Journal of Immunology Research

Molecular Component-resolved Diagnostics in Allergy

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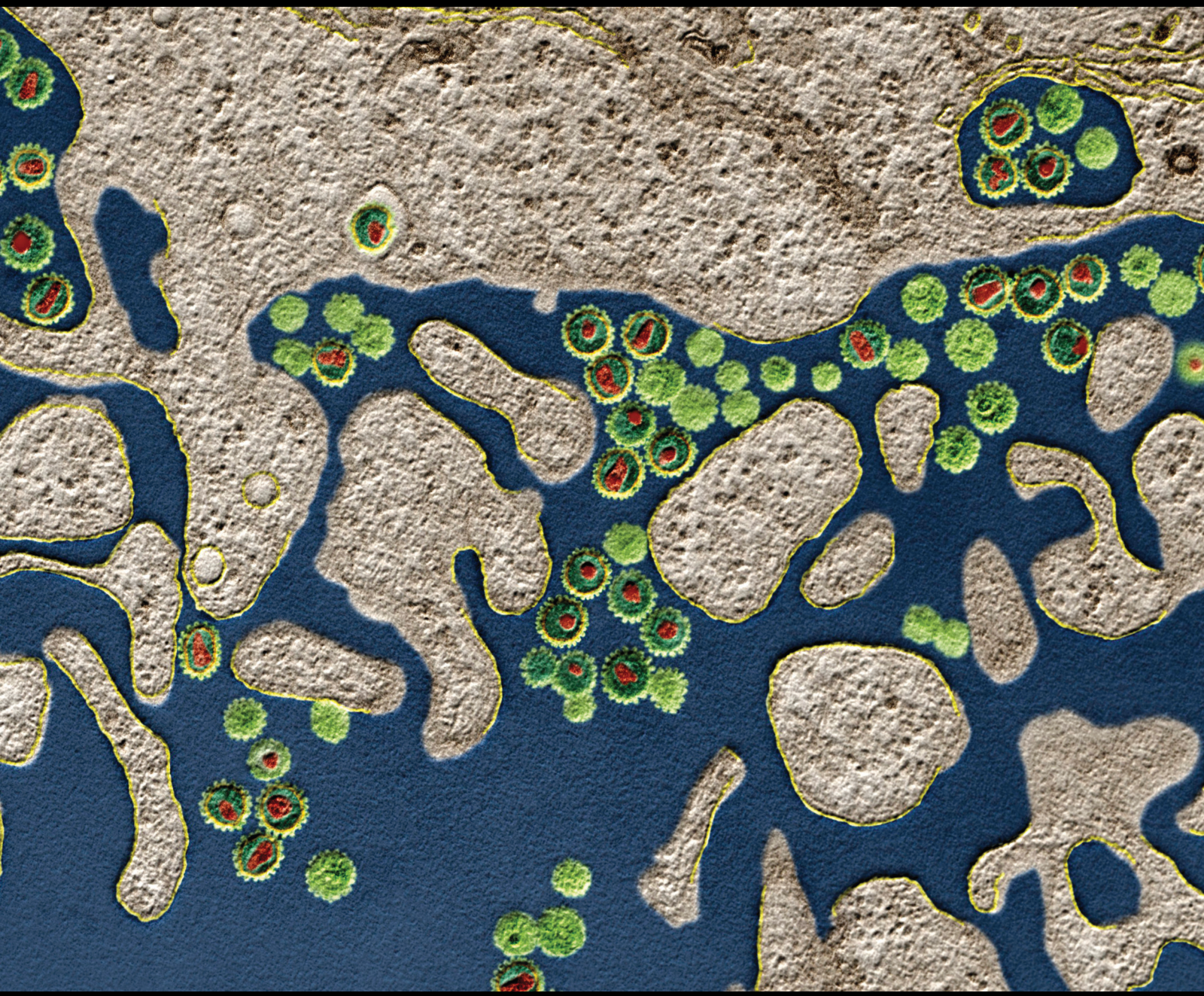

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Contents

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Research Article

Evaluation of Der p 10 in a Cohort of European Children: Role of Molecular Diagnostics and Clinical Features

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Background. Allergy toward the dust mite is steadily increasing on the European continent. This sensitization may be a risk factor for developing sensitization to other mite molecules such as tropomyosin Der p 10. This molecule often correlates with food allergy and the risk of anaphylaxis after ingesting mollusks and shrimps. *Materials and Methods*. We analyzed the sensitization profiles by ImmunoCAP ISAC of pediatric patients from 2017 to 2021. The patients under investigation were being followed for atopic disorders such as allergic asthma and food allergies. The study aimed to analyze the prevalence of sensitization toward Der p 10 in our pediatric population and assess the related clinical symptoms and reactions after ingestion of foods containing tropomyosins. *Results*. This study included 253 patients; 53% were sensitized toward Der p 1 and Der p 2; 10.4% were also sensitized to Der p 10. Assessing patients sensitized to Der p 1 or Der p 2, and Der p 10, we observed that 78.6% were affected by asthma ($p < 0.005$) and had a history of prior anaphylaxis after ingestion of shrimp or shellfish ($p < 0.0001$). *Conclusion*. The component-resolved diagnosis gave us a deeper understanding of patients' molecular sensitization profiles. Our study showed that a fair proportion of children sensitive to Der p 1 or Der p 2 are also sensitive to Der p 10. However, many patients sensitized to all three molecules had a high risk of asthma and anaphylaxis. Therefore, the assessment of Der p 10 sensitization should be considered in atopic patients with sensitization to Der p 1 and Der p 2 to avoid encountering possible adverse reactions after ingesting foods containing tropomyosins.

1. Introduction

House dust mite (HDM) is one of the most common indoor allergens. More than 50% of allergic patients and more than 80% of asthmatic children are sensitized to dust mites [1]. HDMs, especially *Dermatophagoides pteronyssinus* (DP), are considered an important source of allergic sensitization, and they are the main risk factor for allergic respiratory diseases in genetically predisposed patients [2, 3]. Der p 1 and Der p 2 are considered the major allergens of DP, as more than 90% of the mite-sensitized patients are positive for them [4]. Another novel HDM major allergen is Der p 23, with a reported incidence of 74% in individuals with DP sensitization [5]. Recent research suggests that Der p 23 is a major allergen already clinically significant in the first years of life [6, 7]. Furthermore, in HDM-allergic individuals, Der p 23 appears strongly related to asthma, allergic rhinitis, and atopic dermatitis in infancy [8, 9]. These findings support the inclusion of Der p 23-IgEs molecular testing in clinical HDM allergy suspicion.

Der p 10, on the other hand, is one of the minor allergens of HDM, with a reported prevalence among DP-sensitized patients between 5% and 18% [10, 11]. Der p 10 is a tropomyosin, one of the primary thermostable allergenic components responsible for the cross-reactivity across crustaceans, mites, insects, and nematodes [12] (Figure 1). It is regarded as the main invertebrate panallergen that sensitizes susceptible individuals by inhalation or ingestion [13].

In particular, Der p 10 shares high sequence homology with Pen a 1 (shrimp tropomyosin allergen), with an

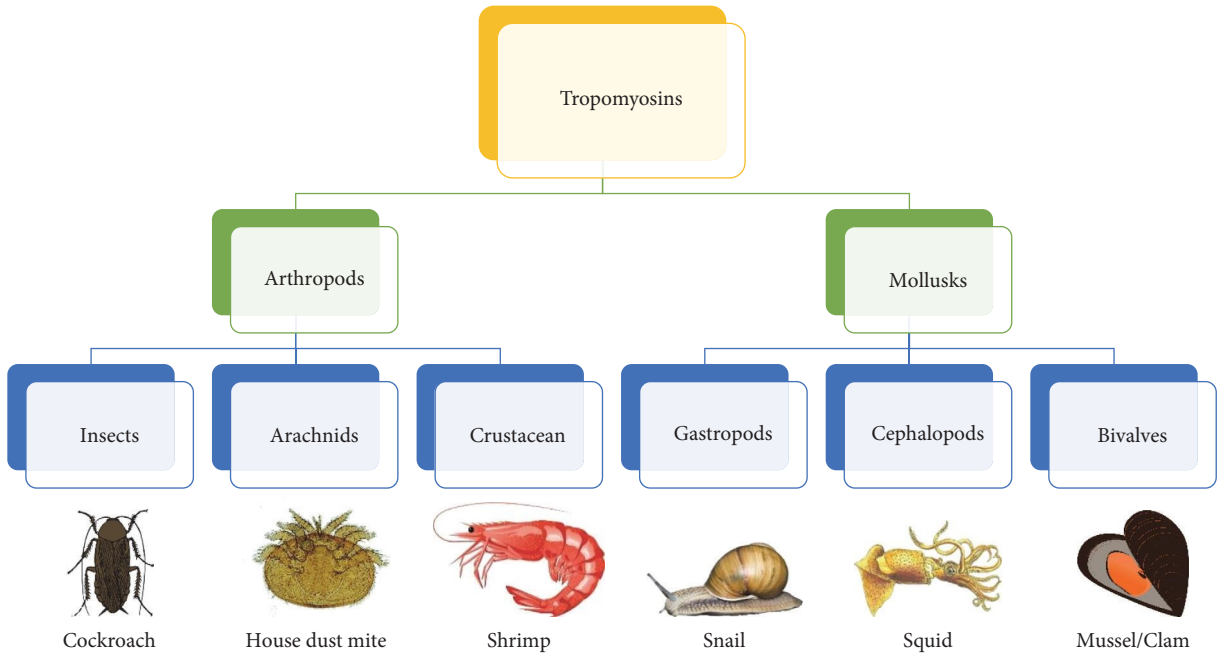

Figure 1: The family of tropomyosins.

aminoacidic sequence similarity of 81% between shrimp and HDM tropomyosins and four identical IgE-binding epitopes [14, 15]. Shellfish is one of the leading causes of persisting throughout life food allergy and is a common cause of food-induced anaphylaxis [16]. Shellfish allergy affects up to 10% of the general population, especially in the Asia-Pacific regions [17–19]. In Western countries, children's self-reported rates of shellfish allergy range from 0.06% to 2%, but the actual prevalence in the general and pediatric population is underestimated [20]. Few studies investigated the clinical and biomolecular role of tropomyosins in HDM-sensitised children. The objective of our study was to evaluate the prevalence of sensitization toward Der p 10 in our pediatric population assessing the associated clinical symptoms and the incidence of allergic reactions after ingesting foods containing tropomyosins.

2. Materials and Methods

2.1. Patients. The study included consecutive children attending the Allergy and Pneumology Unit of Pediatric Clinic University of Campania "Luigi Vanvitelli" from 2017 to 2021. All patients aged between 1 and 18 years were followed for atopic disorders such as allergic asthma, atopic dermatitis, urticaria, allergic rhinitis, and food allergies and performed an ImmunoCAP ISAC.

2.2. Study Design. We analyzed the serum-specific IgE of molecules Der p 1, Der p 2, and Der p 10 retrospectively, and Der p 23 using the microarray method (ImmunoCAP ISAC, ThermoFisher Scientific, Milan, Italy). Molecular sensitization profiles obtained by ImmunoCAP ISAC were evaluated and compared with each other to assess possible cross-reactivity and correlations. Sensitization was defined when the value was higher than 0.3 ISU-E. The study considered patients' clinical data, such as asthma, atopic dermatitis, rhinitis, urticaria, and history of anaphylaxis after food ingestion. The molecular sensitization to Der p 1, Der p 2, Der p 10, and Der p 23 were compared to clinical data to assess differences between sensitized and not-sensitized populations. We evaluated four groups of patients: the first group was sensitized to Der p 1 or Der p 2, the second group was sensitized to Der p 10, the third group was sensitized to both Der p 10 and Der p 1 or Der p 2, and the fourth group was sensitized to both Der p 23 and Der p 1 or Der p 2.

2.3. Endpoint. The primary endpoint assessed the sensitization profile toward the Der p 1-Der p 2 and Der p 10, and Der p 23 molecules and the clinical and laboratory characteristics of sensitized patients. The secondary endpoint was to compare sensitized populations and to evaluate how sensitization to Der p 10 or Der p 23 can affect clinical manifestations in patients.

2.4. Statistical Analysis. Patients' characteristics were defined using descriptive statistics and expressed as a percentage. We used the χ^2 test to compare the data obtained on the clinical and molecular sensitization profiles analyzed during the study. Significance was set for p-values < 0.05. All the analyses were performed using Microsoft Excel for Microsoft 365, Microsoft Inc., Redmond, Washington, USA, and IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.

3. Results

The current study assessed the sensitization to Der p 1, Der p 2, and Der p 10 in 253 consecutive Caucasian children, 167 males and 86 females, ranging in age from 1 to 18 years, between 2017 and 2021. Furthermore, within this group of 253 children, we evaluated sensitization data regarding Der p 23 in a subset of 70 patients, 48 males and 22 females.

Table 1: Clinical characteristics and sensitization to Der p 10 and Der p 1 or Der p 2 of the whole population under study.

	Der p 1/Der p 2 sensitized	Der p 10 sensitized	Asthmatics	Atopic dermatitis	Urticaria	Rhinitis
Total patients	134 (53%)	23 (9.1%)	113 (44.7%)	81 (32%)	105 (41.5%)	111 (43.9%)

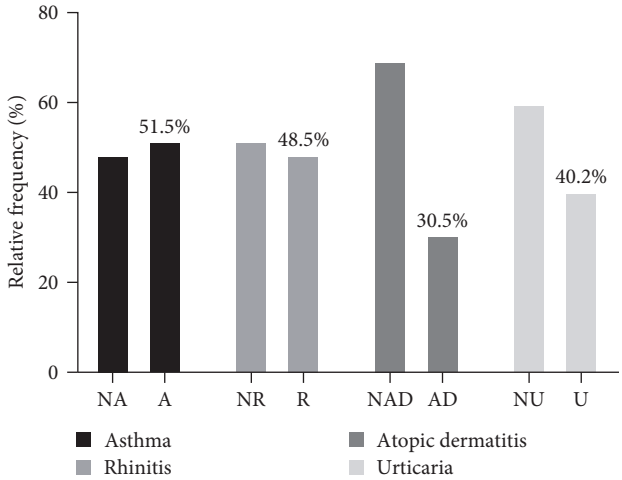

Figure 2: Clinical characteristics in patients sensitized to Der p 1–Der p 2. NA, nonasthmatics; A, asthamatics; NR, nonrhinitic; R, rhinitic; NAD, nondermatitic atopic; AD, atopic dermatitis; NU, nonhorticarial; U, horticarial.

Table 2: Clinical characteristics and sensitization to Der p 10 of the population sensitized to Der p 1 or Der p 2.

	Der p 10	Der p 23	Asthmatics	Atopic dermatitis	Urticaria	Rhinitis
Patients Der p 1/2 sensitized	14 (10.4%)	18 (54.5%)	69 (51.5%)	41 (30.6%)	54 (40.3%)	65 (48.5%)
Patients Der p 1/2 not sensitized	9 (7.6%)	2 (5.7%)	44 (37%)	40 (33.6%)	51 (42.9%)	46 (38.7%)

Table 3: Clinical characteristics of the sensitized population in Der p 10.

	Asthmatics	Atopic dermatitis	Urticaria	Rhinitics	Anaphylaxis
Der p 10 sensitized	17 (73.9%); $p<0.01$	12 (52.2%)	9 (39.1%)	9 (39.1%)	19 (89.6%); $p<0.0001$
Der p 10 not sensitized	96 (41.7%)	69 (30%)	96 (41.7%)	102 (44.3%)	2 (0.9%)

Table 4: Clinical characteristics of the sensitized population in Der p 23.

	Asthmatics	Atopic dermatitis	Urticaria	Rhinitics	Anaphylaxis
Der p 23 sensitized	14 (66.7%); $p<0.05$	4 (19.0%)	9 (42.9%)	12 (57.1%)	2 (9.5%)
Der p 23 not sensitized	19 (38.8%)	18 (36.7%)	25 (51.0%)	12 (24.5%)	0 (0.0%)

The data analyses found that 53% of patients were sensitized to Der p 1 or Der p 2 and 9.1% to Der p 10. About patients' clinical data, 44.7% suffered from asthma, 32% from atopic dermatitis, 41.5% from urticaria, and 4.9% from rhinitis (Table 1). Data analysis of the first group of patients sensitized to Der p 1 or Der p 2 showed that patients with asthma were 51.5%, patients with atopic dermatitis were 30.6%, patients with urticaria were 40.3%, and patients with rhinitis were 48.5% (Figure 2). The 10.4% of patients sensitized to Der p 1 and Der p 2 were also sensitized to Der p 10, and the 54.5% of patients sensitized to Der p 1 and Der p 2 were also sensitized to Der p 23 (Table 2).

Data analysis of the second group of patients sensitized to Der p 10 showed that patients with asthma were 73.9% ($p<0.01$), patients with atopic dermatitis were 52.2%, patients with urticaria were 39.1%, and patients with rhinitis were 39.1%. The 89.6% ($p<0.0001$) of patients sensitized to Der p 10 reported anaphylactic reactions after ingestion of shrimp or shellfish (Table 3). Data analysis of patients sensitized to Der p 23 showed that patients with asthma were 66.7% ($p<0.05$), patients with atopic dermatitis were 19.0%, patients with urticaria were 42.9%, and patients with rhinitis were 57.1% (Table 4). Data analysis of the third group of patients sensitized to Der p 10 and Der p 1 or Der p 2 revealed

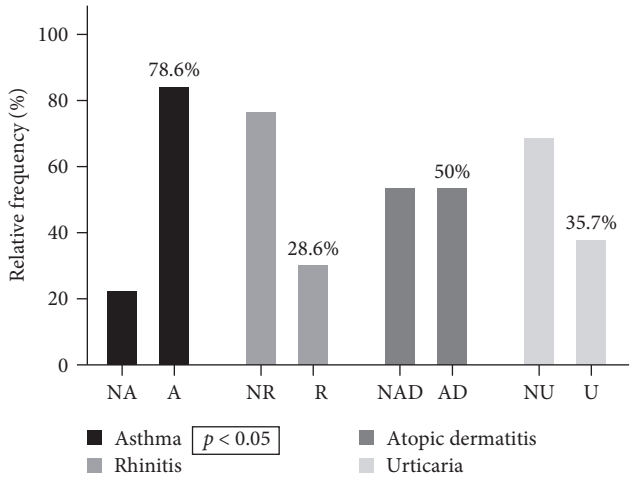

Figure 3: Clinical characteristics in patients sensitized to Der p 1–Der p 2–Der p 10. NA, nonasthmatics; A, asthamatics; NR, nonrhinitic; R, rhinitic; NAD, nondermatitic atopic; AD, atopic dermatitis; NU, nonhorticarial; U, horticarial.

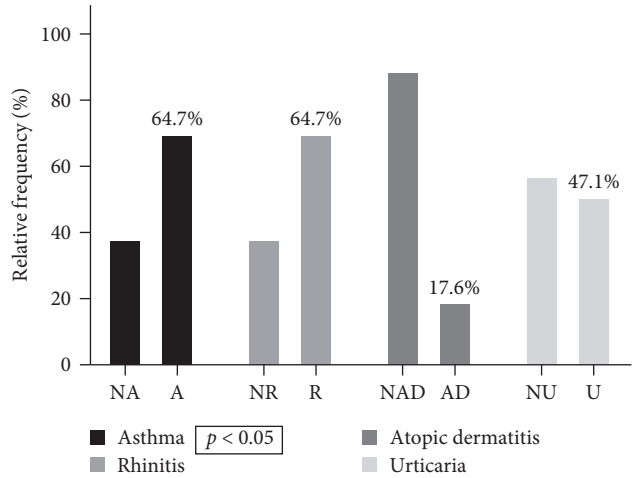

Figure 4: Clinical characteristics in patients sensitized to Der p 1–Der p 2–Der p 23. NA, nonasthmatics; A, asthmatics; NR, nonrhinitic; R, rhinitic; NAD, nondermatitic atopic; AD, atopic dermatitis; NU, nonhorticarial; U, horticarial.

Table 5: Comparison of clinical characteristics of the population sensitized to Der p 10 and Der p 23 and Der p 1 or Der p 2.

	Asthmatics	Atopic dermatitis	Urticaria	Rhinitics	Anaphylaxis
Patients Der p1/2 sensitized	14 (10.4%)	54 (40.3%)	69 (51.5%)	41 (30.6%)	13 (9.7%)
Der p 10 sensitized	17 (73.9%); $p<0.01$	12 (52.2%)	9 (39.1%)	9 (39.1%)	19 (89.6%); $p<0.0001$
Der p 23 sensitized	14 (66.7%); $p<0.05$	4 (19%)	9 (42.9%)	12 (57.1%)	2 (9.5%)
Der p 1-2-10 sensitized	11 (78.6%); $p<0.05$	7 (50%)	5 (35.7%)	4 (28.6%)	11 (78.6%); $p<0.0001$
Der p 1-2-23 sensitized	11 (64.7%); $p<0.05$	3 (17.6%)	8 (47.1%)	11 (64.7%)	2 (11.8%)

that patients with allergic asthma were 78.6% ($p<0.05$), patients with atopic dermatitis were 50%, patients with urticaria were 35.7%, and patients with allergic rhinitis were 28.6% (Figure 3). Der p 1 or Der p 2 sensitization was present in 61.9% of individuals who had previously had anaphylaxis to shrimp or shellfish.

Data analysis of the fourth group of patients sensitized to Der p 23 and Der p 1 or Der p 2 revealed that patients with allergic asthma were 64.7% ($p<0.05$), patients with atopic dermatitis were 17.6%, patients with urticaria were 47.1%, and patients with allergic rhinitis were 64.7% (Figure 4). We performed the χ^2 test regarding sensitization to Der p 10 and anaphylaxis after ingestion of shrimp and shellfish and Der p 10 and asthma, obtaining statistical significance ($p<0.0001$ and $p<0.01$, respectively) (Table 5).

4. Discussion

In our study, we evaluated 253 pediatric patients. More than half of the patients showed sensitization toward HDM, and most were affected by atopic disorders such as asthma and rhinitis. In literature, the prevalence of sensitization to Der p 10 is about 9%–18% in Europe and 5.6% in Spain [21, 22]. Our data analysis showed similar results, 9.1% of patients were sensitized to Der p 10, and 89.6% of these patients reported anaphylactic reactions after shrimp or shellfish ingestion ($p<0.0001$). In comparison, 73.9% were affected by asthma ($p<0.01$), with statistically significant results. In epidemiological research with 48 patients allergic to shellfish, 82% of them appeared to be sensitized to HDM [23]; in our analysis, 61.9% of patients allergic to shellfish were also sensitized to HDM. In our study, the Der p 10 sensitized group suffered more from atopic conditions such as asthma, rhinitis, and atopic dermatitis than Der p 1 and Der p 2 sensitized patients.

Regarding sensitization against Der p 23, we observed that 66.7% of patients were affected by asthma ($p<0.05$). Based on our experience, we have observed that sensitization to multiple dust mite molecules is associated with a higher proportion of individuals with asthma among our patients. According to some studies, in HDM-allergic individuals, the likelihood of developing asthma is influenced by the number of allergen sources other than HDM [8] and the number of mite allergen molecules a person has become sensitized [24]. The importance of HDM sensitization is now taken into greater attention. It has been proposed that the primary sensitizer for shellfish allergies is inhalant exposure to HDM tropomyosin by subsequent IgE cross-reactivity with shellfish tropomyosin, an explanation for the later age of onset and prevalence of oral symptoms seen in the Asia-Pacific area, where HDM is highly common [20]. In general, it has been reported that in shellfish-sensitized children, the prevalence of HDM sensitization is high (~90% in the

Jirapongsananuruk study, ~73% in the Chiang trial) [25, 26]. A Spanish study also supports these data by investigating patients with HDM and shrimp allergies. The authors found an almost complete inhibition of shrimp extract by a mite (*Chortoglyphus arcuatus*) in immunoblot inhibition studies, suggesting that HDMs are the primary sensitizers [20, 27]. Shrimp allergy in the Mediterranean is strictly associated with and almost always dependent upon HDM sensitization [23]. So far, it has focused on the association between Der p 10 and other tropomyosins as risk factors for shellfish and shrimp allergy. In our experience, we observed that about 10% of patients sensitized to HDM were also sensitized to Der p 10. The association between simultaneous sensitization to HDM and Der p 10 and anaphylaxis was statistically significant ($p < 0.0001$), and of this group of patients, 78.6% were asthmatic ($p < 0.05$). In Farioli et al. [28] study, including patients with reported reactions to shrimp, the authors found that the simultaneous positivity of all HDM recombinant sIgE allergens (nDer p 1, rDer p 2, and rDer p 10) corresponded to a 4.8% increase in the odds of developing shrimp allergy. Interestingly, the presence of asthma was associated with a 736% increase in the odds of developing symptoms after shrimp ingestion (Wald test: $p = 0.002$), with a 4.050% increase in the odds of developing asthma (Wald test: $p < 0.0005$) when positivity of anti-nDer p 1, 2, and 10 ($p = 0.085$) IgE levels were considered as single variable [28]. Concerning this finding, we think that patients, in particular asthmatic, sensitized exclusively to Der p 1 and 2 should be followed over time and repeat *in vivo* and *in vitro* tests (component resolved diagnosis (CRD) to check for sensitization to Der p 10 and other tropomyosins, which are the main responsible for the cross-reactivity between mollusks and anthropoids.

5. Conclusions

Our research confirms that dust mite allergy is a common condition in children. Few studies investigate sensitization to seafood and shrimp in children with mite allergies. In our research, we found that not only is Der p 10 statistically associated with anaphylaxis after ingestion of crustaceans and shrimp, but also that sensitization to Der p 10 in children sensitized to Der p 1 or Der p 2 is not uncommon. As evidenced by other studies, the sensitization to multiple dust mite molecules is linked to a higher prevalence of asthma among individuals. In conclusion, in children sensitized to HDM, it is essential to investigate a history of clinical reactions toward crustaceans and mollusks and possibly test for their sensitization *in vivo* and *in vitro* tests. Additionally, in our experience, the use of CRD would be helpful in identifying children who are sensitized to Der p 1, 2, and 10 not only in the context of atopic disorders but also as a risk factor for primary sensitization to crustaceans and shrimp, to prevent severe reactions and anaphylaxis.

Data Availability

Data are available from the corresponding author upon reasonable request.

Consent

Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Sensitization to nsLTP: A Retrospective Study in An Italian Pediatric Population over the Last Decade

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Background. Food allergy is common in the Mediterranean, especially concerning lipid transfer proteins (LTPs) allergy. LTPs are widespread plant food allergens in fruits, vegetables, nuts, pollen, and latex. Also, LTPs are prevalent food allergens in the Mediterranean area. They can sensitize via the gastrointestinal tract and cause a wide range of conditions: from mild reactions, such as oral allergy syndrome, to severe reactions, such as anaphylaxis. LTP allergy in the adult population is well described in the literature, concerning both the prevalence and clinical characteristics. However, there is poor knowledge about its prevalence and clinical manifestation in children living in the Mediterranean. *Materials and Methods*. This study, including 800 children aged from 1 to 18 years, investigated the prevalence of 8 different molecules of nonspecific LTP over time in an Italian pediatric population visited over the last 11 years. *Results*. About 52% of the test population was sensitized to at least one LTP molecule. For all the LTPs analyzed, sensitization increased over time. In particular, using the years 2010 through 2020 as a comparison, the major increases were observed for the LTPs of the English walnut Jug r 3, the peanut Ara h 9, and the plane tree Pla a 3 (about 50%); the increase of the LTP of the Hazelnut Cor a 8 was about 36%, and that of the LTP of the artemisia Art v 3 was approximately 30%. *Conclusions*. The latest evidence in the literature indicates an increase in food allergy prevalence in the general population, including children. Therefore, the present survey represents an interesting perspective about the pediatric population of the Mediterranean area, exploring the trend of LTP allergy.

1. Introduction

Nonspecific lipid transfer proteins (nsLTPs) are ancient and highly conserved pan-allergenic molecules widespread in plant foods [1]. They are the primary food allergy (FA) in adults and adolescents in the Mediterranean Basin and the most important allergens that cause food-induced anaphylaxis in Italy [2]. This characteristic is due principally to their biochemical structure, such as high resistance to low pH, elevated temperature, and gastrointestinal proteolysis. In the review by Costa et al. [3], the physicochemical properties, including those of LTPs analyzed to identify how they influence the allergenic potency of plant allergens: the abundance of LTPs is related to an increased risk of allergic elicitation, while the loss of protein 3D structure does not affect their allergenicity; glycation, aggregation and high temperatures (100°C) do not affect their IgE-binding capacity, while the combination of pressure-heat and pressure-heat-enzymatic hydrolysis treatments is efficient in reducing the IgE-binding capacity of LTP. These implications are still the subject of clinical research. To date, they are classified into two subfamilies based on their molecular weight: nsLTP1 (9 kDa), which includes most of LTPs, capable of causing a specific IgE response, and nsLTP2 (7 kDa) [4]. LTPs are usually localized in the pericarp of the fruits, thanks to their defensive role against phytopathogenic bacteria and fungi. The manifestation and the clinical severity of the LTP-related symptoms vary according to the level of avidity of the IgE implicated.

Many sensitized patients appear completely asymptomatic; others may manifest local symptoms as an oral allergy syndrome (OAS) or contact urticaria or, in more severe cases, significant manifestations like vomiting, asthma, abdominal pain, urticaria-angioedema, and systemic reactions up to anaphylactic shock [5]. The epidemiological and clinical aspects of FA are poorly studied in the pediatric population [1]. FA is an adverse immunologic response, which appears systematically after exposure to a certain food [6]. Symptoms of FA may vary, from common urticaria to anaphylaxis [7]. They can affect the gastrointestinal tract with vomiting and abdominal pain or the skin and mucosa with urticaria and edema. In severe reactions, also the cardiovascular system may be affected, with hypotension, tachycardia, up to cardiac arrest [8]. Ideally, any food can elicit an allergic reaction; the most common cause of FA in children are milk, egg, peanuts, tree nuts, shellfish, and fish [9, 10]. In the Mediterranean area, especially in Italy and Spain, rPru p 3, the first allergen to cause sensitivity in children is the nsLTP from peaches (*Prunus persica*). It may subsequently promote new sensitizations to many nsLTP-containing foods [11, 12]. Namely, rPru p 3 cross-reacts with other LTP molecules contained in many fruits belonging to the Rosaceae family, including apple, peach, apricot, and pollens, such as mugwort, olive, Parietaria, and plane [2, 13, 14]. This cross-reactivity is particularly evident for rPru av 3 (*Prunus avium*, cherry) and Mal d 3 (*Malus domestica*, apple), which have a structural homology of 88% and 80%, respectively. The structural homology of Pru p 3 with Jug r 3 (*Juglans regia*, walnut) appears lower (61%) as well as with Cor a 8 (*Corylus avellana*, hazelnut) (59%), Ara h 9 (*Arachis hypogaea*, peanut) (53%), Tri a 4 (*Triticum aestivum*, wheat) (45%), and it reduces more and more considering the LTP of pollens: Art v3 (*Artemisia vulgaris*, mugwort) (46%), Par j 1 (*Parietaria judaica*, pellitory wall) (29%), and Ole e 7 (*Olea europaea*, olive tree) (19%) [15]. Diagnosis of LTP allergy is based on clinical history, followed and partly supported by the skin prick test (SPT) with extracts or fresh food (prick-by-prick). However, Component resolved diagnosis (CRD) has improved the accuracy of diagnosing IgE-mediated FA [16], assessing sensitization to individual allergen molecules using purified native or recombinant allergens. Basophil activation test measures are helpful to differentiate between tolerant controls and patients with LTP allergies, although neither sensitivity nor reactivity can differentiate the severity of clinical symptoms [17]. The presence of allergen-specific IgE against LTPs could indicate a risk of allergic reactions; generally, the higher the level of IgE detected, the higher the probability of a clinically manifest allergic reaction [18, 19]. Recent data seem to suggest that there is a high probability of LTP-sensitized patients to progress over time to severe allergic reactions: in Betancor et al.'s [20] study, 13.2% of 38 plant-food LTP- sensitized patients experienced allergic reactions, and 31% of 113 plant-food-allergic patients sensitized to LTP reported reactions to new, previously tolerated plant foods. Moreover, several plant-food sensitizations may result from the cross-reaction of the LTPs from various plant foods and pollens, resulting in LTP syndrome; for example, Pru p 3 positivity can cause an allergy to any LTP-related food [21]. Furthermore, the same results for the same allergens may not provoke the same clinical manifestations due to differences in individual patient sensitivities [22, 23]. As there is poor knowledge concerning LTPs sensitization in pediatric populations in the Mediterranean area, the present study aimed to investigate the prevalence of LTPs in children in Campania, a region in southern Italy.

2. Material and Methods

2.1. Patients. This study included 800 consecutive pediatric patients who visited the pediatric allergology clinic at the University of Campania "Luigi Vanvitelli" from 2010 to 2020. All patients were between the ages of 1 and 18 years old and were being followed for atopic disorders such as allergic asthma, atopic dermatitis, and allergic rhinitis. They had a suspected diagnosis of FA proposed by their primary care pediatricians. The study retrospectively analyzed the serum-specific IgE for eight different nsLTPs using the microarray method (ImmunoCAP ISAC, ThermoFisher Scientific, Milan, Italy). First, the data concerning nsLTPs were extracted and then compared. The nsLTPs analyzed were Ara h 9 (peanut), Jug r 3 (walnut), Cor a 8 (hazelnut), Pru p 3 (peach), Tri a 14 (wheat), Art v 3 (mugwort), Ole e 7 (olive tree), and Pla a 3 (plane tree). Sensitization was diagnosed in the presence of a value greater than 0.35 ISU-E.

2.2. Endpoints. The primary objective of our study was to evaluate the trend of sensitizations to eight different nsLTPs in a pediatric population living in the Mediterranean area between 2010 and 2020. The secondary objective of our study was to compare sensitization year by year to assess the trend of each year and compare them.

2.3. Statistical Analysis. All continuous variables were evaluated for normality according to the Shapiro–Wilk test. Differences in not-normally distributed continuous variables were investigated using the Kruskal–Wallis test. Significance was set for p-values < 0.05. The data obtained about the nsLTPs analyzed during our study were compared using the chi-square test. All analyses were performed using Microsoft Excel for Microsoft 365, Microsoft Inc., Redmond, Washington, USA, and GraphPad Prism version 8.0.2 for Windows, GraphPad Software, San Diego, California, USA.

3. Results

Analysis of the results showed that 507 patients (63.4%) were male and 293 patients (36.6%) were female. The sensitization to peach Pru p 3 was the most common, affecting 46% of the population. Additionally, 34.2% of children were sensitized to Jug r 3, 32.4% to Art v 3, 31.9% to Pla a 3, 31.2% to Ara h 9, 30% to Cor a 8, 11.5% to Ole e 7, and 7.3% to Tri a 14. About 52% of the population in the study period was sensitized to at least one LTP (Figure 1).

Over the past 10 years, the prevalence of LTP sensitization in the population has grown. According to the graph (Figure 2), the number of sensitized patients increased significantly for each nsLTP examined, peaking in 2019.

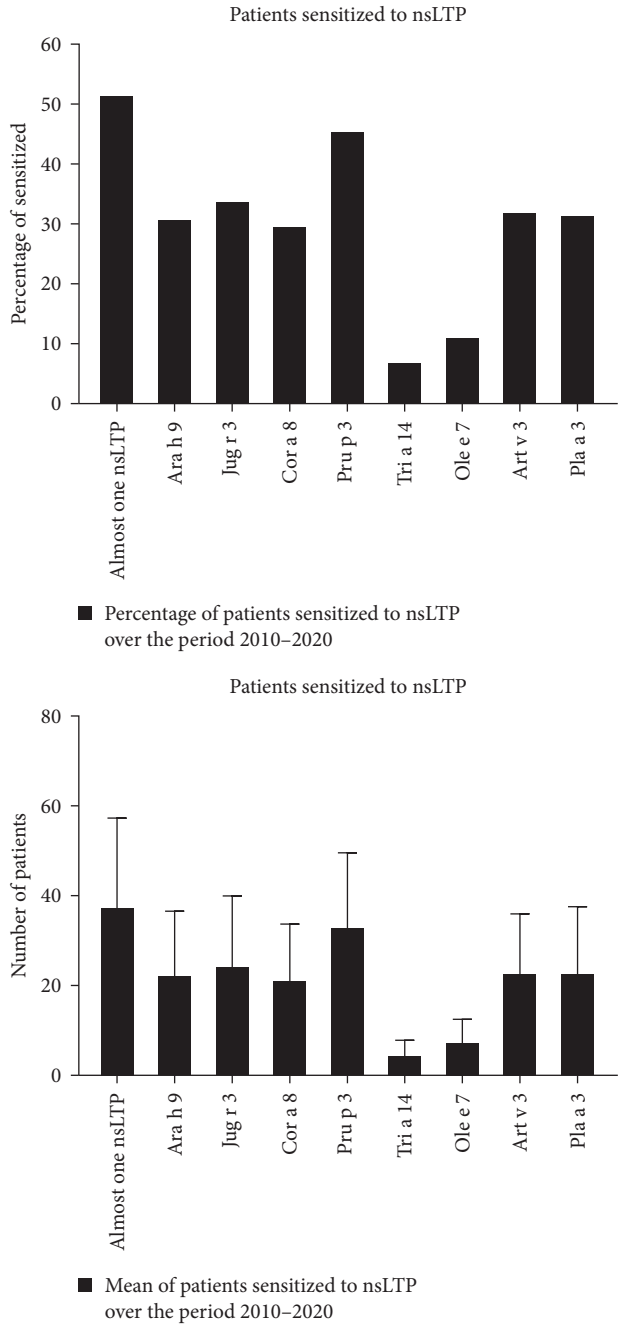

Figure 1: Sensitization to eight nsLTPs in the period 2010–2020.

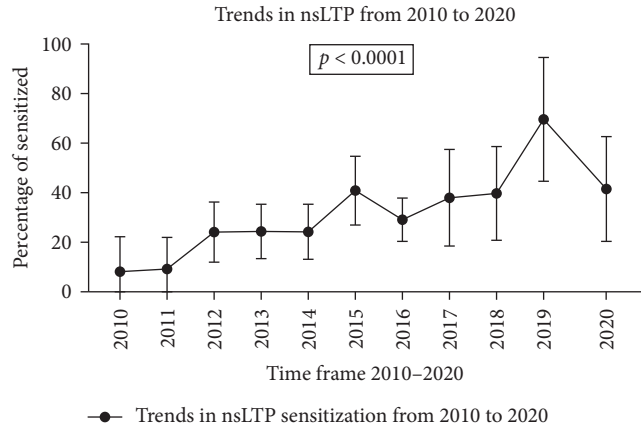

Figure 2: Trends in nsLTP sensitization from 2010 to 2020.

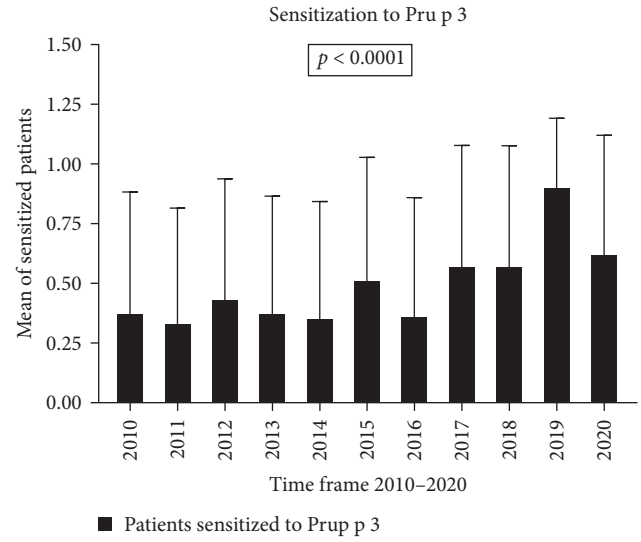

Figure 3: Trends in Pru p 3 sensitization over 2010–2020.

In particular, if we compare the years 2010 and 2020, the increase for the LTP of the English walnut Jug r 3 and the peanut Ara h 9 LTP was about 50%. The LTP of the Hazelnut Cor a 8 increased by about 36% when comparing 2010 to 2020, while during the same period, the LTP of Peach Pru p 3 had an increase of about 23% (Figure 3). The LTP of wheat Tri a 14 increased by about 6%, with a peak increase of about 20% in years such as 2015 and 2019. The LTP of the olive tree Ole e 7 shows an increase of about 11% when comparing 2010 to 2020. The LTP of Artemisia Art v 3 grew by around 30%, whereas the LTP of the plane tree Pla a 3 increased by approximately 50% during the same period (Table 1).

The data collected about the eight LTPs analyzed during our study show a p-value < 0.05 for each LTP analyzed from 2010 to 2020. The age difference of the patients between the single years studied by the multiple comparisons of the Kruskal–Wallis test obtained a nonsignificant value. In each of the years examined, the average age of the patients was consistently between 8 and 10 years (Figure 4).

4. Discussion

The objective of this study was to determine the prevalence of sensitization to LTP molecules among pediatric patients in Campania, southern Italy, from 2010 to 2020. The analysis of patient data revealed a frequency of approximately 52% for sensitization to LTP molecules over the entire period analyzed, with an increase observed in the second half of the decade for most of the molecules tested. To our knowledge, this is the first study to report on the prevalence of LTP sensitization in a pediatric population in Campania, southern Italy, over the past decade. Our findings are consistent

Table 1: Year-to-year percentages of patients sensitized to nsLTP and mean age.

Years	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Mean age	8	8	9	8	8	8	9	10	10	9	9
Ara h 9 (%)	0	0	23	30	26	51	37	56	51	78	50
Jug r 3 (%)	0	1	23	36	32	55	37	54	56	89	55
Cor a 8 (%)	10	17	31	18	25	39	28	31	41	78	46
Pru p 3 (%)	40	33	43	37	36	51	37	58	58	91	63
Tri a 14 (%)	0	2	7	7	5	19	16	7	8	21	6
Ole e 7 (%)	0	0	9	12	11	22	18	14	14	41	11
Art v 3 (%)	15	21	34	26	24	39	25	37	40	79	46
Pla a 3 (%)	0	0	23	29	35	51	35	47	50	80	55

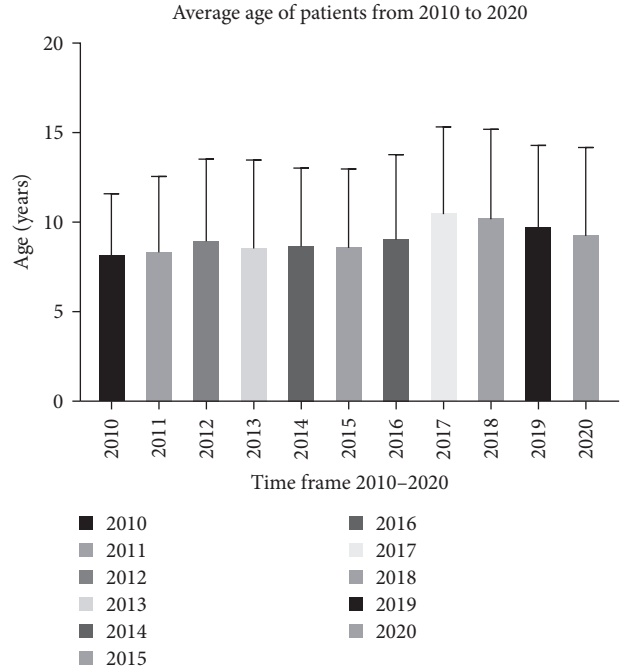

Figure 4: Average age of patients.

with recent studies that have reported an increase sensitization and allergy in the last years [8, 24–27]. In the United States, the proportion of hospital admissions due to food anaphylaxis in children aged 0–18 years increased by more than two-fold between 2000 and 2009 [28]. Between 1998 and 2012, food anaphylaxis admission rates in the United Kingdom increased from 1.2 to 2.4/105, with 0–4 aged children showing the greatest rates [29]. The number of children who visited the American emergency departments for food-induced anaphylaxis increased by 214% ($p < 0.001$) from 2005 to 2014; infants and toddlers had the greatest rates [30]. Currently, the prevalence of FA in children is between 5% and 10% in Western countries and about 7% in China and Korea [25, 31]. The rise in FA and sensitization can be attributed to a number of factors, such as the lack of exposure to microbes necessary for building immune defenses in the early years of life, the polarization of the immune response toward a Th2 phenotype (hygiene hypothesis), vitamin D deficiency, indiscriminate use of antibiotics, pollution, delayed introduction of food allergens, and changes in the microbiota [24]. The principal limitation of our study is that we only discuss the prevalence of LTPs sensitizations in a selected pediatric population, not its relationship to clinical data and allergic symptoms. It is reasonable to assume a link between the increase in LTP sensitization and the rise in allergic reactions. However, it is crucial to differentiate between food sensitization and real FA, i.e., the occurrence of symptoms after ingestion of the sensitizing allergen. For example, in a recent article about 82 pediatric patients with allergic rhinitis due to Parietaria pollen allergy and sensitization to Pru p 3, the LTP of the peach, anaphylaxis after eating foods containing LTP was reported by about one-quarter of children, the other half reported FA or OAS; the remaining one-quarter were merely sensitized [32]. A Spanish study that examined a group of children with nut allergies discovered that clinical symptoms are not always present when a child becomes sensitized to a particular plant-food LTP: in fact, Pru p 3- and Jug r 3-IgEs were present in 69% and 63% of peach- and walnut-tolerant patients, respectively. Like this, 9.1% of hazelnut-tolerant people exhibited positive Cor a 8, compared to 36.8% for peanut (Ara h 9) and 26.2% for wheat (Tri a 14). Therefore, a definitive diagnosis of FA cannot be made based solely on IgE and SPT results without considering the patient's clinical history [33]. In addition, a study by Novembre et al. [34] found that the levels of specific IgE to Pru p 3 in Italian children with peach allergies do not help predict the severity of the allergic reactions. An allergy management strategy based on immunological understanding should be implemented for patients sensitized to LTP molecules. In other words, LTP-sensitized individuals (such as those who did not experience a clinical reaction) could consume any meal they could handle until overt symptoms started to show. This approach is clinically relevant as it helps to maintain both immunological and clinical tolerance. In addition, it is the base of the prevention of FA. To differentiate between tolerance and symptoms, a proper medical strategy should focus on increasing the patient's "engagement" with his actual clinical state [19, 35]. Dietary avoidance of essential nutrients, such as fruits and vegetables, may adversely affect a child's development, health, and quality of life for both the child and their parents. Because of these factors, food avoidance strategies should be based on clinical reactivity rather than only sensitization [6]. Due to the partial similarity of LTP derived from many foods and sensitization does not signify allergy,

LTP-allergic people can consume all tolerated foods until the onset of overt symptoms. This strategy might avoid harmful and needless restrictive diets and possibly promote the growth of natural tolerance as a sort of physiological immunotherapy [19, 36]. The gold standard diagnostic test is still the oral food challenge in circumstances where diagnostic uncertainty persists.

5. Conclusion

LTP allergy is widely described in adults but is also an emerging issue in the pediatric population. The actual prevalence of this sensitization in children is not well known, and it still often remains underdiagnosed in these patients. By comparing literature documents with our experience, these data will have significant importance for subsequent epidemiological studies against some allergic diseases on our national territory and at the European level. LTP allergy can cause not only local allergic reactions such as urticaria and OAS but also more critical reactions such as nausea, vomiting, abdominal pain, angioedema, and systemic reactions such as anaphylactic shock. The analysis of these data allows us to evaluate the actual prevalence of sensitization toward the various LTP. This knowledge could be an important starting point for implementing innovative studies regarding the true prevalence of clinical reactions in the pediatric population and for implementing information and prevention strategies against possible allergic reactions in the pediatric population.

Data Availability

Data are available from the corresponding author on reasonable request.

Consent

Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Molecular Allergy Diagnostics in Children with Cow's Milk Allergy: Prediction of Oral Food Challenge Response in Clinical Practice

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Background. Cow's milk allergy (CMA) is the most common food allergy in early childhood. Children with CMA require a precise and punctual diagnosis. Oral food challenge (OFC) is the gold-standard procedure for diagnosing allergies, but it is laborious and requires a particular setting. The aim of the study was to identify the cutoff value of serum allergen-specific IgE values able to predict a positive response to OFC. *Methods*. Children with suspected CMA performed OFC with cow's milk (CM) or derivatives. Total IgE and specific IgE to raw CM, α-lactalbumin, β-lactoglobulin, and casein were measured. *Results*. Seventy-two children performed OFC, and 30 (41.6%) had a positive response. The significant predictive factors were sensitization to raw CM extract ($p = 0.03$), α-lactalbumin ($p = 0.013$), β-lactoglobulin ($p = 0.09$), and casein ($p = 0.019$). The cutoff was, respectively: 5.13 kUA/L for raw CM, 1.47 for α-lactalbumin, 1.35 for β-lactoglobulin, and 4.87 for casein. *Conclusions*. This study allowed us to define a set of cutoff values for CM protein-specific IgE. However, these cutoffs should be interpreted not as a diagnostic tool for CMA but only predictive of response to OFC in a specific territory. Thus, the practical message may be that a value above the cutoff allows a good approximation to identify children to be started on OFC.

1. Introduction

Food allergy represents a relevant health concern, mainly in childhood; in particular, cow's milk allergy (CMA) is the most relevant in infancy and early childhood, affecting up to 5% of children [1]. However, the actual prevalence significantly varies across countries as well as the severity of clinical manifestations [2]. Accordingly, a long-term UK study pointed to 23 children with CMA who had anaphylaxis after milk ingestion [3]. Therefore, CMA is a common, but not a trivial, medical condition.

From an immunological point of view, cow's milk (CM) consists of different allergenic molecules. The most relevant allergenic molecules are α-lactalbumin (Bos d 4), β-lactoglobulin (Bos d 5), and casein (Bos d 8). The sensitization prevalence of these molecules is hugely variable, whereas the allergy prevalence in sensitized subjects is about 20% [4]. In addition, it has to be underlined that boiling, pasteurization, ultra-high temperature treatment, evaporation, and formula maintain the milk allergenicity [5]. Only extensive hydrolysis significantly affects milk allergenicity. These clinically relevant concepts should be considered in CMA subjects with severe manifestations after milk or its derivatives ingestion.

Another essential characteristic of CMA is the natural history: the onset usually occurs before 12 months of age, at the same time as the introduction of CM [6]. Moreover, CMA commonly disappears over time as most children develop immunological tolerance to cow's milk. However, some subjects maintain CMA even during adulthood [7].

Symptoms usually occur immediately after CM ingestion and involve mainly the skin, gastrointestinal and respiratory tract, and, more rarely, the cardiovascular system [8].

Furthermore, symptoms intensity may range from mild to severe until anaphylactic shock, also fatal [9].

Consequently, CMA patients require adequate management based on thorough workup and close follow-up. Namely, an early and precise diagnosis is mandatory for prescribing a tailored diet and avoiding useless and potentially dangerous milk avoidance. The correct diagnosis of CMA requires a suggestive history, the documentation of sensitization, i.e., production of specific IgE, and consistency between history and sensitization [10].

Sensitization may be investigated by skin prick test, with allergen extracts or fresh milk, and/or serum IgE assay. However, serum assay is more precise and reliable than skin testing [11]. Moreover, serum testing presently is advantaged by the use of molecular diagnostics, which allows the identification of the immunological profile in each patient [12]. In this regard, the most recent guidelines on managing CMA patients reported the diagnostic criteria for interpreting the laboratory outcomes [13–18]. However, it must be emphasized that only the oral food challenge (OFC) makes it possible to acquire a definite diagnosis of CMA [13–18].

Though, OFC should be performed only in particular settings, characterized by trained staff and equipped with adequate tools. Therefore, predictive tools that facilitate CMA suspicion can be handy in clinical practice. In this regard, several studies have attempted to define cutoffs of specific IgE levels, mainly molecular-specific, that could identify subjects with suspected CMA as a first step [19–28]. However, the reported values needed to be more consistent. Based on this background, the present study aimed to evaluate the cutoff of allergen-specific IgE to milk and its molecules in clinical practice.

2. Materials and Methods

This study retrospectively evaluated all children consecutively admitted to performing OFC from 2013 to 2022. The data were extracted from an electronic platform containing demographics, clinical history, and biological data.

Laboratory data included peripheral eosinophils, total serum IgE, and allergen-specific IgE to casein, α-lactalbumin, β-lactoglobulin, and raw milk.

IgE was measured using ELISA assays provided by Thermofisher (Milan, Italy). The total serum IgE normal level is <100 kU/L. Sensitization is defined when the value of allergen-specific IgE is >0.35 kUA/L.

The inclusion criteria were the need to perform OFC for suspected CMA, suggested by symptom occurrence after CM ingestion, sensitization to CM extract and/or milk molecules, and pediatric age. Exclusion criteria were concomitant diseases and medications that could interfere with interpreting results.

The OFC was performed using the following procedure: patients started from doses between 0.05 and 1 mL, progressively incremented. The OFC ended when a clinical manifestation occurred or a cumulative dose of 80 mL had been reached.

Table 1: Baseline characteristics ($n = 72$).

Sex	
Females	20 (27.8%)
Males	52 (72.2%)
Age (months)	71.6 ± 56.61
Sensitisation to other food allergens	50 (69.4%)
Sensitisation to inhalant allergens	43 (59.7%)
Atopic dermatitis	30 (41.7%)
Recurrent wheezing	38 (52.8%)
Allergic rhinitis	21 (29.2%)
Chronic urticaria	5 (6.9%)
Oral allergy syndrome	1 (1.4%)
Other food allergies	17 (23.6%)
Allergy in family	40 (55.6%)
Allergy tests	
Total IgE (kU/L)	476.4 ± 733.0
IgE to cow's milk (kUA/L)	23.2 ± 35.84
IgE to Bos d 4 (α-lactalbumin) (kUA/L)	13.4 ± 26.96
IgE to Bos d 5 (β-lactoglobulin) (kUA/L)	12.8 ± 23.35
IgE to Bos d 8 (casein) (kUA/L)	16.4 ± 27.50

The Ethics Committee of the IRCCS Istituto Giannina Gaslini approved the procedure (10/17; 04/05/2017). Parents signed informed consent.

A univariate logistic regression model was applied to identify which variables best predict response after OFC. Subsequently, a receiver operating characteristic curve was used to assess the accuracy of different IgE classes in predicting the response. For each receiver operating characteristic curve, the area under the curve with the corresponding 95% confidence interval was calculated with the best cutoff point, which provided both the highest sensitivity and specificity.

A 2-tailed p value of 0.05 was considered significant. All statistical analyses were performed using SPSS 23.0 (SPSS, Chicago, Illinois).

3. Results

Seventy-two children, 52 males (72.2%) and 20 females (27.8%), mean age of 71.6 months, performed the OFC to CM, as reported in Table 1. Fifty (69.4%) children were sensitized to other food allergens and 43 (59.7%) to inhalant allergens. In addition, different comorbidities were present: 30 (41.7%) children had atopic dermatitis, 38 (52.8%) recurrent wheezing, 21 (29.2%) allergic rhinitis, 5 (6.9%) chronic urticaria, 1 (1.4%) oral allergy syndrome. Forty (55.6%) children had at least one family member with an allergy.

The mean total IgE level was 476.4 kU/L, the data concerning sensitization to milk and its molecules are in detail reported in Table 1.

Thirty (41.6%) children were positive for OFC with milk (Table 2). The analysis concerned the valuable parameters that could predict the positive response to OFC. The significant predictive factors were sensitization to raw CM extract ($p = 0.03$), α-lactalbumin ($p = 0.013$), β-lactoglobulin ($p = 0.09$), and casein ($p = 0.019$). The cutoff was, respectively:

Table 2: Predictive factors for a positive response to CM challenge.

	Negative challenge ($n = 42$; 58.4%)	Positive challenge ($n = 30$; 41.6%)	p	AUC (95% CI)	Cutoff	Spec.	Sens.
Age (months)	60.8 ± 49.81	86.7 ± 62.71	0.05				
Sex							
Females	11 (26.2%)	9 (30.0%)	0.79				
Males	31 (73.8%)	21 (70.0%)					
Severity of reaction							
Mild	9 (23.1%)	10 (41.7%)	0.27				
Moderate	15 (38.5%)	6 (25.0%)					
Severe	15 (38.5%)	8 (33.3%)					
Total IgE (kU/L)	605.7 ± 1227.47	661.4 ± 1041.39	0.65				
IgE to cow's milk (kUA/L)	8.4 ± 14.72	22.8 ± 30.89	0.03	0.65 (0.52–0.79)	5.13	70.0%	65.5%
IgE to Bos d 4 (α-lactalbumin) (kUA/L)	2.8 ± 5.76	11.1 ± 21.66	0.013	0.68 (0.55–0.81)	1.47	71.1%	62.1%
IgE to Bos d 5 (β-lactoglobulin) (kUA/L)	2.8 ± 5.62	6.5 ± 12.68	0.09	0.62 (0.49–0.76)	1.35	67.6%	58.6%
IgE to Bos d 8 (casein) (kUA/L)	5.5 ± 10.74	18.5 ± 27.57	0.019	0.67 (0.54–0.81)	4.87	81.6%	51.9%
Total daily cumulative dose administered on the last day of the challenge (mL)	23.7 ± 33.03	27.2 ± 43.79	0.17				

Table 3: Predictive cutoff values for raw caw's milk (F 2), α-lactoalbumin (Bos d 4), β-lactoglobulin (Bos d 5), and casein (Bos d 8) reported in the most recent literature. Data are expressed as kUA/L.

Author	Reference	F 2	Bos d 4	Bos d 8	Bos d 5
Castro et al.	[22]	3.06	2.08	1.85	1.47
Cuomo et al.	[24]	≥5			
Petersen et al.	[25]	3.64	0.77	1.59	2.33
Ayats-Vidal et al.	[27]	3.87	2.25	1.6	0.95
Castro Neves et al.	[28]	7	8.8	7	7.3
Present study		5.13	1.47	1.35	4.87

5.13 kUA/L for raw CM, 1.47 for α-lactalbumin, 1.35 for β-lactoglobulin, and 4.87 for casein. In addition, area under the curve (AUC), specificity, and sensitivity values are reported in detail in Table 2.

4. Discussion

CMA is the most frequent food allergy in children under the age of 3 years. Children with CMA require hardworking management by the physician and constant attention from the parents. However, it is quite common to observe children on unnecessary and often harmful exclusion diets at an age when milk is an essential nutrient for growth. Therefore, a precise and accurate diagnosis is the fundamental premise for properly managing CMA. In this regard, OFC remains the gold standard for providing a certain CMA diagnosis [20]. However, OFC is a laborious and potentially risky procedure that should be performed only in highly qualified centers. As a result, children undergoing OFC need a rigorous selection based on a reasonable presumption of CMA. A suggestive history and sensitization to milk molecules may predict CMA with good approximation [10]. Therefore, data on sensitization to CM allergens could represent a valuable tool to corroborate a suspicion of CMA and thus refer a child to the OFC. In this regard, several studies attempted to define a reliable cutoff of specific IgE levels to predict a positive response to OFC and, consequently, identify children with CMA [29].

The present study derived from previous research showing that children with anaphylaxis to CM proteins had higher levels of specific IgE than children with mild CMA [10]. In particular, children with >12.2 kUA/L IgE to casein had an OR of 15 to have anaphylaxis compared to children with IgE levels below this cutoff.

As we recently reported data concerning children with CMA and undergoing oral immunotherapy [29], the present study aimed to identify factors predictive for positive OFC and define the cutoff of specific IgE levels.

The results highlighted that the predictive cutoff for raw CM was 5.13 kUA/L, 1.47 for α-lactoglobulin, 1.35 for β-lactalbumin, and 4.87 for casein. However, these cutoff values are only partially reliable considering the single AUC scores, specificity, and sensitivity outcomes. Moreover, these findings are partially consistent with the most recent literature data, as reported in Table 3. Namely, a Brazilian study reported a cutoff of 3.06 kUA/L for CM (sensitivity 71%; specificity 98%), 2.08 for α-lactalbumin (sensitivity 58%; specificity 98%), 1.85 for β-lactoglobulin (sensitivity 57%; specificity 98%), and 1.47

(sensitivity 66%; specificity 98%) [22]. A Danish study identified different cutoff values for milk proteins: 3.64 for raw CM, 0.77 for α-lactalbumin, 1.59 for β-lactoglobulin, and 2.33 for casein [25]. A Spanish study reported the following cutoff values: 3.87 for raw CM, 2.25 for α-lactalbumin, 1.6 for β-lactoglobulin, and 0.95 for casein [27]. Finally, a Portuguese study identified these values: 7 for raw CM, 8.8 for α-lactalbumin, 7 for β-lactoglobulin, and 7.3 for casein [28]. In addition, Cuomo et al. [24] reviewed 31 studies on this topic and identified the cutoff of 5 kUA/L for CM extract as a likely predictor for CMA.

Therefore, there is no complete agreement between the various cutoffs defined by the various studies. In addition, considering the criteria for validity (e.g., AUC, sensitivity, specificity), all the more reason that each study population has different outcomes. Consequently, the proposed cutoff values can have a somewhat relative reference value primarily because of the country under consideration. In this regard, the relative inconsistency among these studies could depend on different populations and the lack of sIgE/tIgE relationships for a more precise analysis.

Therefore, the results should always be interpreted cautiously and related to the survey location. Furthermore, based on the validity criteria of the tests, it seems necessary to emphasize that the diagnosis of CMA mandatorily requires the performance of OFC.

This study had several limitations, especially the limited number of subjects and the failure to perform a double-blind OFC with a placebo. In addition, the ratio between total IgE and allergen-specific IgE was not performed. On the other hand, this study reflects what can occur in real-life in a level 3 pediatric allergy center.

In conclusion, this study allowed us to define a set of cutoff values for CM protein-specific IgE. However, these cutoffs should be interpreted not as a diagnostic tool for CMA but only predictive of response to OFC in a specific territory. Thus, the practical message may be that a value above the cutoff allows a good approximation to identify children to be started on OFC.

Data Availability

Data are available on demand to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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