

Clinical, immunological and pathological profile of infants suffering from cow's milk protein allergy

ELENA TĂȚĂRANU¹⁾, SMARANDA DIACONESCU^{2,3)}, CAMELIA GEANINA IVĂNESCU³⁾, IOAN SÂRBU^{2,3)}, MARIA STAMATIN^{2,4)}

¹⁾PhD Student, "Grigore T. Popa" University of Medicine and Pharmacy, Iassy, Romania

²⁾Department of Mother and Child, "Grigore T. Popa" University of Medicine and Pharmacy, Iassy, Romania

³⁾"Sf. Maria" Emergency Clinical Hospital for Children, Iassy, Romania

⁴⁾"Cuza Vodă" Clinical Hospital, Iassy, Romania

Abstract

Cow's milk protein allergy (CMPA) is the most frequently encountered form of food allergy in pediatric patients and occurs secondary to cow's milk proteins (CMP) ingestion. The aim of this study is to define the profile of children suffering from CMPA and to describe the associated pathological findings. The authors performed a retrospective case-control study on 160 infants that presented with CMPA symptoms at "Sf. Maria" Emergency Clinical Hospital for Children, Iassy, Romania, between January 2013 and January 2015. Fifty-five infants were diagnosed with CMPA (Group 1 – cases group) and 105 had no proven allergy (Group 2 – control group). Mean age of patients, gender distribution and prevalence of premature birth registered no statistically significant difference between the two groups. The prevalence of familial history of allergy was higher in case of patients with CMPA (36.36% versus 20% in control group). The delay between the introduction of CMP into alimentation and symptoms' onset was significantly shorter in Group 1 (12 days) compared to Group 2 (42 days) ($p=0.0051$), thus pleading for an earlier onset of symptoms in case of CMPA. CMPA usually manifested through an association of gastrointestinal (76.36%), cutaneous and mucosal symptoms (70.91%). Specific IgE were positive values in 49 patients with CMPA (89.09%) and 32 patients (30.48%) without CMPA ($p<0.001$). Endoscopic examinations with tissue sampling were performed in 26 infants with CMPA. Focal erythema, erosions and lymphoid nodular hyperplasia were signaled in 23 cases and eosinophilic infiltration was noticed in 15 cases. In conclusion, specific IgE and pathological changes offer highly reliable methods for CMPA diagnosis.

Keywords: food allergy, cow's milk protein allergy, infant, specific IgE, eosinophilic infiltration.

Introduction

Food allergy represents a set of symptoms related to an immunoallergic response directed against a food allergen after exposure. Clinical manifestations may be digestive, skin, respiratory or general. We distinguish several forms of food allergy, an IgE-mediated immediate allergy, the most common, a delayed non-IgE-mediated allergy and a mixed form when the two types of allergic reactions, immediate and delayed coexist [1].

Cow's milk protein allergy (CMPA) is the most frequently encountered form of food allergy in pediatric patients and is defined as a reproducible reaction, via an immunologic hypersensitivity, following ingestion of cow's milk proteins. It is the consequence of immunization against one of the 30 proteins contained in cow's milk and largely found in milk formulas. As with any food allergy, from the clinical point of view there are immediate, delayed and mixed forms [2, 3].

Besides the challenge of determining the origin of symptoms among the wide range of possible diseases, the physician withstands his diagnostic process complicated by the absence of a simple confirmation test. Indeed, the gold standard, based on the oral provocation test (OPT), is rarely performed given the associated risks [1]. As for allergy testing, prick and patch test are more feasible but not always positive. Endoscopy with biopsy for histological examination is considered an additional method that can

help in the diagnosis of CMPA [4]. Relatively common and well known, the CMPA remains a difficult diagnosis to establish, because of the wide variety of clinical manifestations (digestive, skin, respiratory or general).

Any other reaction having no immunological mechanism is called non-allergic food hypersensitivity (e.g., lactose intolerance). The mechanism involved in lactose intolerance is lactase enzyme defect, forcing the exclusion of lactose containing products. It rarely affects infants less than one year and is more frequently encountered in children 6 to 12 years or young adults [5].

In developed countries, it is difficult to assess the real prevalence of CMPA since there is a large difference between the prevalence of self-perceived CMPA secondary to questionnaire evaluation and confirmed CMPA. The comparative prevalence varies according to the studies, from 1 to 17.5% versus 0.6 to 2.5% among preschool children (<5 years), from 1 to 13.5% versus 0.3% in children of school age (5–16 years) and 1 to 4% versus less than 0.5% in adults [6].

In infants, cow's milk proteins (CMP) are the first and only dietary antigen introduced into the diet until diversification. Therefore, CMPA is a condition that occurs early, mainly in the first year of life with an incidence of 2 to 3% [7]. However, once diagnosed, treatment is simple and effective, based on allergen avoidance, substituting the milk by a protein hydrolysate. The prognosis is excellent; with 85 to 90% of children recovering before

the age of three years, thus allowing the reintroduction of cow's milk [8].

In 2010, a working group, the *Diagnosis and Rationale for Action against Cow's Milk Allergy* (DRACMA), including international experts from the *World Allergy Organization* (WAO), established recommendations for the diagnostic and therapeutic management of CMPA [6]. The goal of elaborating and proposing international consensus recommendations has been complicated by medical practice conditions that vary greatly from one country to another.

Intestinal biopsy has gained interest as a method of obtaining macroscopic and microscopic samples of intestinal mucosa that could prove useful if there are doubts concerning the diagnosis, considering a suggestive finding in favor of CMPA – the presence of more than 60 eosinophils in six high-power fields (HPFs) and/or more than 15–20 eosinophils per field. These histopathological changes may occur throughout the entire gastrointestinal tract (esophagus, stomach, duodenum, sigmoid colon, rectum) and are associated to symptoms according to location [9].

The aim of this study is to define the profile of children suffering from CMPA and to describe the associated pathological findings. A more precise knowledge of children at risk of developing CMPA would facilitate their identification, enhance the diagnosis and lead to better management.

Patients and Methods

The authors performed a retrospective case-control study on 160 infants that presented with CMPA symptoms at "Sf. Maria" Emergency Clinical Hospital for Children, Iassy, Romania, between January 2013 and January 2015. Fifty-five infants were diagnosed with CMPA (Group 1 – cases group) and 105 had no proven allergy (Group 2 – control group). Endoscopic procedures with tissue sampling were performed in 26 cases with severe gastrointestinal symptoms after OPT. Biopsies were considered positive for CMPA in the presence of more than 15–20 eosinophils per field or more than 60 eosinophils in six HPFs. The presence of specific IgE antigens was evaluated in all cases. A specific IgE value >5 kIU/L was considered pathological.

For each of the patients, the following data were registered: birth date, gender, prematurity, the presence of gastroesophageal reflux, familial history of allergy (first degree relatives), personal history of allergy, breast feeding history, age (months) when CMP were introduced, milk formula(s) used, age (months) at symptoms' onset, symptoms, clinical signs, laboratory data (including specific IgE), paraclinical investigation data (upper gastrointestinal endoscopy, rectosigmoidoscopy) in selected cases, biopsy data in selected cases.

Informed consent from parents or legal caregivers was obtained in all cases. Patients with moderate or severe malnutrition, primary or secondary immunodeficiency, metabolic, endocrine and neurological diseases were excluded from the study. Statistical analysis was performed using SPSS 21.0 for Mac by means of univariate and multivariate statistical tests (*chi-square*, Student's *t*-test, logistic regression). Statistical significance level was considered 5%.

Results

The mean age of patients, gender distribution and prevalence of premature birth registered no statistically significant difference between the two groups. The prevalence of the gastroesophageal reflux was significantly higher in control group and could be responsible for symptoms mimicking CMPA. At the opposite, the prevalence of familial history of allergy was higher in case of patients with CMPA compared to control group. A higher percentage of patients were fed with milk formulas including CMP at presentation in control group compared to the group of infants with CMPA (Table 1).

Table 1 – Patients' characteristics

Parameter	Group 1	Group 2	P
Age [years]	4.2 (2–12)	3.9 (2–11)	n.s.
Gender (M:F)	1.12:1 (29/26)	0.75:1 (45/60)	n.s.
Premature birth (N, %)	6 (10.9%)	23 (21.9%)	0.003
Gastroesophageal reflux (N, %)	13 (23.6%)	46 (43.8%)	0.002
Familial history of allergy (N, %)	20 (36.36%)	21 (20%)	0.012
Cow's milk formula since birth (N, %)	8 (14.55%)	31 (29.52%)	0.011
Alimentation including CMP at presentation	41 (74.55%)	91 (86.67%)	0.03

M: Males; F: Females; N: No. of cases; n.s.: Not significant.

In most cases, alimentation diversification was not initiated prior to presentation (40 patients from Group 1 – 72.73%, 70 patients from Group 2 – 66.67%) with no statistically significant association between the presence of CMPA and alimentation diversification ($p=0.35$). In cases with alimentation diversification, this process was initiated at an average of 4.7 months for Group 1 and 6.2 months for Group 2.

Mean age when CMP were added to alimentation registered statistically significant differences between the groups (2.12 months in Group 1, 0.96 months in Group 2, $p=0.0039$).

First symptoms occurred at an average of 2.5 months for Group 1 and 3.65 months for Group 2, with no statistically significant difference ($p=0.0620$), and the delay between the introduction of CMP into alimentation and symptoms' onset was significantly different between the two groups (12 days for Group 1 and 42 days for Group 2, $p=0.0051$), thus pleading for an earlier onset of symptoms in case of CMPA compared to gastrointestinal symptoms mimicking CMPA.

Gastrointestinal symptoms dominated the clinical picture in both groups (Table 2) and comprised the following symptoms (Table 3).

Cutaneous and mucosal symptoms (hives, eczema) occurred more frequently in patients with CMPA compared to respiratory or ENT symptoms (asthma, rhinitis) that were registered mainly in control group. Infants with CMPA presented an average of 1.7 organs/systems involved compared to 1.3 in Group 2 ($p<0.001$) with a maximum of 3 for both study groups. Twenty-one (38.18%) of the infants in Group 1 presented with symptoms related to a single organ/system (13 cases with digestive symptoms – 61.9%) compared to 78 of Group 2 (74.29%) (54 with digestive symptoms – 69.23%). Thus, CMPA usually manifests through an association of gastrointestinal, cutaneous and mucosal symptoms.

Table 2 – Frequency of symptoms in the two study groups

Symptom	Group 1	Group 2	P
Gastrointestinal	42 (76.36%)	76 (72.38%)	n.s.
Cutaneous and mucosal	39 (70.91%)	19 (18.09%)	<0.001
Respiratory/ENT	5 (9.09%)	22 (20.95%)	0.02
General symptoms (malaise, crying, shock)	7 (12.73%)	16 (15.24%)	n.s.

n.s.: Not significant.

Table 3 – Frequency of symptoms in the study groups

Symptom	Group 1	Group 2	P
Vomiting	31 (56.36%)	28 (26.67%)	<0.001
Diarrhea	15 (27.27%)	19 (18.09%)	n.s.
Rectal bleeding	5 (9.09%)	20 (19.05%)	n.s.
Constipation	2 (3.64%)	5 (4.76%)	n.s.
Bloating/abdominal pain	6 (10.91%)	17 (16.19%)	n.s.
Alimentation refusal	2 (3.64%)	10 (9.52%)	n.s.
Failure to thrive	5 (9.09%)	29 (27.62%)	<0.001

n.s.: Not significant.

Patients with CMPA generally presented with immediate allergic reaction (44 cases – 80%), followed by delayed reaction (nine cases – 16.36%) and mixed forms (two cases – 3.64%).

Laboratory test results included specific IgE determination at an average age of 4.93 months for Group 1 and 5.67 months for Group 2 (no statistically significant difference). Specific IgE (anti-cow milk, anti- α -lactalbumin, anti- β -lactoglobulin and anti-casein) were positive (0.1 kIU/L threshold) in 49 (89.09%) patients with CMPA and 32 (30.48%) patients without CMPA ($p < 0.001$). Thirty-three of the 49 patients with CMPA and positive specific IgE presented with antibodies against all the four proteins.

Six patients with CMPA presented specific IgE values <0.1 kIU/L (absent). Higher positivity rates and higher specific IgE values were noticed in case of infants with CMPA (Table 4).

Table 4 – Specific IgE positivity rate and mean value

IgE type	Mean value Group 1 [kIU/L]	Group 1	Mean value Group 2 [kIU/L]	Group 2	P
Anti-cow milk	18.12	49 (89.09%)	0.61	32 (30.48%)	<0.001
Anti- α -lactalbumin	11.77	38 (69.09%)	0.43	3 (2.86%)	<0.001
Anti- β -lactoglobulin	14.92	41 (74.55%)	0.23	7 (6.67%)	<0.001
Anti-casein	17.04	38 (69.09%)	0.19	3 (2.86%)	<0.001

For multivariate analysis (logistic regression), there were considered three qualitative variables (atopic terrain, breast feeding, cutaneous and mucosal signs) and two quantitative variables (the delay between the introduction of CMP into alimentation and symptoms' onset, number of affected organs/systems). The presence of cutaneous and mucosal signs was associated to a 6% chance of presenting CMPA [OR (odds ratio) 0.062, 95% CI (confidence interval) 0.022–0.172].

Endoscopic examinations (upper gastrointestinal endoscopy, colonoscopy) were performed in 26 infants with CMPA and pathologic aspects with more than 15–20 eosinophils per field or more than 60 eosinophils in six HPFs were noticed in 15 cases: esophageal – one case, duodenal – three cases (Figure 1), duodenal and rectal – two cases, rectal – nine cases. Focal erythema, erosions and lymphoid nodular hyperplasia (Figures 2 and 3) were signaled in 23 cases (five with rectal bleeding).

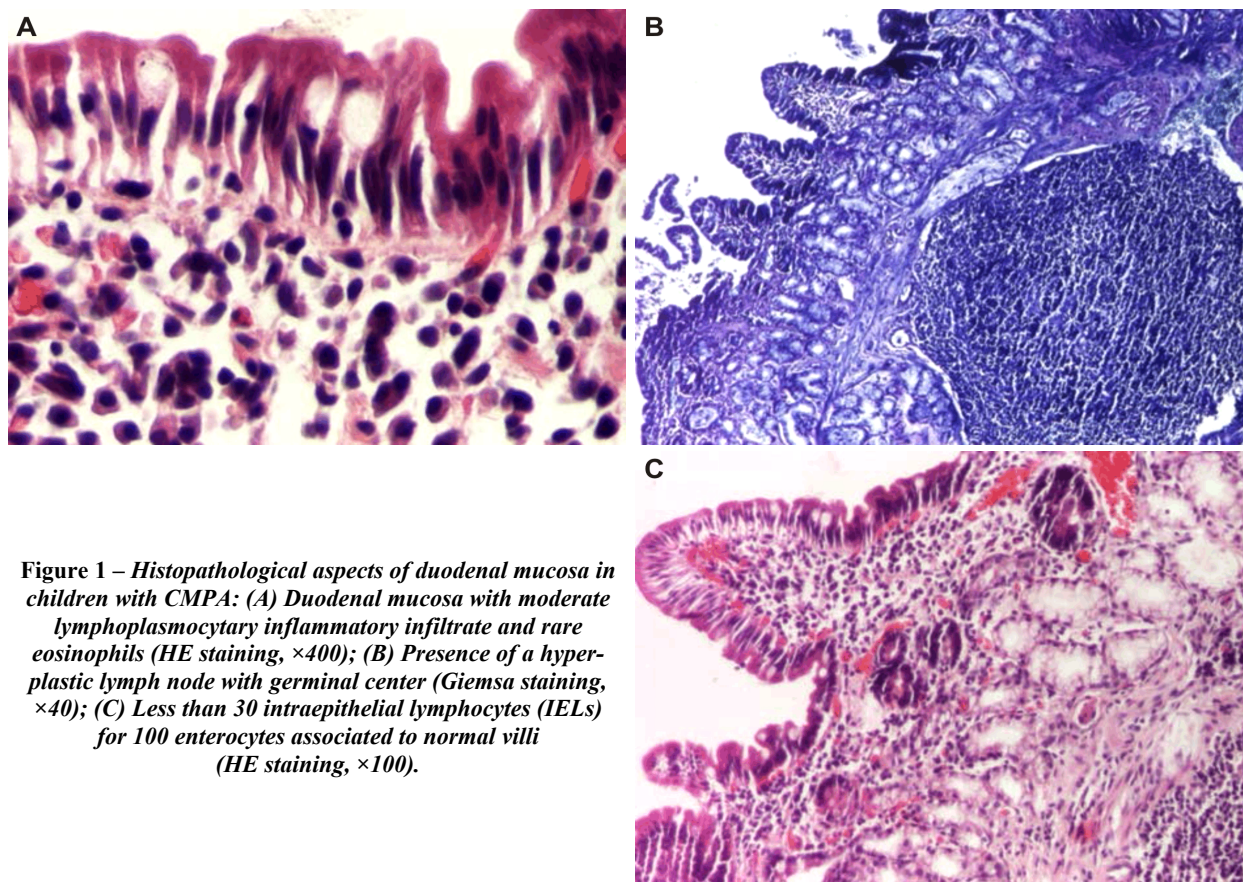


Figure 1 – Histopathological aspects of duodenal mucosa in children with CMPA: (A) Duodenal mucosa with moderate lymphoplasmocytary inflammatory infiltrate and rare eosinophils (HE staining, $\times 400$); (B) Presence of a hyperplastic lymph node with germinal center (Giemsa staining, $\times 40$); (C) Less than 30 intraepithelial lymphocytes (IELs) for 100 enterocytes associated to normal villi (HE staining, $\times 100$).

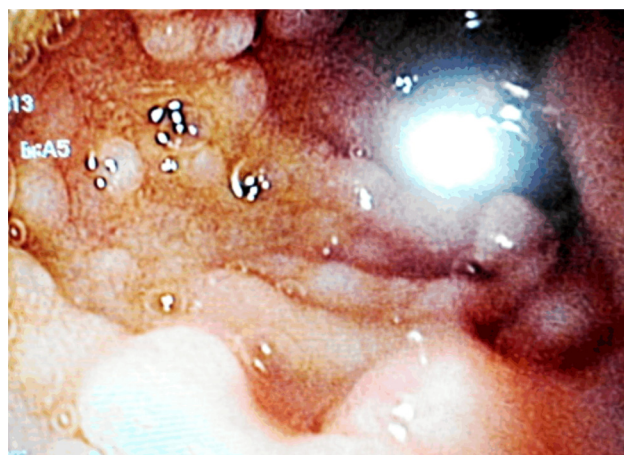


Figure 2 – Upper digestive endoscopy in a 3-year-old boy. Nodular lymphoid hyperplasia of the duodenum.

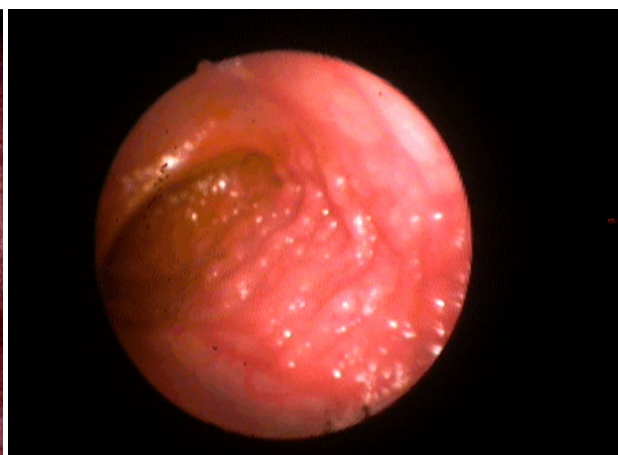


Figure 3 – Retrograde ileoscopy in a 5-year-old boy. Nodular lymphoid hyperplasia of the distal ileum.

Discussion

The univariate analysis performed in the current study identified several factors associated with CMPA and multivariate analysis allowed a calculation of the risk of developing CMPA based on an adjusted comparison of different parameters. Atopic terrain proved to be an important risk factor for CMPA (36.36% infants from Group 1 presented familial history of allergy compared to 20 from Group 2) as confirmed by several consulted studies that identified a frequency of familial atopy between 20–78% in case of infants with CMPA [10]. Relative risk of developing CMPA was evaluated as 2.3 higher if both parents are atopic and increases even more if there is a familial history of food allergy according to Paupe *et al.* [11].

In the literature, the age at first contact with CMP is considered a determinant factor for CMPA as sensitization to CMP could appear *in utero* (maternal alimentation), mediated by maternal breast milk, or triggered by milk formula administered during the first three days of life [12]. All breastfed newborns whose mothers consumed more than half a liter of milk per day are exposed to β -lactoglobulin [13]. Adding to breastfeeding as a supplement a milk formula increases 1.5 times the CMPA risk.

Mean age when CMP were introduced into alimentation registered statistically significant differences between the groups ($p=0.0039$). The delay between CMP introduction and the onset of symptoms was 30 days shorter in case of infants with CMPA possible due to immediate allergic reactions (80% of cases in our study). Analyzed studies state that CMPA occurs faster if cow's milk is introduced into alimentation immediately after birth [14]. Most infants with CMPA become symptomatic before the age of one month, often in the week following the introduction of cow's milk [7] and the prevalence of the disease diminishes in the 2nd year of life.

Gastrointestinal symptoms occurred in more than 70% of cases in both study groups and only cutaneous (hives, eczema) and mucous symptoms were registered four times more frequently in Group 1 compared to Group 2 and were associated to a 6% risk of presenting CMPA. CMPA is the leading cause of hives in children less than six months and 30% of infants with eczema have CMPA. Food allergy symptoms in general are mostly cutaneous

(62.7%), followed by digestive (30.3%), respiratory (6.9%) and anaphylactic (4.9%) [15]. Infants with CMPA usually presented two organs/systems involved (54.55%) compared to control group that presented a single organ/system involvement (74.29%).

Digestive symptoms associated to CMPA vary and are non-specific both in our study in the analyzed literature [16]. Diarrhea and vomiting were the most frequently accounted symptoms, and constipation occurred rarely. Failure to thrive is a classic but inconstant sign with a plurifactorial origin (pain, vomiting, diarrhea, malabsorption).

In our study, asthma was essentially a symptom observed in non-allergic infants. Rhinitis and/or conjunctivitis were rather rare symptoms in both groups. In literature, respiratory events and/or ENT symptoms are infrequent in CMPA [7] because trigger antigens are swallowed and not inhaled.

In our study, anaphylactic shock was only present in allergic infants. Malaises were distributed in roughly the same proportions between the two groups. Crying was most often associated with other symptoms but sometimes occurred isolated. In literature, anaphylactic shock represents 4–9% of clinical manifestations encountered in CMPA [11]. It can occur within a few minutes to three hours and manifests through hypotension, respiratory pauses, cyanosis, erythema or generalized hives. Digestive manifestations appear in the second time. CMPA would be responsible for 11% of fatal anaphylactic reactions in infants [6].

Specific IgE have a real diagnostic and prognostic value, particularly for acute forms. They can be negative at an early age and become secondarily positive in 27% of cases at one year [17]. Taking the threshold value of the laboratory (0.1 kIU/L), the average number of different positive specific IgE was 2.7 times higher for allergic infants compared to non-allergic ones. Severity of CMPA was proportional to the number of positive specific IgE. Using the same threshold value, specific IgE proved 89% sensitivity and 69% specificity in CMPA diagnosis. Several authors stated large IgE sensitivity and specificity ranges (51–58% and 88–98% respectively) by using different threshold values [18]. In our particular case, the CMPA diagnosis threshold of 5 kIU/L was largely surpassed for each of the specific IgE in allergic

infants. In the control group, all values were inferior to 0.8 kIU/L.

Several studies analyzing endoscopic and histological findings in children with CMPA reported that the most frequently encountered anomalies are focal erythema, erosions and nodular lymphoid hyperplasia, alterations that occur in 40–90% of cases [19, 20]. Regarding the histological findings of esophageal, gastric, duodenal and rectal biopsies, most authors agree that the presence of more than 60 eosinophils in six HPFs and/or more than 15–20 eosinophils/HPF is highly suggestive for CMPA, although there are some reports in the literature that suggest that 6–10 eosinophils/HPF may be suggestive of this condition [20, 21].

Odze *et al.* analyzed 20 patients with CMPA related proctocolitis and found focal nodular hyperplasia and erythema in 95% cases and characteristic eosinophilia infiltration in 60% of cases [22]. Hwang *et al.* found endoscopic abnormalities (94.7% nodular lymphoid hyperplasia, 5.3% focal erythema) and more than 60 eosinophils in the lamina propria on 10 HPFs in all 38 patients with allergic proctocolitis studied [23].

Conclusions

CMPA diagnosis is difficult because of varied and specific symptoms. The existence of two types of allergy, immediate and delayed, complicates the diagnosis, as the causality relation is not always obvious. OPT remains the gold standard in diagnosing CMPA but its potential dangerousness causes a difficulty in implementation and a significant cost. Wearing a certain diagnosis is fundamental to any therapeutic approach in order to avoid the harmful consequences of over or under diagnosis. Specific IgE and pathological changes offer highly specific and sensitive methods for CMPA diagnosis.

Conflict of interests

The authors declare that they have no conflict of interests.

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Corresponding author

Smaranda Diaconescu, Lecturer, MD, PhD, Department of Mother and Child, “Grigore T. Popa” University of Medicine and Pharmacy, 16 University Street, 700115 Iassy, Romania; Phone +40744–922 161, e-mail: smaranda.diaconescu@umfiasi.ro