96 h². However, in case of infants whose
131 symptoms do not resolve within that time frame, the clinician needs to know for how long the
132 elimination diet should be applied, and how broad it should be, in order to identify infants with
133 other triggering proteins or MFA, or those with no food allergy. In addition, some infants whose
134 rectal bleeding disappears with a diagnostic elimination diet do not re-bleed with a challenge
135 diet⁵.

Although blood and mucus in stool are the principal manifestations of FPIAP, other symptoms have been identified in previous studies, such as diarrhea, colic, vomiting, and pain during defecation^{1,6}. These reports are largely based on retrospective data. However, irritability, feeding difficulty, vomiting, and diarrhea were also reported in a very recent prospective birth cohort study⁷. Symptom severity and time to resolution of each symptom with an appropriate elimination diet are still unclear. As well as these additional symptoms, concurrent atopic dermatitis (AD) and a relationship with IgE sensitization has also been reported in recent years⁸.

The purpose of this prospective study was to clarify the little-known clinical characteristics of FPIAP by identifying useful tools for structuring diagnostic elimination and challenge diets, especially in patients with MFA, via coexistence patterns of triggering foods, and risk factors for MFA. Another aim was to determine the time needed for the resolution of each symptom, to document the symptom spectrum in patients with FPIAP, to evaluate patients with no re-bleeding after an elimination diet followed by the food challenge test, and to investigate the relationship between FPIAP and IgE-mediated food allergies.

Methods

Study group

This prospective cross-sectional study was conducted in a tertiary reference center, the Pediatric Allergy and Pediatric Gastroenterology Clinics of the Kocaeli University Medical Faculty, Turkey, between March 2019 and March 2020. All children under one year of age referred with visible blood in stool were enrolled consecutively in the absence of other conditions such as infections, anal fissure, necrotizing enterocolitis, inflammatory bowel diseases, coagulation defects, intussusception, volvulus and Hirschsprung disease. Etiologies other than FPIAP had been already ruled out by the Pediatric Gastroenterology Clinic. Patients referred with occult blood in stool were not included.

The study was approved by the Ethical Committee of the Medical Faculty (KÜ GOKAEK 2019/1), and written informed consent was obtained from the parents of participating patients.

Diagnosis of FPIAP

FPIAP was diagnosed according to the international guidelines with consecutive elimination and challenge diets^{9,10}. Symptom resolution achieved by elimination of offending foods, followed by reintroduction with symptom deterioration, confirmed the diagnosis. Cases whose symptoms did not recur during the challenge were regarded as transient colitis (TC).

Study design

Initial allergy evaluation with specific immunoglobulin E (sIgE) (cow's milk, egg, beef, wheat, and peanut) and skin prick test (SPT) for cow's milk, egg, wheat, hazelnut, peanut and walnut were performed in all cases on admission. Baseline symptoms and clinical characteristics (visible blood in stool, mucus in stool, vomiting, loose stools, painful flatus, persistent perianal erythema, persistent diaper dermatitis, infantile colic, feeding refusal, poor weight gain, AD, urticaria, age at symptom onset, and dietary status) were recorded. Symptom severity of blood and mucus in stool, vomiting, infantile colic, perianal erythema and persistent diaper dermatitis was graded on admission. The patients were followed up at two-week intervals, and time to improvement for each symptom was recorded. The same physician was in charge of the patient visits throughout the study. All parents were given the option to contact this physician by phone and to instantly report any symptom which resolved.

Offending food has been described as the food that causes visible blood in stool for both single and multiple allergies. The detection of two or more offending foods causing FPIAP in the same patient was regarded as representing MFA. Foods causing either AD flare or IgE-mediated reactions were not considered offending unless they caused visible blood in stool. Symptom severity grading and evaluation of sIgE and SPT are shown in the repository material.

Elimination procedure

Maternal elimination diet was applied among infants who were exclusively breastfed. For formula-fed infants, the elimination diet was applied with amino acid-based formula. In case of infants consuming complementary foods, the offending foods were also strictly avoided. Cow's milk was the first food eliminated from the diet. The first elimination diet also covered other foods accompanying visible blood in stool in the history. Since one of the aims of the present study was to investigate the relationship between FPIAP and IgE-mediated food allergies, we additionally eliminated those foods exhibiting positive sIgE and/or SPT during the first elimination diet as a methodology specific to this study. The other details are given in Figure 1.

Challenge procedure

Challenge protocols were implemented based on published guidelines¹¹. Oral food challenges (OFCs) were performed in hospital in cases with IgE-mediated food allergies such as the presence of urticaria in the history accompanying FPIAP. In case of patients whose only symptom was visible blood in stool without food sIgE, OFCs were carried out at home.

OFCs were performed four weeks after the disappearance of visible blood in stool¹². Challenges were started with the last food to be eliminated (Figure 1). For FPIAP, the OFC was considered positive if visible blood in stool re-appeared during the following two weeks. For IgE-mediated food allergies, the OFC was considered positive in the presence of skin reactions (urticaria, angioedema), gastrointestinal (vomiting), or respiratory (rhinoconjunctivitis, bronchospasm, cough, or dyspnea) manifestations within two hours of ingestion of the offending food. For AD flare, an increase of at least 10 SCORAD points within six hours following ingestion of the last dose was considered an immediate reaction^{13,14}.

The challenge was regarded as positive in cases with a history of visible blood in stools related to an incidental food intake. In cases of negative OFCs, regular dietary consumption of the offending food was performed for at least two consecutive weeks after OFC.

Symptom resolution times

The duration of a symptom from the onset of the diagnostic elimination diet to improvement was regarded as symptom resolution time. The elimination diet was considered "diagnostic" when visible blood in stool resolved by eliminating the suspected food. Each symptom was evaluated individually for improvement. Time elapsed from the first day of bleeding to resolution was considered symptom resolution time in the subjects with TC.

Statistical analyses

Statistical analyses were conducted on IBM SPSS v21.0 software (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to evaluate the distribution of groups containing more than 50 cases, and the Shapiro Wilk test for groups with fewer than 50 cases. Continuous variables were expressed as median (interquartile range, IQR) and categorical variables as counts (percentages). The Chi-square test or Fisher's exact test were used for assessing categorical variables. The non-parametric Mann-Whitney U test was used to compare continuous variables between two groups. A two-sided p value <0.05 was considered statistically significant. Logistic regression analysis was performed to determine the risk factors for MFA and concomitant IgE reactions. Multivariate analysis included p values of 0.20 and below identified at univariate analysis. The results were expressed as odds ratio and 95% confidence interval.

Results

One hundred and nine infants meeting the inclusion criteria were enrolled. However, seven patients were lost to follow-up, and the final study population thus consisted of 102 infants (53 boys, 52%). Ninety-one (89%) children were diagnosed with FPIAP. Eleven (11%) children did not bleed during the challenge and were identified as TC cases. Additionally, 16 patients diagnosed with intussusception (n=12), Hirschsprung disease (n=2) and acute bloody diarrhea (n=4) among infants younger than 12 months admitted with visible bloody stools during the study period were excluded.

239

240 *Clinical characteristics of FPIAP and TC*

241 The main clinical characteristics of the patients with FPIAP and those with TC in terms
242 of age, sex, dietary status and concomitant atopic disorders are shown in Table 1. Age at onset
243 of visible blood in stools was lower in the infants with TC compared to those with FPIAP
244 (median 1.5 months [IQR 0.5-3.5 months] versus median 3.0 months [IQR 2.0–4.0 months],
245 respectively; $p = 0.036$). Analysis showed that visible blood in stool began before two months
246 of age in 63.6% of the infants with TC, compared to 18.9% of the patients with FPIAP
247 ($p=0.003$).

248 All patients with FPIAP and TC had visible blood and mucus in stool. Colic ($n:41$,
249 45.1%) and painful flatus ($n:52$, 57.1%) were the most frequent accompanying complaints in
250 patients with FPIAP. Vomiting, loose stools and persistent perianal erythema were also
251 observed to some extent (Table 2). Only four patients had no symptoms accompanying blood
252 and mucus in stool.

253 *Offending foods*

254 The most common offending foods were cow's milk 94.5% ($n:86$), egg 37.4% ($n:34$)
255 and beef 11% ($n:10$) (Table 3). FPIAP was also caused by wheat and nuts in 5.5% ($n:5$) and
256 3.3% ($n:3$, all hazelnut) of patients, respectively.

257 Fifty-seven percent ($n:52$) of the patients with FPIAP reacted to a single food (49 to
258 cow's milk, three to hen's egg). Beef allergy and wheat allergy were never observed alone.
259 MFA was determined in 42.9% ($n:39$) of patients (Table 3). The two most common offending
260 foods documented together in the same individual among the patients with MFA were cow's
261 milk and egg 32.9% ($n:30$). Univariate analysis identified concurrent AD as a risk factor for
262 allergies of cow's milk and egg in the same patient (OR 2.74, 95% CI 3.09-6.87, $p=0.032$).

Risk factors for multiple food allergies

The median (IQR) absolute eosinophil counts (cells/ μ L) in patients with multiple and single allergies were 313 (218-460) versus 211 (100-328) ($p=0.013$). AD and an absolute eosinophil count of 300 cells/ μ L or greater were determined in 51.3% (20/39) and 53.8% (21/39) of the patients with MFA, versus 28.8% (15/52) and 32.7% (17/52) of the patients with single allergy ($p=0.029$ and $p=0.043$, respectively) (Table 4).

Multivariate logistic regression analysis identified concurrent AD [OR 2.98, 95% CI 1.18-7.55, $p=0.021$] and an absolute eosinophil count ≥ 300 cells/ μ L [OR 2.72, 95% CI 1.09-6.80, $p=0.032$] as independent risk factors for MFA (Table 5).

Concomitant IgE-mediated reactions

Positive sIgE and/or SPT values (Eight egg sIgE and three cow's milk sIgE values >0.35 kU/L; seven egg-SPT and three cow's milk-SPT ≥ 3 mm) were obtained in nine patients with FPIAP. IgE-mediated reactions alongside visible blood in stool were observed in eight of these nine patients. Symptoms in patients with positive challenges included vomiting (n:1), AD flare (n:3), vomiting and AD flare (n:2), vomiting and urticaria (n:1), and vomiting, urticaria and AD flare (n:1). No anaphylaxis was observed in any patient.

The frequency of moderate-to-severe AD was higher in patients with IgE-mediated food allergy compared to those without such allergy (62.5% (5/8) vs. 18.1% (15/83), respectively, $p=0.012$). The frequency of vomiting and urticaria were also higher in patients with IgE-mediated food allergy ($p=0.02$ and $p=0.02$, respectively). Characteristics of the patients with food protein-induced allergic proctocolitis with and without IgE-mediated food allergy are given in the repository material (Table E1). Multivariate analysis identified moderate-to-severe AD as a risk factor for IgE-mediated food allergy (OR 6.85, 95% CI 1.27-37.03, $p=0.025$) (Table 6).

Symptom resolution times

The median duration of individual symptoms from the onset of the diagnostic elimination diet to symptom improvement, together with symptom scores, are shown in Table 2. Visible blood in stool disappeared within a median time of three days (IQR: 1-14.5 days) with the diagnostic elimination diet. Resolution of visible blood in stool occurred within 15 days in 86% (79/91) of patients, within three days in 55% (50/91), and within one day in 38% (35/91). The median duration of visible blood in stool was one day (IQR:1-6 days) in subjects with TC. Visible blood in stool was observed for one day in 72% (8/11) of the patients with TC. The duration of visible blood in stool was 10 days in two patients and 30 days in one patient among the infants with TC.

Mucus in stool improved in a median of 30 days (IQR: 8-75 days), and was the last symptom to resolve. Disappearance of mucus was observed after the onset of complementary feeding in 64.8% (59/91) of patients. The longest median time to resolution after mucus was observed with painful flatus. Three-quarters of feeding refusal, poor weight gain, loose stools and persistent perianal erythema improved within 30 days. Poor weight gain and loose stools improved in all subjects within 60 days. Resolutions of AD and vomiting occurred in three-quarters of cases within approximately two weeks.

Discussion

The present study was conducted to clarify the clinical characteristics of FPIAP in order to augment the diagnostic strategies employed in daily practice. The prospective design, in contrast to previous studies, made it possible to document the age group in which TC clustered most, and to evaluate the time to improvement with elimination diet for each individual symptom and risk factors for MFA. Visible blood in stool disappeared within a median time of three days with the diagnostic elimination diet. Resolution of visible blood in stool occurred within 15 days in 86.3% of patients. Mucus was the last symptom to disappear. Some less prominent additional symptoms such as loose stools, colic, vomiting, painful flatus, feeding refusal, transient poor weight gain, and perianal erythema were also documented. Cow's milk and egg were the most common triggering foods. MFA was determined in 42% of the patients.

AD was a risk factor for MFA specifically to cow's milk and egg. Additionally, moderate-to-severe AD was identified as a risk factor for concomitant IgE-mediated food allergy.

Several studies in recent years have reported that visible bright red rectal bleeding with eosinophilic infiltration in the rectosigmoid mucosa in non-sick neonates can disappear spontaneously^{5,15}. The reported age range at symptom onset is between one and 43 days, the majority of cases occurring soon after birth^{5,15,16}. Although this condition, known as neonatal transient eosinophilic colitis, is reported as a distinct entity from FPIAP, distinguishing between neonatal transient eosinophilic colitis and FPIAP is problematic¹⁵. Although there are some differences in the clinical manifestations of these two conditions, they likely represent the spectrum of the same clinical entity. Both are known to be self-limited conditions. Our observations suggested that there is a high probability of visible blood in stool in a healthy-appearing infant under two months of age being transient in nature. No bleeding was observed with reintroduction of the offending food in 11% of our patients. Nearly two-thirds of these were under two months of age. Such a presentation may be due to an ultra-short natural history of allergic mechanism. A one-month 'watch and wait' approach before an elimination diet has recently been proposed for monitoring whether spontaneous resolution occurs in some patients¹⁷. Due to the transient nature of bloody stools before two months of age in the present study, this approach may indeed be appropriate, especially during this period. Moreover, our results emphasize the importance of the challenge steps of the diagnostic process following elimination.

In order to accurately manage the dietary steps in FPIAP, it is important to know the time to resolution of the symptoms with appropriate elimination. Current guidelines recommend that the diagnostic elimination diet for FPIAP be maintained for two to four weeks^{6,9,10,18,19}. However, prospective data supporting these recommendations are lacking, except for one recent prospective observational study which addressed that point and found that almost all children with non-IgE mediated gastrointestinal food allergies improved within four weeks following a diagnostic elimination diet¹². However, the time required to improvement was not

evaluated separately for each symptom, and the median age of the patients was above the classic period for FPIAP. The present study was therefore designed to investigate the disappearance of each symptom in FPIAP using structured elimination and challenge steps in a prospective manner. Visible blood in stool, the main symptom of FPIAP, resolved in a median three days with elimination. Resolution occurred within 15 days in 86.3% of patients. Three quarters of AD and vomiting in patients with FPIAP also improved within nearly two weeks. These data support the idea that two weeks are sufficient to confirm the resolution of visible blood in stool, AD and vomiting in a patient with suspected FPIAP or else to proceed with elimination with another food.

The presence of mucus in stool is one of the common symptoms of FPIAP. For this reason, clinicians may sometimes over-diagnose FPIAP in infants without blood in stool, or may over-extend the elimination diets according to the mucus itself. Mucus in stool was present in all of our patients, and the prospective follow-up of mucus in stool showed that it was the last symptom to disappear in FPIAP. Resolution of this symptom took place within a median 30 days and was not observed until complementary feeding in approximately 65% of patients. This observation supports the idea that elimination diet should not be extended on the basis of the mucus itself in cases in which blood has already disappeared. Otherwise the persistence of isolated mucus in stool may lead to unnecessary elimination diets. Additionally, three-quarters of feeding refusal, poor weight gain, loose stools and persistent perianal erythema in patients with FPIAP improved within 30 days. The symptom resolution periods documented in the present study, in line with the findings of Lozinsky et al., indicate that 30 days are needed to evaluate the resolution of the symptoms mentioned above by eliminating the suspected food¹².

Although cow's milk is the most common inciting food in FPIAP, other food allergens, such as egg, wheat, beef, and nuts, can also be involved^{2,4,20}. Soy and egg are among the second most commonly reported foods, worldwide^{2,20}. Offending foods are known to vary by geographic regions, and depending on dietary habits and genetic background. In Turkey,

traditional complementary foods do not contain soy, and there is no commercially available soy-based infant formula. Studies from Turkey have only very rarely reported soy as an offending food in FPIAP^{3,21-25}. Soy was also not reported in the food-related symptom histories of the patients in the present study. No patients also failed to improve, and soy was not therefore included in this study.

Varying rates of MFA in FPIAP between 5% and 35% have been reported in previous studies^{2,3,21-25}. Forty-two percent of the patients in the present study were affected by two or more food allergens. The two foods most commonly identified together in patients with MFA were cow's milk and egg, in agreement with other studies from Turkey^{3,21-25}. Three or more food allergies were found in approximately 9% of our patients. Different clinical practices are observed among pediatricians, such as starting the diagnostic process with a wide elimination diet that covers all of the most common allergenic foods in the first step. The low prevalence of three or more offending foods in the present study represent evidence against this practice.

The management of diagnostic elimination steps may sometimes be confusing. Since cow's milk is the most common trigger, elimination of this alone is sufficient in most patients. However, in infants with MFA, symptoms do not resolve until all offending foods have been eliminated. Under such conditions, a prior knowledge of risk factors for MFA will be a useful guide for the clinician. AD and an absolute eosinophil count ≥ 300 cells/ μ L were identified as independent risk factors for MFA in the present study. For these reasons, elimination may be started with the two most likely foods, cow's milk and egg, in infants with these risk factors. Similarly, Vandenplus *et al.* proposed starting diagnostic elimination with cow's milk and egg in infants with concomitant moderate-to-severe AD¹⁸. In patients with no risk of MFA a linear order of elimination, step by step according to the frequency of the offending foods, represent a reasonable approach. When evaluated from this perspective, the frequency of offending foods in our study is compatible with general application of elimination order.

An association between non-IgE mediated allergies and atopic co-morbidities has been highlighted in recent years⁸. Meyer *et al.* reported 41.5% AD and 29.9% sIgE positivity in a

large retrospective study of children with food-induced gastrointestinal allergies²⁶. Conversely, some previous studies have reported gastrointestinal co-morbidities in patients with AD²⁷. Approximately 30% of infants with food protein-induced enterocolitis syndrome (FPIES), another non-IgE mediated food allergy, develop atopic diseases, 24% of cases have positive sIgE levels to the food triggering FPIES, and up to 40% may exhibit evidence of IgE positivity to other foods^{4,28}. The presence of IgE antibodies has been shown to indicate a persistent disease⁴. Such a relationship with IgE sensitization is also being investigated for FPIAP, and AD has been documented as a common allergic comorbidity^{8,23,24}. One recent study reported a positive IgE reactive rate of 23%, while IgE sensitization and MFA were identified as risk factors for a persistent course in FPIAP²⁴. AD was more common among children in the MFA group in that research compared to the single food allergy group²⁴. Another study reported that infants with MFA had a higher frequency of positive sIgE and/or positive SPT results than those with a single food allergy²³. Approximately 10% of the patients with FPIAP in the present study exhibited sIgE positivity, and AD was a widely seen allergic comorbidity, with a frequency of 40%. AD was also found to be a risk factor for MFA in FPIAP. Moreover, moderate-to-severe AD was associated with an increased risk of IgE-mediated food allergy. Although laboratory methods have no place in the diagnosis of FPIAP, our data and other recent reports concerning concomitant IgE reactions suggest that performing sIgEs in FPIAP patients with moderate-to-severe AD may be a rational strategy. Our data support the idea that FPIAP may be involved in the early stages of non-IgE allergic march and indicate atopic comorbidities^{7,8}. The spectrum of documented symptoms and clinical manifestations of FPIAP indicates an ongoing need for collaboration between pediatric gastroenterologists and allergists.

In addition to these reports of coexistence patterns of IgE positivity and FPIAP, one recent large healthy-infant cohort study reported that children with FPIAP were at increased risk of developing IgE-mediated food allergies. Eleven percent of children with FPIAP were reported to develop IgE-mediated food allergy compared to 5% of children without FPIAP²⁹. The early introduction of allergenic foods is known to be capable of reducing the risk of IgE-mediated

food allergy³⁰. The authors of the cohort study hypothesized that the antigen elimination diet used to treat FPIAP in the first year of life may have increased the risk of IgE allergy²⁹. A shared immunopathogenic food allergy response in both FPIAP and IgE-mediated food allergy also has been postulated as a second potential mechanism²⁹. Other studies have also revealed a significant risk of developing an acute IgE-mediated reaction to food after elimination in children with AD with food sensitization³¹. Given the association between elimination diets and developing IgE-mediated reactions, it is crucial to manage the diagnostic steps in line with the international guidelines with consecutive elimination and challenge diets, and to avoid unnecessary extended elimination diets in the follow-up period.

Clinicians are aware that visible blood in stools in a well-appearing infant suggest FPIAP. However, less prominent additional symptoms such as loose stools, colic, vomiting, pain during defecation, feeding refusal, poor weight gain, and perianal erythema may also be seen^{1,2,6,7}. Lake observed painful defecation in 22% of patients with FPIAP². This finding was later described as “colicky behavior with pain on defecation”¹. We also observed prospectively that painful flatus with loose stools in a crying baby is a frequent complaint in FPIAP, at a rate of up to 60%. Perianal erythema was another accompanying finding in 30% of our patients. Our data highlight the coexistence of AD and other symptoms in FPIAP, with a prospective design contrast to previous studies^{3,23}.

One particular strength of the present study lies in its prospective design. The structured elimination and challenge steps also permitted reliable documentation of the time required for recovery for each symptom and uncovering the whole symptom spectrum. The frequency of a positive sIgE response in a patient with FPIAP is low. These patients constituted only a small proportion of our patients. Similarly, the number of patients who recovered with the first elimination and who did not bleed with challenge was also naturally limited. The small sample size of these patients also makes the interpretation of these results problematic. Since we did not perform colonoscopy in these cases, we were unable to provide any discussion based on eosinophilic infiltration with which to compare these patients with neonatal transient

eosinophilic colitis cases. Finally, since this study was conducted in a tertiary reference center, it did not reflect the general population.

In summary, cow's milk and egg are the most common foods found together in patients with FPIAP with MFA. AD is a common allergic comorbidity and a risk factor for MFA in FPIAP patients. Given these data, it may be a reasonable strategy to simultaneously perform cow's milk and egg elimination in the first step of the diagnostic elimination diet in the presence of accompanying AD in FPIAP patients. Moreover, there is a high probability that moderate-to-severe AD will exhibit IgE-mediated reactions. These issues should now be evaluated in further studies. Visible blood in stool, the main symptom of FPIAP, resolves in a median three days with elimination and resolution occurs within 15 days in most patients. A two-week duration of elimination for blood in stool is sufficient to arrive at a judgment of suspected foods for FPIAP. Mucus in stool is a long-lasting symptom even if blood disappears. It should not therefore be considered a follow-up criterion that is directly related to the recovery or persistency of FPIAP. There is a tendency for re-bleeding not to occur with challenge in infants presenting before two months of age. This predisposition needs to be investigated in further prospective studies.

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Figure 1. Structured elimination and challenge steps of diagnostic diet in food protein induced allergic proctocolitis. OFC; oral food challenge.

*Since one of the aims of our study was to investigate the relationship between FPIAP and IgE-mediated food allergies, we additionally eliminated the foods exhibiting positive sIgE and/or SPT during the first elimination diet as a methodology specific to this study.

Table 1. Characteristics of patients with food protein-induced proctocolitis and those with transient proctocolitis

	Patients with food protein-induced proctocolitis (n=91)	Patients with transient proctocolitis (n=11)	p
Age at admission (months), median (IQR)	5.0 (4.0-6.0)	4.0 (1.7-4.8)	0.020
Age of onset of visible blood in stool (months), median (IQR), [minimum-maximum]	3 (2-4) [0.5-6.5]	1.5 (0.5-3.5) [0.5-5]	0.036
Patients with onset of visible blood in stool < 2 months, n (%)	17/91 (18.9%)	7/11 (63.6)	0.003
Male sex, n (%)	48/91 (53%)	5/11 (45%)	0.647
Dietary status at time of onset of symptoms, n, (%)			0.811
Exclusively breast-fed	70/91 (75%)	8/11 (%73)	
Breast-fed and formula-fed	13/91 (13%)	3/11 (%27)	
Exclusively formula-fed	1/91 (1%)	0	
Complementary food	7/91 (11%)	0	
Concomitant atopic/allergic diseases/conditions			
Atopic dermatitis	37/91 (40.7%)	2/11 (18.2%)	0.198
Urticaria	3/91 (3.3%)	0	1.000
Absolute eosinophil count (cells/microliter), median (IQR)	250 (182-404)	285 (231-413)	0.566
Eosinophil %, median (IQR)	2.9 (1.9-4.5)	3 (2.5-4)	0.955

IQR, interquartile range

Table 2. Symptoms on admission, age at onset, and resolution times of individual symptoms in response to diagnostic elimination diet, and symptom severity scoring in patients with food protein-induced allergic proctocolitis

	Symptoms on admission n, (%)	Age at onset of symptoms (months), Median (IQR)	Scoring	Time to symptom resolution (days), Median (IQR), [minimum-maximum]
Visible blood in stool	91 (100)	3 (2-4)	2 (2-5)	3 (1-14.5) [1-105]
Mucus in stool	91 (100)	2 (1-3)		30 (8-75) [1-240]
frequency			3 (3-3)	
Density			3 (2-3)	
Colic	41 (45.1)	1 (0.5-2)	4 (3-6)	7 (7-45) [1-90]
Vomiting	25 (27.5)	1 (0.5-2)	6 (2-6)	7 (1-15) [1-30]
Persistent perianal erythema	29 (31.9)	1 (0.5-2.5)	1 (1-2.5)	7 (7-30)
Persistent diaper dermatitis	5 (5.5)	4 (0.9-5.9)	2 (1.5-3)	7 (7-7)
Atopic dermatitis	37 (40.7)	2 (1-3.6)	28 (18-35)*	7 (3-16.5) [1-120]
Loose stools	32 (35.2)	1.5 (0.5-3)	-	10 (7-30)
Painful flatus	52 (57)	1 (0.5-2)	-	21 (7-60)
Feeding refusal	20 (22)	2 (0.8-3)	-	18.5 (3.5-30)
Poor weight gain	17 (18.7)	3 (1.8-5.1)	-	15 (7-30)

*Scoring atopic dermatitis (SCORAD) scores were used to assess the severity of atopic dermatitis. IQR, interquartile range
 Loose stools, painful flatus, feeding refusal and poor weight gain were not graded.

Table 3. Offending foods in patients with food protein-induced allergic proctocolitis

Offending foods	Frequency n (%)
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Cow's milk	86/91 (94%)	
Egg	34/91 (37.4%)	
Beef	10/91 (11%)	
Wheat	5/91 (5.5 %)	
Nuts	3/91 (3.3%)	
Single food	52/91 (57.1%)	49 CM 3 egg
Two foods	31/91 (34.1%)	23 CM & egg 5 CM & beef 2 CM & wheat 1 egg & nuts
Three foods	7/91 (7.7%)	3 CM, egg & beef 2 CM, egg & wheat 1 CM, egg & nuts 1 CM, beef & wheat
Four foods	1/91 (1.1%)	1 CM, egg, beef & nuts
CM, cow's milk		

Table 4. Characteristics of FPIAP patients with single allergy and multiple food allergies

	Patients with single allergy	Patients with multiple allergies	p
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	(n=52)	(n=39)	
Age at admission (months), median (IQR)	5.2 (4.1-6.0)	4.8 (4-6.5)	0.587
Age of onset of visible blood in stool (months), median (IQR)	3 (2-4)	2.5 (1.5-4)	0.122
Male sex, n (%)	25 (48.1)	23 (59.0)	0.303
Dietary status at time of onset of symptoms, n (%)			0.583
Exclusively breast-feeding	40 (76.9)	32 (82.1)	
Breast-feeding and formula	7 (13.5)	6 (15.4)	
Exclusively formula-feeding	1 (1.9)	0	
Complementary food	4 (7.7)	1 (2.6)	
Symptoms on admission n (%)			
Visible blood in stool	52 (100)	39 (100)	
Mucus in stool	52 (100)	39 (100)	
Colic	20 (38.5)	21 (53.8)	0.144
Vomiting	13 (25.0)	12 (30.8)	0.542
Loose stools	17 (32.7)	15 (38.5)	0.568
Painful flatus	25 (48.1)	27 (69.2)	0.044
Feeding refusal	10 (19.2)	10 (25.6)	0.465
Poor weight gain	10 (19.2)	7 (17.9)	0.877
Persistent perianal erythema	13 (25.0)	16 (41.0)	0.104
Persistent diaper dermatitis	1 (1.9)	4 (10.3)	0.084
Concomitant atopic/allergic diseases/conditions, n (%)			
Atopic dermatitis	15 (28.8)	20 (51.3)	0.029
Urticaria	2 (3.8)	1 (2.6)	0.735
Absolute eosinophil count (cells/ μ l)	204 (100-330)	300 (227-430)	0.013
Median (IQR)			
Eosinophil %, median (IQR)	2.5 (1.7-3.9)	3.8 (2.4-5.4)	0.059
Eosinophil count ≥ 300 (cells/ μ l), n%	17 (32.7)	21 (53.8)	0.043
Concomitant IgE-mediated reactions, n (%)	2 (3.8)	6 (15.4)	0.070

IQR, interquartile range

Table 5. Risk factors for multiple food allergies in patients with food protein-induced allergic proctocolitis

Univariate

Multivariate

	OR	95% CI	p	OR	95% CI	p
Age of onset of visible blood in stool (months)	0.79	0.58-1.06	0.113			
Colic	1.87	0.80-4.33	0.146			
Painful flatus	2.43	0.98-5.81	0.056			
Persistent perianal erythema	2.09	0.85-5.11	0.107			
Persistent diaper dermatitis	5.83	0.63-54.38	0.122			
Atopic dermatitis	2.60	1.09-6.19	0.031	2.98	1.18-7.55	0.021
Absolute eosinophil count (cells/ μ l)*	1.002	1.00-1.004	0.048			
Eosinophil %	1.00	1.00-1.00	0.083			
Eosinophil count $\geq$ 300 cells/ μ L	2.40	1.02-5.65	0.045	2.72	1.09-6.80	0.032
Concomitant IgE-mediated reactions	4.55	0.87-23.90	0.074			

IgE, immunoglobulin E; OR, Odds ratio; CI, Confidence interval

* Not included in multivariate analysis as it is highly correlated with "Eosinophil count $\geq$ 300 cells/ μ L"

Table 6. Risk factors for IgE-mediated food allergy in patients with food protein-induced allergic proctocolitis

	OR	Univariate 95% CI	p	OR	Multivariate 95% CI	p
Age at admission (month)	1.10	0.94-1.30	0.247			
Age of onset of visible blood in stool (month)	1.61	0.99-2.61	0.053			
Vomiting	5.25	1.15-23.94	0.032	4.00	0.67-23.75	0.127
Atopic dermatitis	5.03	0.96-26.49	0.057			
Moderate-to-severe atopic dermatitis	7.56	1.63-35.13	0.010	6.85	1.27-37.03	0.025
Urticaria	27.33	2.16-346.42	0.011	6.91	0.36-133.81	0.201

CI, Confidence interval; OR, Odds ratio

