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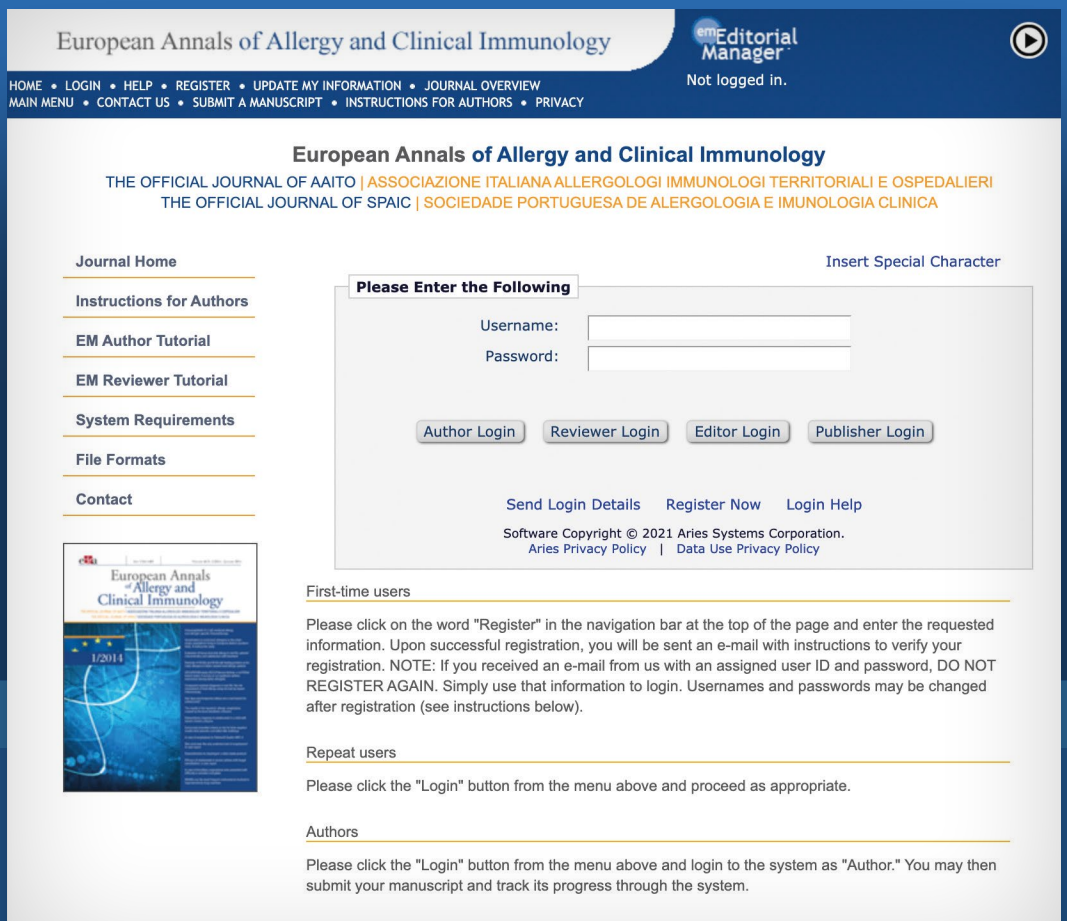

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edra REVIEW | Eur Ann Allergy Clin Immunol | Vol 57, N.3, 99-106, 2025

Gennaro Liccardi[1], Matteo Martini[2,3], Maria Beatrice Bilò[2,3], Lorenzo Cecchi[4], Manlio Milanese[5], Antonino Musarra[6], Ermanno Puxeddu[7], Paola Rogliani[1,7]

A narrative review on allergy and exposure to domestic and non-domestic animals: favorable and unfavorable effects

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Allergic rhinitis; animals; bronchial asthma; cat; dog.

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Impact statement
It is evident the need for further studies encompassing prevention aspects, standardization of pet allergens, enhancement of the efficacy of various AIT modalities as well as new immunological approaches.

Summary
The aim of this contribution was to highlight the "favorable" and "unfavorable" roles of domestic and non-domestic animals on airway sensitization processes and on the type / severity of the clinical symptoms induced by their exposure. We performed a literature research in MEDLINE for allergic manifestations and animals.
Pets can be "allergy friends" through mechanisms related to hygiene hypothesis and translational aspects, the dual role of IgG4 antibodies for pets, and their promising role as healthcare service animals (dogs).
On the contrary, animals can be "allergy enemies" when inducing allergic sensitization and respiratory symptoms (sometimes leading to severe reactions), and also due to cross reactivity with other pets' allergens, indirect exposure and ubiquity of their allergens, cross reactivity between Can f 5 and human prostate-specific antigen (PSA). Moreover, in some cases they can trigger anaphylaxis, induce occupational asthma, and act as pests. Finally, we must outline the modest efficacy of allergen immunotherapy (AIT) for their allergens. From a strictly allergological perspective, it is evident that the "negative" aspects resulting from exposure to domestic / non-domestic animals outweigh the "positive" aspects. As a consequence, it is up to humans to seek new ways to balance the pros and cons by exploring research areas that can allow the best possible coexistence with subjects at risk of allergy with domestic and non-domestic animals.

Introduction

Dogs and cats are the most prevalent pets globally. Recent data indicate that 44.7% of families own a dog, while 35.4% have a cat at home in Italy (1), while allergic sensitization frequency to cats and dogs is reported as 16% and 9%, respectively (2).

Beyond the emotional relationship with humans (including their role in pet therapy), common pets drive relevant economic activities, including breeding, veterinary services, and new pet-related professions (*e.g.*, pet sitters, groomers, pet shop workers, military/police units) and pet product industries (*e.g.*, pet-food, accessories). Furthermore, the increasingly widespread habit of having

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exotic animals as pets has also increased the frequency of allergic sensitization to these creatures (3, 4). In the strictly allergy context, unlike other allergenic sources, animals can play a dual role in the development of allergic sensitization, either inhibiting or facilitating this process.

The aim of this contribution was to highlight the "favorable" and "unfavorable" roles of domestic and non-domestic animals on airway sensitization processes and on the type/severity of the clinical symptoms induced by their exposure.

Data sources and study selections

We performed a literature research in MEDLINE for randomized and non- randomized trials, systematic reviews and meta-analyses. We selected studies according to the following key words: allergic rhinitis, animals, bronchial asthma, cat, dog, horse, guinea pig, pig, or mouse.

How pets can be "allergy friends"?

The controversial of early exposure to pets on the subsequent development of allergy

Early exposure to animals on the development of allergic sensitization and asthma is a rather controversial topic as it is possible to observe both a "preventive" and a "facilitating" effect on this process. Furthermore, the "protective" effect appears to be more significant in rural environments, compared to urban areas. The rural lifestyle, characterized by contact with farm animals (including cattle), greater presence of microorganisms, less pollution, *etc*., constitutes a set of factors considered "protective" on the development of allergic pathologies. On the contrary, the urban lifestyle characterized by indoor living, high hygiene standards, and significant pollution, represents several "facilitating" factors. This theory is known as the 'hygiene hypothesis' (5).

In a recent review, Indolfi *et al*. (6) found a relationship between the number of owned pets and a protective effect against allergy and asthma. The likelihood of developing allergy and asthma seems to be influenced by various factors in children, including the genetic background and early exposure to different environmental factors, including allergens that may interact with the gut microbiota and the immune system. In fact, microbiota detected in nasal fluids (7) and indoor dust (8) of individuals with pets at home are likely to be associated to a reduced risk of allergic sensitization. On the contrary, Park *et al*. (9) have found that early-life exposure to dog reduces atopy but increases the risk of nonatopic bronchial hyper-reactivity (BHR) and nonatopic asthma at 7 years old.

Indolfi *et al*. (6) and others (10) demonstrated that exposure to pets during early infancy can also reduce the incidence of food allergies.

Dual role of IgG4 antibodies in pet allergy

The clinical significance of IgG4 in allergic diseases is complex and controversial. However, published data indicate that allergen-specific IgG4 plays a multifaceted role in allergic diseases that is protective or pathogenic depending on different allergens or exposure conditions (11). In particular, acute allergen exposure may lead to IgE-mediated histamine-induced hypersensitivity reactions by high-affinity receptor FcεRI whereas chronic allergen exposure may induce IgG4 formation and allergen binding by IgG4. The IgG4 binding to the inhibitory receptor FcgRIIb prevents hypersensitivity reactions and induces allergen tolerance (11).

Translational aspects

A better understanding of the pathophysiology of asthma in humans by utilizing animal models of asthma is aimed at improving diagnostics and treatments. Depending on the research question and budget, certain animal models of asthma may be more applicable for use. For this purpose, various animals have been used such as rodents, guinea pigs, rabbit, dogs, sheeps, cats, horses, non-human primates. Naturally occurring animal models of asthma have the benefit of investigating the aspect of chronicity, collecting longitudinal data, and evaluating novel treatments (12).

Role as service animal in healthcare (especially dogs)

Although dogs can induce respiratory allergy, we would like to cite the potential role of service dogs in certain clinical conditions at high risk of anaphylaxis such as surgical interventions in subjects with high allergic risk. Tew and Taicher (13) documented the first report of a service dog used to detect mast cell mediator release in a patient with mastocytosis. This service dog was used not only in a family-centered care model, but also as an additional perioperative monitoring tool to predict the potential occurrence of perioperative mediator release (13). We think that this type of "service" provided by dogs towards subjects at high risk of anaphylaxis should be strongly encouraged.

How pets can be "allergy enemies"?

Induction of allergic sensitization and cross reactivity of pet allergens

At present, eight main allergens characterized by different molecular weights, biochemical properties, and biological functions have been identified both in dogs and cats (14-17) (**table I**). Notably, the primary cat allergen (Fel d 1) is responsible for over 90% of allergic sensitizations to cats, while dogs can have various allergens involved, such as Can f 1, Can f 2, and Can f 5 (18,19). In Italy, the frequency of allergic sensitization to cats and dogs were reported to be 16% and 9%, respectively (2). In Northern Europe and the United States, a high frequency of pet ownership leads to a much higher risk of sensitization (up to 50%) (20). Conversely, China exhibits sensitization frequencies similar to those

in Italy (14.9% for dogs and 9.3% for cats) (21). As expected, it has been shown that the poly-sensitization to dog and cat allergen components is associated with a high likelihood of allergic symptoms during pet exhibition shows (22).

Allergens from furry animals belong to a restricted number of protein families, mostly lipocalins, albumins, secretoglobins, latherins or cystatin-A (4). Animal dander contains cross-reactive molecules and current efforts aim at defining species-specific allergens with high diagnostic sensitivity (4).

The degree of cross-reactivity between animal allergens varies by species. For example, dog-Can f 1 and cat-Fel d 7 show a moderate risk of cross-reactivity; dog-Can f 3, cat-Fel d 2, horse-Equ c 3 and domestic pig-Sus s 1 have a high risk of cross-reactivity with other serum albumin; dog-Can f 6, cat-Fel d 4, horse-Equ c 1, guinea pig-Cav p 6 and mouse-Mus m 1 demonstrate a moderate cross-reactivity risk with some lipocalins; dog-Can f 8 and cat-Fel d 3 pose a moderate risk; horse-Equ c 6 and horse-Equ a 6 present a high risk (15). The cross-reactivity of animal allergens causes important difficulties both from a diagnostic and therapeutic point of view, for example in the choice of the allergen to treat with AIT. These problems can be mitigated using the Component-Resolved Diagnosis (CRD), which allows to discriminate genuine sensitizations from cross-sensitizations.

A particular condition is the pork-cat syndrome in which patients who are allergic to cat epithelium develop symptoms of allergy after the ingestion of pork meat. Primary sensitization is presumed to be caused by inhaled Fel d 2 (cat serum albumin), with predominantly respiratory symptoms and subsequent reactions to pork due to cross-reactivity between Fel d 2 and pork serum albumin (Sus s 1) (23). Moreover, it has been shown that some cat immunoglobulins carry alpha-GAL and are believed to play a role in sensitization to this allergen (24, 25)

Mild to severe respiratory symptoms

Studies in cat allergy using controlled methodology of exposure (*e.g.*, natural exposure cat rooms, allergen exposure chambers) have been very useful to demonstrate short- and long-term effects of cat allergen inhalation, thereby providing a more holistic representation of cat allergen-induced respiratory symptoms (26). It is essential to underline that the percentage of pet allergens carried on small particles (about 0.5-2 μm) becomes easily airborne, under normal domestic ventilation, and it constitutes the main material able to trigger respiratory symptoms, in sensitized patients (27). In fact, Zeidler *et al.* (28) demonstrated that common exposure to cat allergens results in significant small airways obstruction and hyperresponsiveness persisting for at least 23 hours. Beyond this timepoint, these changes were no longer detected by conventional physiologic measures.

Sensitization to dog is also an important risk factor for asthma in children. In fact, Kack *et al.* (29) showed that polysensitization to furry animal allergens and high IgE levels to the dog lipocalins Can f 2, Can f 4, and Can f 6 are associated with asthma severity in dog dander sensitized children. Molecular allergy diagnos-

Table I *- Main cat/dog allergens.*

Allergens source	Allergen	Biochemical family	Prevalence of specific IgE in patients (%)	MW (kDa)
Cat (*Felis domesticus*)	Fel d 1	Secretoglobin	> 90	18
	Fel d 2	Serum albumin	14-23	69
	Fel d 3	Cystatin	10	11
	Fel d 4	Lipocalin	63	22
	Fel d 5	Immunoglobulin	20-40	400
	Fel d 6	Immunoglobulin	-	800-1,000
	Fel d 7	Lipocalin	38	17.5
	Fel d 8	Latherin	19	24
Dog (*Canis familiaris*)	Can f 1	Lipocalin	50-90	23-25
	Can f 2	Lipocalin	22-35	19
	Can f 3	Serum albumin	25-60	69
	Can f 4	Lipocalin	35-59	18-23
	Can f 5	Kallicrein	31-76	8
	Can f 6	Lipocalin	56	27-29
	Can f 7	Nieman Pick type C2	10-14	14
	Can f 8	Cystatin	10-14	14

tics may thus help the clinicians to evaluate the impact of allergic sensitization on asthma morbidity (29).

Ubiquity of pet allergens

Cat and dog allergens should be considered as ubiquitous because they are found not only in indoor environments where these animals are kept, but also in other indoor private or public places where they have never been kept (27). Public spaces include nurseries, offices, hospitals, hotels, schools and means of public transport (*e.g.*, buses, cars, airplanes) (20). These indoor environments, contaminated by pet allergens, are able to induce allergic sensitization in susceptible individuals and trigger respiratory symptoms in already highly sensitized subjects (30). In fact, in these contaminated environments, especially schools, the amount of pet allergens is higher than threshold values generally recognized as sufficient to induce sensitization (1 μg of allergen/g) or trigger respiratory symptoms (8-10 μg of allergen/g) for dust (31). In developed countries, the consequence of pet allergen ubiquity is a persistent stimulation of airways like the one induced by dust mite, that may increase the risk of allergic sensitization either directly or by a cross-reaction mechanism involving albumins and lipocalins (15, 32).

Indirect exposure

Accumulation of pet allergens in indoor environments without animals has been demonstrated to correlate with the number of visitors owning a pet or with those who are in regular contact with these animals. Therefore, the higher the frequency of pet ownership in a community, the higher the presence of pet allergens in apparently pet-free spaces (33). We and others have shown that clothing and other items, such as human hair, constitute a means of transferring pet allergens into pet-free indoor environments (34, 35).

As previously described, the percentage of pet allergens carried on small particles (about 0.5-2 μm) becomes easily airborne under common domestic ventilation and it constitutes the real risk either for allergic sensitization or, in some circumstances, exacerbation of symptoms in already sensitized patients (27). However, it has been shown that the indirect modality of exposure to allergens of domestic animals can also include other species, such as rabbits (36) or horses (37, 38).

Cross reactivity between Can f 5 and human prostate-specific antigen (PSA)

Can f 5, a newly described dog allergen, is a prostatic kallicrein, an androgen-regulated protein expressed in the prostate and detectable only in male dogs (39). Some studies have shown that the frequency of a prevalent or exclusive allergic sensitization to Can f 5 is high, ranging from 37% to 52% (40). A high rate of allergic sensitization to Can f 5 has been recorded in the North East of Italy. Specifically, 69.02% of dog-sensitized patients showed IgE against Can f 5, and 57.92% were exclusively sensitized to Can f 5 (41). Moreover, we have also shown that mono-sensitization to Can f 5 is strongly associated to a prevelent esposure to male dogs, compared to female dogs (42). A moderate potential for cross-reaction between Can f 5 and human Prostate Specific Antigen (PSA), contained in human semen, has been demonstrated, and this cross-reaction may be clinically significant (although very rare) after sexual intercourse in women allergic to dogs (43, 44).

Inducers of anaphylaxis

Pets have been reported to induce anaphylaxis after contact or inhalation of allergens (45, 46), as well as following a bite (47, 48). Anaphylaxis is more frequent in the literature after laboratory animals' bites (mostly mice and rats), compared to pets (49, 50). Recently, an anaphylactic shock due to a slow loris' bite has been described (51). Slow loris is a venomous monkey, sometimes kept as a pet, that combines its brachial gland exudate (which serves as a defensive tool) with saliva and bites to deliver the venom (51). Interestingly, homology has been shown between this brachial gland protein and the main allergen of cat dander (52). Laboratory data demonstrate that Fel d 1 may satisfy some criteria to be considered a toxin and thus suggesting the possibility that cat-induced reactions could also include a form of toxic reaction (53). A similar model is provided by hymenoptera stings, possibly leading to allergic or non-allergic (*e.g.*, toxic) severe reactions (54).

Occupational asthma

Allergies to common pets (or other furry animals) may also occur in some occupational settings where workers are intensively exposed to animal dander during most of their working time (55, 56). We have also described a case of respiratory allergy induced by an unusual occupational exposure to rabbit in a part-time magician (57). In fact, a few minutes after the rabbit was pulled out from the top hat, the patient experienced the onset of upper and lower airway symptoms, and in some occasions, he was forced to stop the show and to use short acting $\beta_2$agonists and intramuscular steroids (57). In case of contact with domestic and non-domestic animals, workers should apply prevention measures to avoid the passive transport of allergens from workplaces to private homes, for the consequent risk of allergic sensitization of family members (58).

Animals as pests

Among animals defined as "pests", cockroaches and rodents (mouse and rat) represent the most common cause of airway allergic sensitization and bronchial asthma worldwide (2).

It has been shown that residence in poor and urban areas, exposure to outdoor / indoor pollutants and tobacco smoke, poverty, material hardship, poor-quality housing, and differences in health care quality contribute to increase pest-related allergic sensitization and asthma morbidity (2).

Liccardi *et al.* (59) found that, although their prevalence in Italy, is lower compared with other countries, it is not negligible for cockroaches (8%) while for mouse and rat is rather low (3% and 1% respectively). The risk factors for sensitization to pests, in Italy, are completely comparable to those found in other geographical areas (59).

Modest efficacy of Allergen Immunotherapy (AIT)

Liccardi *et al.* (60) conducted an online survey encompassing both manufacturing companies and allergy centers evenly distributed across Italy. The trends of pet AIT prescriptions, over the last decade, were notably higher for cat allergy, compared to dog. Clinical efficacy is reported as more favorable in patients receiving cat allergenic extracts than those receiving dog allergenic extracts.

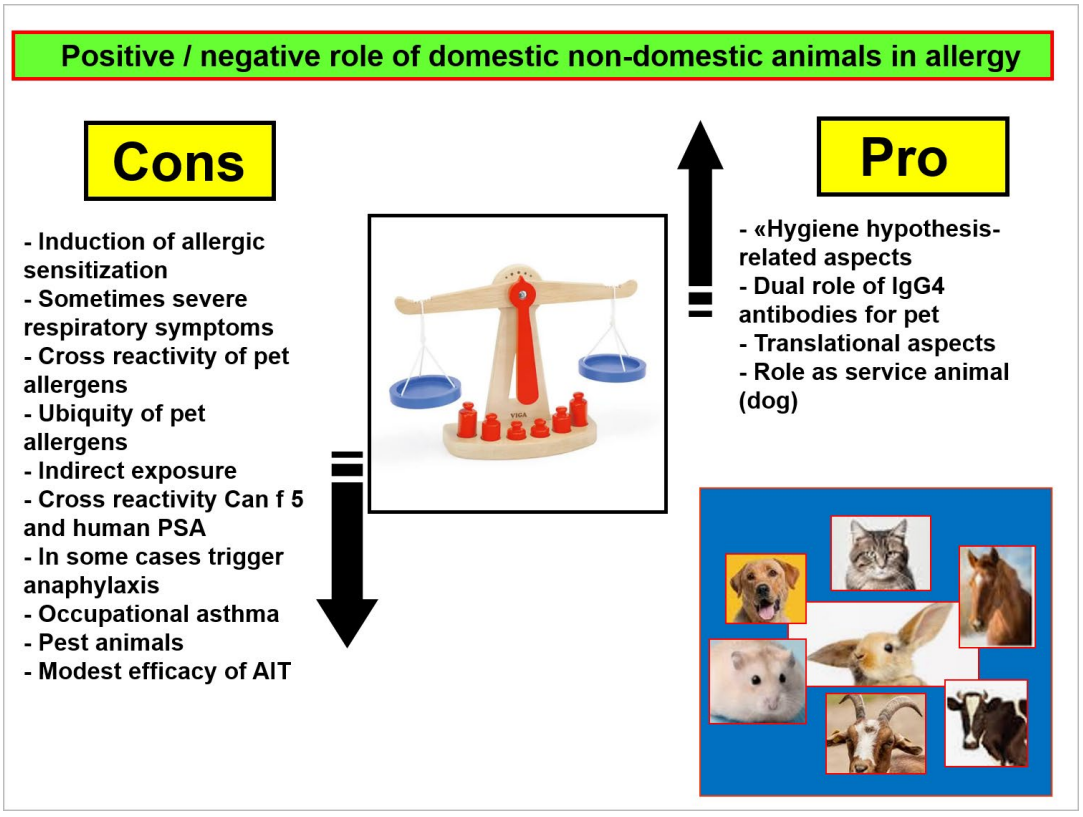
The limited standardization of allergenic materials, especially for dogs, remains the primary factor contributing to the overall unsatisfactory clinical efficacy of traditional AIT, AIT with modified allergens, as well as intralymphatic allergen-specific immunotherapy (ILAIT) (55, 56, 61).

Concluding remarks

The data presented in this review show that, from a strictly allergological point of view, the "negative" aspects resulting from exposure to domestic / non-domestic animals outweigh the "positive" aspects (**figure 1**). Different negative aspects (such as the transmission of infectious agents, invasiveness due to overpopulation, *etc.*) or positive ones (such as service or pet therapy animals, *etc.*) were obviously not considered. It has been well established that allergic sensitization to animals, particularly, but not only, to dogs and cats, is a complex puzzle influenced by many different factors related to individuals, living environments, modes of exposure, and the animals themselves. Considering the high percentage of atopic subjects in the general population and the importance of maintaining the human-animal relationship for ethical, behavioral, social, and psychological reasons, among others, it is up to humans to seek new ways to balance the pros and cons of pet relationship.

There is a clear need for further studies encompassing prevention aspects, standardization of pet allergens, improvement of the efficacy of various AIT modalities as well as new immunological

Figure 1 *- Positive and negative role of domestic non-domestic animals in allergy.*



approaches (*e.g.*, the use of anti-Fel d 1 monoclonal antibodies in humans, immunization of cats to induce neutralizing antibodies against Fel d 1, and feline diet with an egg product containing anti-Fel d 1 IgY antibodies). We hope that the findings of these research areas will allow the best possible coexistence between subjects at risk of allergy and domestic and non-domestic animals.

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None.

Contributions

All authors contributed equally to this work.

Conflict of interests

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Diagnostic accuracy of patch testing based on clinical response to contact allergen restrictions in allergic contact dermatitis

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Impact statement

The PT had moderate diagnostic accuracy. A positive test could be useful as a screening, but it does not confirm the clinical relevance of a contact allergen.

Summary

Background. *Patch testing (PT) is used to identify substances that cause allergic contact dermatitis (ACD). However, the clinical effects of allergen restrictions following PT have not been thoroughly investigated. This study aims to assess the diagnostic accuracy of PT in patients suspected of having ACD.* ***Methods.*** *Prospective study. PT was performed in patients with clinical diagnosis of ACD. Patients with a positive PT (case group) had a strict restriction of the suspected substance for one month. In patients with negative patch testing (control group), allergen restriction was based on clinical history. Clinical reduction (CR) of at least 50% in disease activity (CR50%) after one month of allergen restriction was considered clinically relevant. Total control was defined as clinical reduction of at least 90% (CR90%).* ***Results****. Of 400 patients, 66.2% had a positive PT. The sensitivity of PT according to CR50% was 84%, specificity 47%, PPV 53%, and NPV 81%. Only 10.5% of patients achieved CR90%.* ***Conclusions.*** *The PT had moderate diagnostic accuracy. It could be useful as a screening, but a positive result should be confirmed with controlled allergen restriction. The low number of patients who achieved a 90% CR requests to reconsider the allergens included in PT and the mechanistic processes of the disease.*

Introduction

Contact dermatitis is a common, noninfectious inflammatory skin condition resulting from direct or indirect skin contact with exogenous substances. It typically is revealed by the appearance of lesions, usually eczema, following exposure to various substances (1-3). Contact dermatitis is often divided into irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD). ICD is a nonspecific skin response to direct chemical skin damage involving the release of inflammatory mediators, while ACD is a hypersensitivity reaction to allergens, including immune responses (4). It has been observed that some professions, due to the greater

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contact with certain substances, carry a higher risk of developing ACD. For instance: construction workers, hairdressers, and healthcare professionals, develop ACD secondary to potassium dichromate, PPD, and rubber chemicals, respectively (5, 6).
Diagnosis obstacles arise in establishing the contribution of exogenous substances in the skin disease. The clinical relevance of a substance in ACD can be defined in different ways, but in general we must consider clinically relevant those substances that worsen or cause a patient's symptoms upon exposure and symptoms improves when contact with the substance stops. Patch testing (PT) has been positioned as the gold standard test to establish the diagnosis of ACD and to identify suspects substances potentially associated with the disease (7, 8). Most studies have evaluated the diagnostic performance of PT based on clinical history but in this way is not possible to assess correctly false positives (positive PT without clinical relevance) and this could explain the wide variation in diagnostic performance observed for the PT in the different studies (9); sensitivity ranges from 50-90% and specificity from 40-90% (10, 11). Additionally, several studies suggest a high frequency of positive PT (20-40%) in the general population, which can be explained by an underdiagnosis of the disease or a high frequency of false positives (12, 13).
Clinical guidelines suggest that once the identification of a suspicious substance producing the ACD is made with PT, strict restriction must be carried out (14-17). If the suspected substance is the cause of the problem, with restriction measures there should be significant control of the symptoms, however, there are currently no specific clinical scales to assess ACD activity. This article evaluates PT performance by comparing the ACD activity before and after allergen-restriction using the skin extension and skin severity as clinical parameters. This prospective evaluation offers several advantages over other studies allowing assessment not only of PT´s diagnostic accuracy but also the clinical impact of allergen restrictions in ACD. Additionally, in this study we propose a clinical scale to measure ACD severity.

Materials and methods

Study design

Prospective study with case and control assignation. The main objective of the study was to evaluate the diagnostic performance of the PT in ACD patients. Participants with a positive PT (case group) had a strict restriction of the suspected substance for one month and not change in topical or systemic therapy during evaluation period. Patients with negative PT (control group), allergen restriction was based on clinical history. ACD diagnosis was established by dermatologists or allergists. The gold standard for evaluating PT diagnostic performance was the clinical response after one month of restriction (**figure 1**).

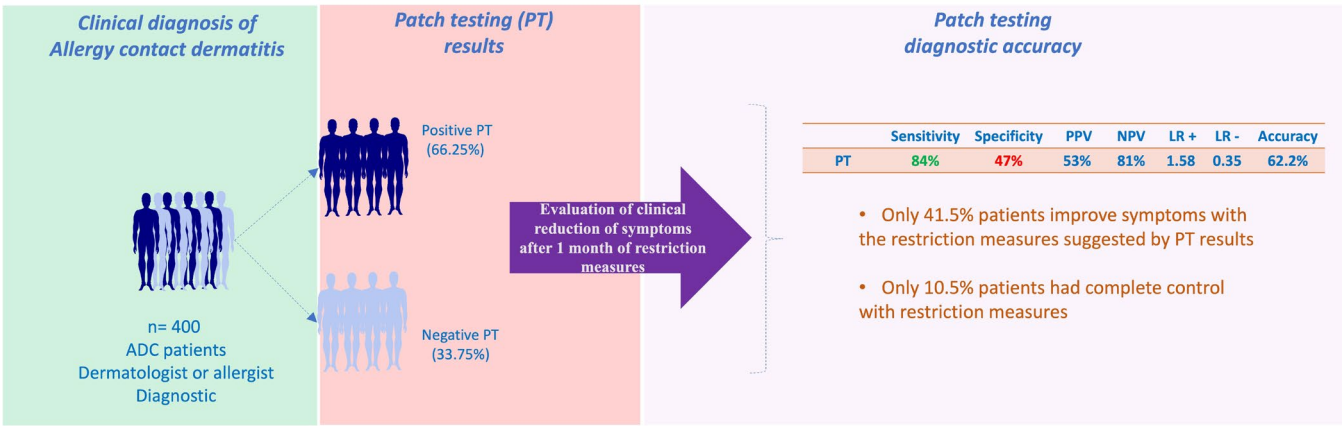
Patient selection

The recruitment of patients was carried out on three centers located in Colombia. Patients with no age limit were included. Patients should not be taking drugs that could affect the interpretation of the test. Patients with other skin conditions were excluded.

Patch testing

The PT was performed in accordance with international recommendations using a standard series (LA-100) from "Chemotechnique diagnostics" laboratory (**table IS**). Forty allergens, enclosed in plastic chambers were applied to each patient back. After forty-eight hours, the patches were removed for a first reading. The second reading was performed at 96 hours. A positive test was determined based on the results of the second reading (15, 16,

Figure 1 *- To evaluate the diagnostic accuracy of patch testing (PT) in patients with clinical diagnosis of allergy contact dermatitis (ACD) we use the clinical response to contact allergen restriction as comparator.*



18). To mitigate measurement biases, a consensus on interpreting the patch tests was reached during an initial meeting with all investigators. Each test was independently reviewed by at least two researchers, with discrepancies resolved by a third researcher.

Assessment of clinical response

To our knowledge, there is not a specific scale to evaluate the activity of the ACD. We evaluated clinical response of allergen restrictions using three parameters; extent of affected skin, pruritus intensity, and investigator global assessment (IGA); the assessment tool is presented in **table I**. This evaluation was carried out one day before and 30 days after allergen restriction. We considered significant clinical reduction in symptoms (CR), a decrease of at least 50% (CR50%) in the assessment tool.

On the first visit, a photographic record of the patient's entire skin surface was captured. Weekly, patients documented their skin's evolution through weekly photographs. Throughout the one-month follow-up, patients were recommended to use only skin hydration as active treatment to assess the clinical response to the restriction. If the intensity of the lesions was not tolerated and required the use of additional topical treatment, the primary outcome was measured the last day before initiating pharmacotherapy.

Considering that there is not specific clinical tool for assessing ACD, we conducted an exploratory analysis to evaluate the correlation between the proposed assessment tool in this study, the quality of life according to the dermatological index of quality of life (DLQI) and the Atopic Dermatitis Disease Control (ADCT).

Restriction measures

All patients underwent a training to identify potential sources of exposure for each substance. Patients could contact the centers to resolve any questions during the restriction month. The objective was to achieve a total restriction during the study period, however this is not always feasible, so at the end of the month the patients were asked to rate from 0 to 100% the rigor of the restrictions to each allergen compared to the period before the study started.

Statistical analysis

Considering the study's objective, we opted not to perform matching between case and control groups. Based on the frequency of exposure reported in previous studies (1, 12, 13) and case definition, at least 80 patients in each group were sufficient to assess diagnosis performance. We pre-established a goal of 400 patients for a greater precision of the results.

Results of the index test (PT) and the reference standard (Contact allergen restriction) were classified in a 2 × 2 contingency table. From this table, standard measures of discrimination, including sensitivity, specificity, predictive values, and likelihood ratios, along with unitary measures (correct classification accuracy), were calculated with 95% confidence intervals. Patients with missing data regarding PT results or the clinical response to the restriction measures were excluded.

Bioethical considerations

The study protocol was approved by the institutional ethics committee (code IN57-2021 # acta 177 Hospital "Alma Mater de Antioquia" and University of Antioquia) and is in line with the Helsinki declaration. Each participants signed to indicate their informed consent.

Results

General characteristics

Of 418 who accepted to participate, a total of 400 patients were included (**table II**). 10 patients were excluded because follow-up

Table I *- Evaluation score.*

	Extension	Pruritus	IgA
CR90%	Reduction ≥ 90%	Reduction ≥ 90% or less than 3 points in pruritus intensity (0 to 10 points).	Reduction ≥ 90% or less than 1 point
CR50%	Reduction ≥ 50%	Reduction ≥ 50% or less than 3 points in pruritus intensity (0 to 10 points).	Reduction ≥ 50% or less than 1 point
No control	Reduction was under 49%	Reduction ≤ 49% (or increase)	Reduction ≤ 49% (or increase)

Extension, pruritus, and investigator global assessment (IGA) were evaluated before and after allergen restriction; criteria for clinical reduction 50% (CR50%) and clinical reduction 90% (CR90%) were based on these three parameters. Pruritus was evaluated with the question "From 0 (none) to 10 (high intense) How was itch in the past 24 hours?". IGA points were defined: 0 clear: NO inflammatory signs of Contac dermatitis (no eczema, no erythema, no induration/papulation, no lichenification, no oozing/crusting). Post inflammatory hyperpigmentation and/or hypopigmentation may be present. 1 Almost clear: Barely perceptible eczema erythema, barely perceptible induration/population, and/or minimal lichenification. No oozing or crusting. 2 Mild: slight but definite eczema, slight but erythema (Pink), slight but definite induration/papula, and/or slight but definite lichenification. No oozing or crusting. 3 Moderate: Clearly perceptible eczema, clearly perceptible erythema (dull red), clearly perceptible induration/papulation, and/or clearly perceptible lichenification. Oozing or crusting may be present. 4 Severe: Marked eczema, marked erythema (Deep or bright red), marked induration/papulation, and/or marked lichenification. Disease is widespread in extent. Oozing or crusting may be present.

was not possible and 8 were excluded after identifying a second skin disease that could affect the interpretation of the results. The female gender was predominant (67.8%); most of the patients were older than 18 years (n = 378, 94.5%) (**table II**). Most patients had an office work (47.25%). A total of 190 (47.5%) patients had lesions in skin areas of high clinical and emotional impact (face, hands, or intimate area); 91 (22.75%) patients with lesions in these high impact areas had also lesions in other body sections. In most patients the PT was done during the first year of the symptom's onset.

Table II *- General characteristics.*

	n = 400 (100%)
Females	271 (67.8%)
Age (years)	48 min 8 max 90
< 18	22 (5.5%)
19 To 40	115 (28.75%)
41 to 60	190 (47.5%)
> 60	73 (18.25%)
Asthma	24 (6%)
Rhinitis	123 (30.7%)
Chronic urticaria	0
Atopic dermatitis	0
Workplace	
Home	70 (17.5%)
Office	189 (47.25%)
Health	13 (3.25%)
Construction	7 (1.75%)
Rural work	24 (6%)
Cosmetic work	18 (4.5%)
Other	79 (19.75%)
Affected body area*	
Face	72 (18%)
Hands	86 (21.5%)
Intimate area	32 (8%)
Other	301 (75.25%)
Disease duration before patch test (years)	
1 year	243 (60.75%)
1 to 5 years	83 (20.75%)
More than 5 years	74 (18.5%)

Continuous variables were presented as median and range (minimum, maximum). *Some patients (22.75%) had more than one affected body area. Unemployed patients were categorized in the area where they spent most of their time.

The most frequent potential allergen triggers according to clinical history were nickel (58%), palladium (43%), and fragrances (18%). Some patients associated certain substances from work (23%) or recreational activities (18%).

Patch testing results

A total of 265 (66.25%) patients had a positive PT. In 142 (53.6%) patients more than one allergen was positive in the PT. Nickel was the most prevalent followed by palladium (**table III**). We explore the relationship between workplace and sensitization patterns but there was not significant association with any of the most common allergens.

Clinical response

Of the 265 (66.25%) patients with positive PT, 166 (41.5%) had a CR50% after performing the restriction measures and in 140 of these patients the allergens were detected with the PT (test sensitivity 84%, 95%CI 77.9% to 89.5%). Twenty-one patients with negative PT had clinical improvement following allergen restrictions based on clinical history and five patients with negative PT who did not carry out an adequate restriction despite the recommendations had a spontaneous improvement. A total of 234 (58.5%) patients had no improvement with restriction

Table III *- Patch testing results.*

	n = 400 (100%)
Positive path test	265 (66.25%)
Monosensitization	123 (46.4%)
Polysensitization	142 (53.6%)
Negative path test	135 (33.75%)
Most common allergens according to the patch test	
Nickel sulphate	110 (41.5%)
Palladium	92 (34.71%)
Fragrance mix	25 (9.43%)
Thimerosal	23 (8.67%)
Cobalt chloride	18 (6.79%)
Neomycin	13 (4.9%)
Potassium dichromate	12 (4.52%)
Methylisothiazolinone	12 (4.52%)
Methyl-dibromo glutaronitrile	11 (4.15%)
Formaldehyde	10 (3.7%)
Others	118 (44.52%)

From the 40 contact allergens probed, only 5 have positivity in at less 5% of patients.

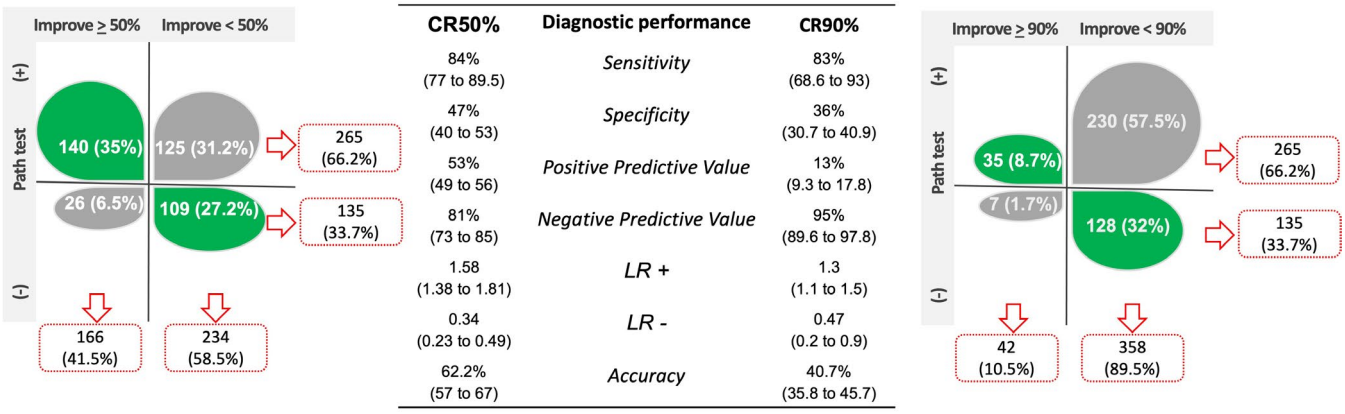
measures; in 109 of them the PT was negative (47% specificity 95%CI 40% to 53.1%).
When evaluating compliance with the restriction measures, there were no statistically significant differences between those who clinically improved *versus* those who did not improve clinically in the case group (improvement 83%, 95%CI 75 to 94% *versus* no improvement 81%, 95%CI 72 to 91% p = 0.7) nor in the control group (improvement 83% 95%CI 75% to 94% *versus* no improvement 81%, 95%CI 72 to 91% p = 0.7).
According to CR50%, the PT correctly classified 249 patients (diagnostic accuracy 62.2%) (**figure 2**). The positive and negative predictive value were 53% and 81% respectively. A positive PT increases the probability of CR50% after restriction (OR 4.6 95% CI 2.8 – 7.6).
According CR90%, the PT had lower diagnostic performance; only 42 (10.4%) patients reached this level of control.

Exploratory comparison of CR assessment tool, DLQI, and ADCT
When we compared the results of CR score and DLQI, 83% of patients with DLQI over 10 points had no control according to CR score; 71% of patients with DLQI under 10 points had CR50% according to CR score.
When we compared the results of CR score and ADCT, 89% of patients with ADCT over six points had no control according to CR score; 68% of patients with ADCT under six points had CR50% according to CR score. This exploratory evaluation suggests a good sensitivity of CR score to evaluated in ACD patients' different domains of clinical control.

Discussion and conclusions

Since its description by Jadassohn (18), done more than 100 years ago, PT is considered the gold standard test for ACD diagnostic (19). The PT is performed using a series of allergens, which means that multiple tests are performed at the same time, which increases the risk of false positives and decision making difficult regarding which restraint measures are relevant in each patient (12, 19, 20). Different studies have evaluated the diagnostic accuracy of PT but to our knowledge this is the first prospective study evaluating diagnostic accuracy based on the clinical result of restriction measures.
Our study presents interesting results: 1) the sensitivity of the test was moderate and according to clinical impact we found that the specificity of the test is low, with a high number of false positives. 2) Many patients achieved partial improvement (CR50%) after restriction measures but few achieved complete improvement (CR90%). 3) A potentially specific clinical scale is proposed to evaluate disease activity in patients with ACD.
Clinical relevance of PT must always be carefully evaluated because positive reactions may indicate sensitization but not significant relation with the disease. The request for unnecessary restrictions can have a high burden on the quality of life of patients. Studies from unselected population from North American and European found that the median prevalence of positive PT to at least one contact allergen was 21.2% for North American and 27% for Europe (range 12.5% to 40.5%) with a higher prevalence in women (35.5% *vs* 17.1%) (12, 13). The interpretation of these studies in the light of our results seems to indicate that the PT has a high frequency of false positives, which explains its high sensitivity but low PPV. Therefore, PT alone cannot confirm the diagnosis of ADC and its clinical relevance needs to be evaluated. However, there is no global agreement on what clinical relevance is in ACD (21); the clinical relevance has been analyzed mainly retrospectively based on the clinical history, environment, work, hobbies of the patient, and identification of the positive allergen in these contexts using PT (22), but little has been studied pro-

Figure 2 *- Diagnostic performance of patients according to clinical reduction ≥ 50% (CR50%) or ≥ 90% (CR90%).*



The parenthesis in table are 95% confidence interval of each parameter; LR: likelihood ration.

spectively regarding the identification and elimination of the allergen and the subsequent evaluation of the clinical response, which constitutes the main strength of this work. Gallo *et al*. (23) evaluated through telephone calls the remission of contact dermatitis in patients who carried out restriction measures based on the result of PT. The authors report a high rate of remission or significant improvement (85.2%, 431/506), much higher than that observed by us. However, the authors performed avoidance measures in only 506 patients out of 1,397 who had a positive test, based on the clinical probability that the PT was relevant, confirming our observation that the PT is useful as a screening test, but a positive result does not confirm clinical relevance. PT allows us to identify substances potentially related to the clinical manifestations of our patients, however, multiple factors can induce false positives or false negatives (*e.g*., new allergens not included in the test; positive sensitizations to old exposures currently not relevant, *etc*.). Therefore, the PT should be accompanied by a detailed anamnesis and an evaluation of the possible substances to which the patient is exposed to identify what additional substances should be included in the test that are not present in the standard battery. These points highlight the importance of carrying out controlled avoidance measures to define the clinical relevance of the substances identified with the PT.

Bearing in mind that there is no validated specific clinical tool for ACD, we used three parameters to talk about clinical relevance. According to these parameters, patients improved with restriction measures (CR50%), but few achieved complete control (CR90%). Considering that contact dermatitis is defined by the appearance of lesions upon exposure by a contact, the low rate of complete control could be explained because the patients did not strictly carry out the avoidance measures or maybe, we must reconsider what we understand about the disease mechanism. Traditionally, it has been proposed that the mechanism for ACD is caused by a type IV delayed hypersensitivity reaction in the skin and is initiated when an allergen enters the skin and activates the innate and adaptive immune system cells (24). However, experimental studies suggest that depending on the allergen multiple mechanisms exists in ACD, and inflammatory profiles could be present in ACD patients even without contact exposition (24, 25). These results imply that the PT could have different diagnostic performance according to the type of allergen exposed and the underlying mechanism (1, 26).

Recent advances in the understanding of contact dermatitis mechanisms suggest that ACD is more complex than previous thought (4, 27). Our results indicate that despite strict restriction, complete remission occurs in a minor number of patients with ACD diagnosis. A high number of patients reach a CR50% but less than 20% of patients reach CR90%. This fact can have two explanations; 1) the PT series that we use does not detect all the allergens involved in the patient's illness. 2) Contact allergens can aggravate the disease but are not always a decisive factor in its persistence, indicating underlying skin damage that can persist even after removing environmental triggers. Despite the fact that this second hypothesis has little evidence and goes against what we popularly accept in ACD, it is in line with the new knowledge about the pathogenesis of the disease (4, 27) and it is similar to what we now know in other skin diseases like atopic dermatitis (28).

ACD in children has been scarcely studied and in general evaluations have been done in patients with atopic dermatitis. Similar as what has been reported in other studies, we observed that the prevalence of ACD diagnosis was higher in patients over 30 years. We explored if there was difference in diagnostic performance of PT in patients under 15 years but there was not significant difference to what we report in adults.

Rajagopalan and Anderson demonstrated a benefit in most domains of the DLQI in a group of contact dermatitis patients who underwent the PT compared with a group who did not (29). They observed that even in patients with a negative test, ruling out the causality of common contacts can lead to an improvement in quality of life. However, in this study it is not clear the clinical impact that restriction to suspected substances has on quality of life.

Our study has some weaknesses and strengths. The low frequency of sensitization and/or exposure to some tested substances makes their correct evaluation difficult. Additional series, patient materials or photopatch test could increase the sensitivity and relevance of the test, mostly in occupational cases. Nevertheless, we included a large number of patients, so we consider that the evaluation was adequate for most of the allergens tested. Additionally, patients were selected because they required a standard PT as a first evaluation because there was little likelihood that their ACD was photoinduced. A possible limitation of the study is the restriction time. We chose a month of avoidance considering the skin cycle (30); however, we cannot rule out that a longer period of time would be better to evaluate the clinical improvement. Despite we educated patients to contact allergen restriction measures, we could no guarantee 100% that all patients fallowed restriction measures all the time. However, considering the support network offered and the weekly contact with the clinical centers, we believe that the restrictions were stricter than what most patients do in real life. Other strengths of the study were its prospective design and the photographic evaluation that allowed us to objectively evaluate the changes reported by the patient. One of the limitations of the PT is the different interpretation of the results since it depends on the experience of the person doing the PT. To reduce this variance in the study, each test was interpreted by at least two clinicians trained in PT, so this potential measurement bias was controlled.

In conclusion, the PT can be useful to identify substances that aggravate ACD, however the high frequency of false positives makes it necessary to evaluate the relevance with adequate assessment

of allergen restriction. The low number of patients who achieved a clinical improvement greater than 90% makes it necessary to reassess the concepts of the disease regarding its pathophysiology.

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Contributions

JSC, MVL: methodology, project administration, resources, software, supervision, validation, visualization, writing - review & editing. LAR: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing - original draft, writing - review & editing. SDZ, JMM: conceptualization, data curation, formal analysis, investigation, methodology, resources, software, validation, visualization, writing original draft.

Conflict of interests

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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The autologous serum skin test predicts the response to anti-IgE treatment in chronic spontaneous urticaria patients: a prospective study

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Key words

Chronic spontaneous urticaria; autologous serum skin test; omalizumab; IgE; endotype.

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Impact statement

In patients with chronic spontaneous urticaria a positive autologous serum skin test is strongly associated with a good but delayed response to omalizumab.

Summary

Background. *Chronic spontaneous urticaria (CSU), characterized by recurrent itchy wheals and angioedema for > 6 weeks, is a quite common disease that may heavily impair the quality of life. Omalizumab, an anti-IgE mAb, has much improved the management of CSU but patients' response to the drug may vary and predictive markers are still largely missing. We investigated the predictive value of the autologous serum skin test (ASST) on omalizumab response.* ***Methods.*** *15 patients with severe CSU eligible for omalizumab treatment were prospectively studied submitting them to ASST and to complete blood count, D-dimer, anti-thyroid peroxidase antibodies, and total IgE measurement before the start of the treatment.* ***Results.*** *14/15 (93%) responded brilliantly to omalizumab at 3 months assessment. 7 responded in less than 1 month ("early responders") and 7 only after multiple administrations ("late responders"). Of 9 patients scoring positive on ASST, 7 (78%) were late, and 2 (22%) early responders to omalizumab (p = 0.021). Of 6 patients scoring negative on ASST, 5 were early omalizumab responders and 1 did not respond. The PPV and NPV of the ASST for a "late" response to omalizumab were 78% and 100%, respectively. Total IgE were significantly higher in early responders.* ***Conclusions.*** *Although larger prospective studies are needed to confirm these results, this study confirms previous retrospective investigations that the positive ASST appears to predict a slow response to omalizumab in CSU patients.*

Introduction

Chronic spontaneous urticaria (CSU), defined as the recurrent occurrence of itchy wheals often with angioedema for more than six weeks, is a common disease that may heavily impair the quality of life. More than 30 years ago, Grattan and co-workers (1) found that a significant proportion of CSU patients respond with a wheal-and-flare reaction at the site of the intradermal injection of a small amount of autologous serum (autologous serum skin test, ASST). This observation represented the first step towards a better understanding of the pathogenesis of chronic spontaneous urticaria (CSU) as an autoimmune disease. The clinical significance of ASST as well as its methodology and interpretation were reviewed and defined by task forces of the European Academy of Allergy and Clinical Immunology (EAACI) about 15 years ago (2, 3). The task forces stated that, although its neg-

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ative predictive value is high, the ASST should be regarded as a test for autoreactivity rather than a specific test for autoimmune urticaria, as it shows only moderate specificity as a marker for functional autoantibodies against IgE or the high-affinity IgE receptor (FcεRI) (2).

Omalizumab, an anti-IgE mAb, has been the second-line therapy for antihistamine-refractory CSU since 2014 (4). Despite its generally high efficacy, variability in patient response to this biological therapy has been evident since the start of its use, with most patients experiencing symptom control, either promptly or after several months of treatment, and a small subset showing only partial response or no response at all.

Although the ASST has been considered a possible marker of IgG-mediated (type IIb) autoimmune CSU along with the direct detection of IgE and FcεRI autoantibodies (5), no study so far investigated prospectively its possible predictive value in the light of omalizumab response. In the present study, we addressed the predictive significance of ASST for omalizumab response in patients with severe CSU.

Materials and methods

We performed a prospective study aiming to assess the predictive value of the ASST on the clinical response to omalizumab in patients with severe urticaria. To this end, 15 adult patients (aged between 18 and 75 years and with a disease duration ranging from 6 weeks to > 10 years) with severe CSU eligible for omalizumab therapy were recruited from the allergology outpatient clinics of the Department of Internal Medicine and Clinical Immunology and the Dermatology Department of Policlinico Umberto I in Rome between October 2022 and August 2023. The study participants, all refractory to second-generation antihistamines at higher than licensed dosage (4), signed an informed written consent to undergo the ASST before starting the biological treatment. The autologous serum skin test was performed and read following the current recommendations, and was classified as positive or negative (2, 3). UAS7 was assessed at baseline when it exceeded a value of 30 in all cases, and then monthly after the start of omalizumab treatment. Upon enrollment, patients underwent also complete blood count, as well as D-dimer, anti-thyroid peroxidase antibodies, and total IgE measurements.

Omalizumab was administered at a monthly fixed dose of 300 mg and had to be stopped in case of no response after 3 months of treatment, as per the current Italian legislation. Patients were classified as "early responders" if they showed a drop of UAS7 to < 16 one month after the start of the treatment, "late responders" if such drop of UAS7 was detected within 3 months of treatment, or non-responders if no significant change in UAS7 was detected after 3 administrations of omalizumab.

Results were analyzed SPSS version 27.0 and JASP version 0.18.1.0. A confidence interval of 95% was set, and correlations were considered significant at a P-value below 0.05. Specific tests such as Fisher's exact test, Student's t-test, PPV, NPV, and logistic regression models were employed as appropriate.

The study was approved by the Ethical Committee of the Policlinico Umberto I in Rome (ID 7097 prot. 0366/2023).

Results

Results are summarized in **table I**. Of the 15 patients studied, 14 (93%) exhibited a clear clinical benefit 3 months after the start of omalizumab treatment while 1 patient (7%) was classified as "non-responder". Of the 14 omalizumab responders 7 (46.5%) responded already after the first administration ("early responders"), and 7 (46.5%) responded only after multiple administrations, ("late responders").

Nine patients (60%) scored positive on the ASST, while 6 (40%) scored negative. Grouping patients based on omalizumab response, 5/6 ASST-negative individuals responded promptly to the drug, and 1/6 did not show any response, whereas 7/9 (77%) ASST-positive patients were "late responders" and 2/9 (22%) exhibited an "early" response (p = 0.021). The PPV and NPV of the ASST for a "late" response to omalizumab were 78% and 100%, respectively.

The "early response" group showed a mean total IgE value of 601 kU/L (IQR 458-813), while in the "late" response group mean total IgE was 50 kU/L (IQR 14.5-180) (p = 0.029). This result

Table I *- Summary table of the differences between the means of the two groups of patients divided according to the response to therapy.*

						95%CI for mean difference	
	t	df	p	Mean difference	SE difference	Lower	Upper
D-dimer (ng/ml)	1.366	12	0.197	658.571	482.291	392.251	1.709.393
Total serum IgE (kU/l)	2.348	12	0.037	415.171	176.815	29.924	800.419
Eosinophils (cells/µl)	0.322	12	0.753	31.429	97.485	180.974	243.831
Anti-TPO IgG (UI/ml)	-1.629	12	0.129	-33.286	20.438	-77.817	11.245

Student's t-test.

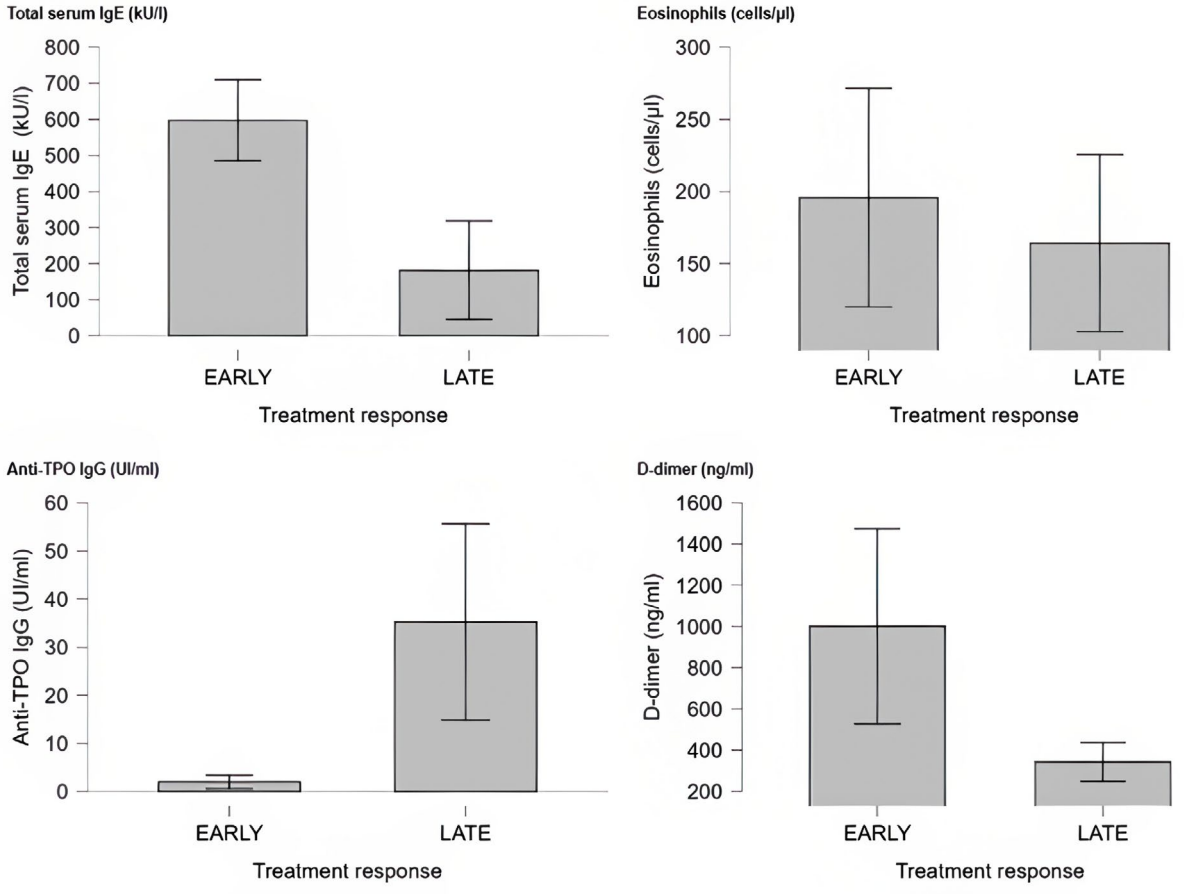
was confirmed also by Student's t-test (p = 0.037) (**table I**). The other continuous variables investigated did not differ significantly between the two groups (**figure 1**).

The only patient who did not show any response to omalizumab scored negative on ASST, and showed a low total IgE level (31.6 UI/ml), as well as negative D-dimer, TPO IgG, and a normal Eosinophil count.

Discussion and conclusions

The present prospective study confirmed the efficacy of Omalizumab in the treatment of CSU (6, 7), and the association between elevated total IgE and a faster response to the therapy (8). In a recent review article, Fok and coworkers (9) remarked that the data of the link between ASST and omalizumab response are still inconsistent, and that prospective studies are needed to confirm it. We observed that most of our patients showing a wheal-and-flare skin response upon the injection of autologous serum were eventually classified as "late responders" to omalizumab, thus fully confirming in a prospective fashion previous observations by Gericke *et al.* (10) who found that ASST-positive CSU patients were 5.5 times more likely to show a slow response to omalizumab than ASST-negative patients, and by Nettis *et al.* (11), whose patients with a positive autologous serum skin test (ASST) were significantly more likely to be "slow responders" to omalizumab treatment. Similar results were also obtained by Chinese researchers in their population (12). Interestingly, only a minority of our "early" omalizumab responders scored positive on ASST. Since an early response to anti-IgE therapy has been associated with type I ("autoallergic") CSU, which is most likely characterized by the presence of IgE specific for several potential auto-allergens, our findings seem to suggest that the ASST does not score positive in the presence of circulating, autoreactive IgE. In contrast, a positive ASST seems to identify patients with IgG-me-

Figure 1 *- Graph of the different means with confidence interval of the continuous variables examined in this study in the two groups divided by treatment response.*



diated autoimmune (type IIb) CSU, characterized by IgG specific for IgE or for high-affinity IgE receptor, in whom the late response to omalizumab is possibly based on a slow downregulation of the IgE-receptor expression on mast cell surfaces (13). The observation of a positive ASST in some early responders might be due to the co-occurrence of auto-reactive IgG and IgE (14). Another interesting point is that the only patient refractory to omalizumab treatment scored negative on the ASST and showed low total IgE, thus showing a discrepancy between the predictive value of these two biomarkers.

The prevalence of positive ASST has been extremely variable throughout the various studies of CSU (15) possibly due to differences in populations studied, and positive ASST results have been frequently recorded also in patients with conditions other than CSU (16). Nonetheless, one point that has been always clear is that the ASST scores positive only in a proportion of CSU patients and that it only partially overlaps with the direct measurement of IgG autoantibodies to the high affinity IgE receptor or IgE (5) or with the basophil histamine release assay (17). Now, in the light of the recent findings about the different endotypes of CSU, these older "strange" observations appear much clearer. Altogether, our findings suggest that the ASST maintains its clinical validity both in detecting patients with a probable IgG-mediated autoimmune pathogenesis and in predicting a late response to omalizumab.

The main limitation of this study is certainly its reduced sample size. Nonetheless, all the main data, including the significantly higher total IgE levels in early responders, the proportion of patients scoring positive on ASST, and the proportion of positive omalizumab responses observed were perfectly in line with those found in most previous studies, thus substantiating our conclusions. Further, although larger prospective studies will undoubtedly be necessary to confirm our observations, the results of this study confirmed those of other observational retrospective ones (10-12) and were so clear-cut to demonstrate statistically significant differences even in the presence of a population as small as 15 individuals.

Fundings

None.

Contributions

AP, FV, EP, MV: data curation, formal analysis, investigation, writing - review & editing. RA: conceptualization, methodology, project administration, writing - original draft, writing - review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Clinical spectrum of patients diagnosed with childhood mastocytosis: a retrospective single center experience

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Key words

Cutaneous mastocytosis; urticaria pigmentosa; mast cell; tryptase; Darier's sign.

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Impact statement

Although symptoms are difficult to control in cases of pediatric mastocytosis and are sometimes associated with significant morbidity, in most cases the symptoms are limited to the skin and follow a mostly benign course in children.

Summary

Background. *Pediatric cutaneous mastocytosis patients diagnosed and followed up by our specialist were enrolled in this study, and clinical and laboratory evaluations were retrospectively analyzed from patients' archived files.* ***Methods.*** *Patients, who applied to the Division of Pediatric Allergy And Immunology Unit of a University Training and Research Hospital between 01 January, 2010 and April 28, 2021, were enrolled in this study.* ***Results.*** *Of the 33 patients included in the study, 11 (33.3%) were female and 22 (67.7%) were male. The median age of onset of the patient's complaints was 7 (0-60) months. The median age at diagnosis was 11 (2-64) months. Their complaints' median regression age was 54 (6-192) months. Resistant clinical findings were followed in 13 (39.4%) patients. Itching, redness, gastrointestinal symptoms, and maculopapular eruption were the most common complaints. The rashes were mostly polymorphic and larger than 1 cm. Heat was the most common trigger. Darier's sign was positive in 97% of the patients. Antihistamines were the most commonly used drug for prophylaxis and treatment. The autoinjector prescription rate was 24.2%.* ***Conclusions.*** *Quality of life was mildly affected in 48.5% of the patients based on the CDLQI scores. Thus, patients should be followed up through adolescence for the development of systemic signs and symptoms.*

Introduction

Mastocytosis refers to a rare group of diseases. It is a disease characterized by an increase in abnormal morphology of mast cells in one or more tissues (1, 2). Mastocytosis usually occurs due to activation mutations that affect the c-Kit tyrosine kinase receptor and give function to this receptor (2). Patients diagnosed with mastocytosis are classified as cutaneous mastocytosis (CM) and systemic mastocytosis (SM) based on the involvement of the affected organ. There are three main groups of childhood mastocytosis (CHM), which are mostly seen in the childhood age group and progress only with skin involvement: urticaria pigmentosa (UP)/maculopapular cutaneous mastocytosis (MPCM), solitary mastocytoma and diffuse CM (1, 3).

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Childhood mastocytosis can be seen both in the pediatric age group and in adults if it does not regress with age. 80% of patients are diagnosed in the first year for the pediatric patient group. Generally, the findings are self-limiting and almost completely recovered in adolescence (4, 5). Mastocytosis in children follows a bimodal distribution. The frequency of disease peaks in the first three years, followed by a decline, and a second much smaller increase in CHM cases is observed after age 15 (2, 6). Although there is a slight predominance of males in pediatric cases, it is more likely to occur in females after puberty. There may be a familial history of mastocytosis, but there is no history in family members in most cases (7, 8).

This study aimed to retrospectively evaluate the demographics, clinical characteristics (type of rash, size, age of onset of the lesion, complaints, *etc*.), laboratory characteristics, and treatment plans of patients diagnosed with CHM.

Materials and methods

Between 01 January, 2010 and April 28, 2021, 33 patients diagnosed with CHM at a University Training and Research Hospital were included. Approval was obtained from the local, non-invasive Clinical Research Ethics Committee (date: October 10, 2021, document no: 74636-469). Again, consent was obtained from the child and/or parent to use the related case pictures here. The study started by distinguishing the patients aged 0-21 years with a diagnosis of mastocytosis (Q82.2 in the ICD10 coding system). Then, the patients who were followed up with the diagnosis of CHM in the Pediatric Immunology and Allergy Division after the physical examination [typical skin involvement with positive Darier sign (major criterion)] and clinical findings and/or biopsy results performed by us with the diagnosis of CHM were included in the study. In this study, the diagnosis was confirmed by skin biopsy in approximately 25% of our patients, and biopsy was performed in suspected cases. c-Kit mutation could not be examined in any of our cases.

The criteria for exclusion in the study can be listed as being over 21 years old, having a history of co-morbid disease, conversion to SM, and having a history of regularly used drugs due to SM or chronic disease.

In patients who applied to our clinic and were diagnosed with CHM, the age of onset, duration and frequency of complaints, types and distribution of skin lesions (plaque, macule, papule, bullae, *etc*.), accompanying complaints related to other systems, triggering factors, demographic characteristics of patients, chronic medication use, additional allergic disease, other chronic disease states, *etc*., family history (family history of atopy/allergic disease) were questioned in the anamnesis. Angioedema/anaphylaxis histories and treatment plans of the patients, whether they needed adrenaline autoinjectors or not, were retrospectively scanned from their files. After recording all this history, physical examination findings, CDLQI (Children's Dermatology Life Quality Index) score, and the results of laboratory tests performed during routine evaluation were evaluated using the 'Statistical Package for Social Science' (IBM SPSS Statistics, Chicago, IL) v21 program.

Statistical analysis

The frequency and percentage ratios of the categorical variables in the study are given together with the tables. Numerical findings are tabulated with mean, standard deviation, and minimum-maximum values. Whether the categorical variables were evenly distributed was evaluated with the chi-square test. Whether the laboratory findings differed according to categorical variables was checked with the Likelihood ratio P-value test, Mann Whitney U, and Kruskal Wallis H tests. Whether there was a relationship between the numerical findings was evaluated with the Spearman correlation test. The study accepted the significance level as ($p < 0.05$).

Results

Of the patients diagnosed with CHM, 11 (33.3%) were girls and 22 (66.7%) were boys. The male/female ratio was found to be 2/1. The median age of onset of complaints in patients with CHM was seven months, and the median age of diagnosis was 11 months. The median age of regression of their complaints was 54 months. Clinical findings continued in 13 (39.4%) patients followed (**table I**).

When the additional allergic diseases of the patients diagnosed with CHM were questioned, sixteen (48.5%) had no concomitant atopic disease, and seventeen (51.5%) had a concomitant allergic disease. Allergic rhinitis in nine (52.9%) patients, allergic asthma in four (23.5%), atopic dermatitis in two (11.8%), food allergy in one (5.9%), food allergy and asthma were found to be together in one case (5.9%) as well.

When the family history of mastocytosis was questioned, it was learned that fourteen (42.4%) had a family history of allergic disease. It was learned that nine (64.3%) of the family mem-

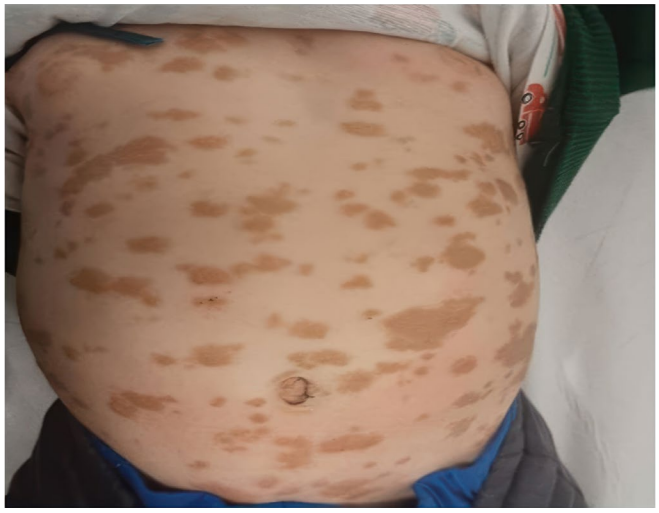
Table I *- Demographic and clinical characteristics of the cases.*

Variables	
Girl	11 (33.3%)
Boy	22 (66.7%)
Median age of onset (month)	7 (0.00-60.00)
Median age at diagnosis (month)	11 (2.00-64.00)
Age of regression (month)	54 (6.00-192.00)
Tryptase	3.94 (1.00-20.50)
History of allergic disease	17 (51.5%)
Allergic disease in family history	14 (42.4%)

Parenthesis shows minimum-maximum or percentage values.

bers with a history of allergic disease had asthma, two (14.3%) allergic rhinitis, two (14.3%) urticaria/eczema, and one (7.1%) food allergy. Nevertheless, there was no mastocytosis case in the families of the patients. No significant correlation was observed when examining the relationship between additional allergic disease and patient family history ($p > 0.05$).

Figure 1 *- Polymorphic maculopapular cutaneous mastocytosis appearance in one of our patients.*



In the patients' physical examinations, cervical lymphadenopathy (LAP) smaller than 1 cm was reported in 3 patients. Abdominal ultrasonography was performed in 23 of 33 patients; hepato-splenomegaly was not detected, and only one patient had abdominal lymphadenopathy smaller than 1 cm.

In our patients, one (3%) of the rashes was only on the head and neck, three (9.1%) only on the trunk, one (3%) only on the extremities, seven (21.2%) on the head, neck and trunk, nine (27.3%) on trunk and extremities, three (9.1%) on extremities and head-neck, nine (27.3%) on head-neck, trunk and extremities. While rashes were detected in one body area in five (15.2%) patients, they were detected in two or more in thirty (84.8%) patients. As the rash type, macule was seen in nine (27.3%) patients, papule in eight (24.2%) patients, and bullae in two (6.1%) patients (**figures 1** and **2**). There are two cases of diffuse CM in our series. We did not have one patient with only nodules and plaque-type lesions (**figure 3**). In patients with more than one type of rash, the maculopapular rash, the most common type, was seen in fourteen (42.4%) patients. Most of the cases diagnosed with polymorphic MPCM is shown in **figure 1**. When lesion sizes and similarities were compared, the lesions of twenty-six (78.8%) patients were larger than 1 cm, and seven (21.2%) were millimetric. While one (3%) of the patients had monomorphic lesions, thirty (97%) had polymorphic type of MPCM lesions. Darier's sign was positive in 32 of 33 (96.9%) patients diagnosed with CHM.

Considering the itching and redness (rash) rates on the skin from the complaints and findings of the patients, itching was positive in 66.7% and redness in 81.8%. Headache was observed in

Figure 2 *- Diffuse cutaneous mastocytosis appearance in CM patients.*

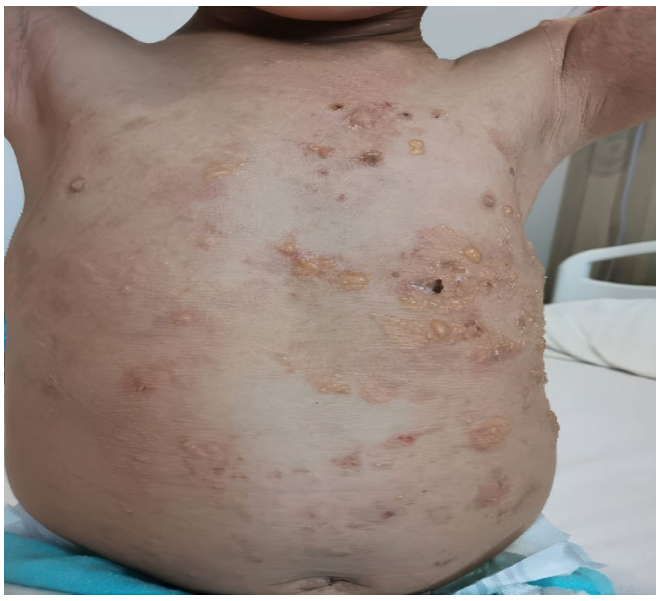
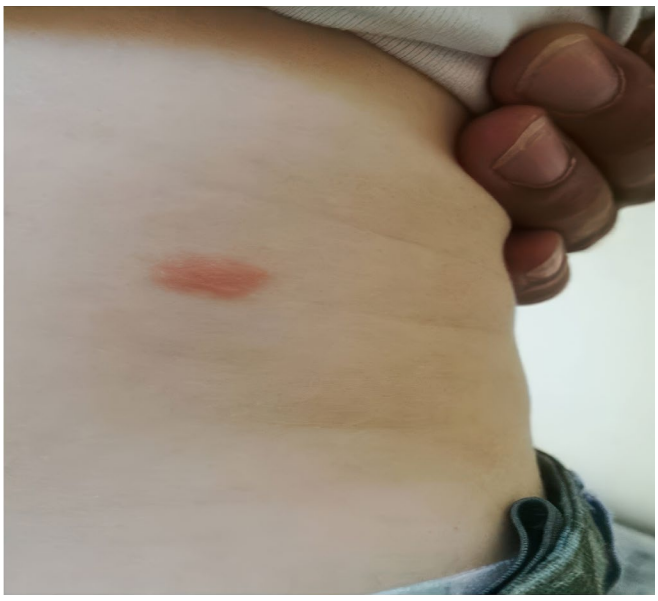


Figure 3 *- Solitary mastocytoma appearance in CM patients.*



only one patient (3%) as a neurological symptom. When gastrointestinal system findings were questioned, they were absent in 29 (88%) patients. One patient (3%) had abdominal pain, one (3%) had nausea and vomiting, one (3%) had reflux, and one (3%) had abdominal pain and nausea. When gastrointestinal complaints were questioned, it was learned that these complaints were of short duration and regressed over time. There were no symptoms or signs of other organs and systems.

Laboratory values were found within the normal range in all routine biochemistry tests (kidney/liver function tests, serum electrolytes, *etc*.). When peripheral smears of patients diagnosed with CHM were examined in terms of systemic involvement, no atypical cells were found in the smear. A hemogram was not obtained from one of the patients. When the hemograms of 32/33 patients diagnosed were examined, 20 (60.6%) were normal. Three (9.4%) patients had anemia, one had neutropenia, six (18.8%) had eosinophilia, and two (6.2%) had thrombocytosis.

Of the 33 patients included in the study, total IgE was measured in 19. 7/17 patients had additional allergic disease and whose total IgE was checked. We had two patients under age 6 with an IgE value of over 100. No statistical significance was found in comparing total IgE values according to additional disease, and there was no correlation between additional allergic disease and total IgE. Sensitization was detected in specific IgE or skin prick tests in six of the patients. Cat sensitivity was detected in one patient, stinging nettle and ash pollens in one patient, grass pollen in one patient, hazelnut in one patient, egg and milk in one patient, and cat with milk sensitivity in one patient.

Vitamin D levels were measured in 15 of the patients with CM. The median vitamin D level of the patients examined was 14.90 ng/ml (7.88-53.0 ng/mL). Vitamin D level is below 12 ng/mL in six patients with vitamin D deficiency; a diagnosis of vitamin D insufficiency was made in 2 patients with a 12-20 ng/ml range. Vitamin D was found in the normal range in 7 (47%) patients, between 20-100 ng/ml. None of the patients had a history of bone-joint pain or spontaneous bone fracture. No clinical findings suggestive of osteoporosis/osteopenia was detected in the patients.

There were 16 patients whose tryptase levels were measured in the study. The median tryptase level of the patients whose tryptase levels were measured was 3.88 ng/ml. In one diagnosed patient, the serum tryptase level was above 20. Bone marrow aspiration was performed with the suspicion of SM in the patient, whose serum tryptase level was above 20 ng/mL, and no atypical cells were found in the patient's bone marrow examination, and the patient's annual follow-up was continued. When the patients with tryptase levels were compared according to gender, there was no significant difference between the mean of boys and girls. When the lesion size groups are separated as < 1 cm and ≥ 1 cm, there is no significant difference between the patients' mean in comparing the lesion size. When the tryptase value is compared according to the lesion type in the patients, it can be said that there is no significant difference between the averages of all lesion types. There was no significant difference between the mean of the normal and lymphadenopathy groups in comparing tryptase values in patients.

Skin biopsy was taken in ten (30.3%) of 33 patients with a diagnosis of CM, and the diagnosis was shown to be CM. In the report of the patients whose skin biopsy was taken, an increase in the number of mast cells was found at a rate that met the diagnostic criteria. Immunohistochemical staining was positive in the patients. When the patients' rashes were questioned regarding the triggering factor/agent, it was understood that 19 (57.6%) patients had a trigger factor. When the factors triggering the complaints of the patients were questioned, it was heat increase (fever) in 6 (18.2%) of them, heat increase (fever) and infection in 5 (15.2%), heat increase (fever) and stress were seen as triggering factors in 3 (9.1%) and only one patient, along with heat increase (fever) and food (chocolate). Sun exposure in one of the remaining four patients, heat increase (fever) and sun exposure in one, stress in one, and infection and stress in one were found to be triggering factors.

The patients who were followed up with the diagnosis of CHM and those who needed autoinjector prescription and treatment were examined. There were 8 (24.2%) patients who had an autoinjector report. There were four patients with prophylactic drug use; 2 of them were prescribed antihistamines, and 2 of them were prescribed montelukast. No medication was ever prescribed for 7 (21.2%) patients. Antihistamine was prescribed for 12 (36.4%) patients, local steroids were prescribed for 5 (15.2%), and antihistamine and local steroids were prescribed for 8 (24.2%) patients in whom treatment was initiated in case of active disease. Systemic steroids, cromolyn sodium, omalizumab, and PUVA were not used in any of our patients, and the symptoms regressed with the treatments given.

Discussion and conclusions

Although males are slightly predominately in CHM, females are more affected after puberty (7, 8). In some studies, the incidence rate in boys was 1.4 times higher than in girls (2, 6). In the study of Ben-Amitai *et al*. (7), the male-female ratio was 1,8/1 in patients diagnosed with CHM. In the study of Kiszewski *et al*. (9), the male-female ratio was 1,8/1; Akoğlu *et al*. (10) reported the male-female ratio as 1,5/1. Some sources argue that CM is seen at an equal rate of male-female in children (11, 12). In the study conducted by Wiechers *et al*., with 144 patients, 73 (50.7%) of the patients were female and 71 (49.3%) were male (11). The patients diagnosed in our study were under 15, and the disease was more common in males. Of the patients diagnosed with CM, 11 (33.3%) were female and 22 (66.7%) were male. The male-female ratio in our study was 2/1, consistent with the literature.

The incidence of CM in the pediatric age group increases in the first two years of life and after the age of fifteen; therefore, a bimodal

distribution is observed (12). The age of onset of CM in children is between birth and two years of age in 55% of patients, typically in the first six months of life. 35% of the patients are diagnosed over the age of 15, and the remaining 10% are diagnosed under the age of 15 (2). Hannaford *et al.* detected skin findings at birth in 39 patients, before the 6th month in 102 patients, at 6-12 months in 8 patients, and after the 12th month in 12 patients (13). Kiszewski *et al.* found that the lesions started in 92% of the patients in the first 12 months, and there was no difference in age of onset between the types (9). In their study, Akoğlu *et al.* found that the lesions appeared in the first six months in 41.8% of the patients and by the age of 13 months in 78.2% of the patients; however, they reported that they did not find a congenital form in any of the patients (10). In the study of Heinze *et al.*, the age of onset of complaints was 2.8 (0-42) months, and the age of disappearance of complaints was 10 (3-19) years (14). In our study, there was no patient whose rash started at birth. The age of onset of complaints in all of the patients in our study was under 15. The earliest rash in our patients appeared in the first month. The median age of onset of the complaints of our patients with MPCM was 7 (0.0-60.0) months. The median age at diagnosis of the patients was 11 (2-64) months. Consistent with the literature, 27 (81.8%) of 33 patients were younger than two years of age. Their complaints' median age of regression was 54 (6-192) months. In 13 (39.4%) patients followed up, rashes continued.

Mastocytosis is a rare disease, and there is usually no familial transmission. However, rarely familial cases have been described (12). In the study conducted by Hannaford *et al.*, 3/173 patients had a family history of mastocytosis (13). Being twins was found in 1.8% of these patients (6). In the study of Ben-Amitai *et al.*, 117 patients were examined, and a family history was found in 13 (11%). In other studies, CM was not found in family members (14, 15). There was no history of mastocytosis in other family members of the patients examined in our study. When the studies were examined, there was no data on allergic diseases in the patients' families. A family history of allergic disease was observed in 14 (42.4%) patients with CM in our study.

In the study of Gonzalez *et al.*, additional allergic diseases were found in 21 (44.7%) of 45 pediatric patients diagnosed with mastocytosis. It was reported that 9 (19.1%) of these patients had allergic conjunctivitis, 7 (14.9%) allergic rhinitis, 5 (10.6%) asthma, and 3 (6.4%) atopic dermatitis (16). In a study by Brockow *et al.* in which pediatric and adult patients were included, the frequency of allergic disease was similar to the population, and concomitant allergic disease was found in 31% of the patients (17). In the study of Azaña *et al.*, the history of concomitant allergic disease in patients diagnosed with mastocytosis and their families was 6% (4). In the study of Caplan *et al.*, it was found to be 47% (18). In our research, concomitant allergic disease was found in 17 (51.5%) of our patients, which is compatible with the literature but at a higher rate. Of those with a history of additional allergic disease, 9 (52.9%) had allergic rhinitis, four (23.5%) had allergic asthma, two (11.8%) had atopic dermatitis, one (5.9%) had a food allergy, one (5%), were found to have a food allergy and allergic asthma.

Itching is among the most common complaints and symptoms in patients with mastocytosis due to excessive secretion of mast cell mediators. Blistering and sudden redness of the rash are other common complaints (6, 14, 15). Although it is said that itching is a common symptom, Hannaford *et al.* included 173 patients in their study. Sudden redness and blistering complaints were common in these patients, and itching was found in only five patients (13). Kiszewski *et al.* reported that 61% of their patients had itching in their study (9). Similar to other studies, in the study of Lange *et al.*, itching was observed in 68% of the patients, sudden rash in 29%, diarrhea in 22%, headache in 18%, hypotension in 16%, and anaphylaxis in 6% (15). In the review of Meni *et al.*, itching was 4% in patients; blistering in skin lesions was 34.5%; sudden redness was 24.5%; findings related to the gastrointestinal tract were found in 19.5% and bone pain in 13.7% (6). In our study, when the itching and rash rates of the patients were analyzed, itching was positive in 63.2% of patients and rash in 81.6% of patients. Symptoms suggestive of respiratory, cardiovascular system, and oncological disorders were absent in patients. Headache was detected in only one patient (3%) as a neurological symptom. When the gastrointestinal system findings were questioned, symptoms were detected in 4 patients (12%). It was found that one of the patients (3%) had abdominal pain, one (3%) had nausea, one (3%) had reflux, and one (3%) had abdominal pain and nausea.

Skin manifestations consist mainly of maculopapular lesions; lesions are brownish or reddish. Among the findings, plaques and nodules may coexist. MPCM lesions can be monomorphic or, more often, polymorphic. Polymorphic lesions are more common and have a better prognosis. In MPCM, large lesions are usually seen at less than seven months, and small lesions are seen in patients over two years of age. Small-sized skin rashes take longer to disappear (≥ eight years) than large-sized skin rashes in children with MPCM (11). In a study by Lange *et al.* in which 101 patients were included, it was reported that 45% of the lesions were plaque, 37% were maculopapular, 25% were bullae, 2% were nodules, and in 6% of the patients, the lesions completely infiltrated the skin (diffuse) (15). In their study with 71 patients, Kiszewski *et al.* examined the rash type of CM patients and found that the most common was macular rash in their patients. Macules in 46 patients, 30 plaques, 27 papules, 16 bullae, and 5 nodules were detected (9). In our study, it was observed that there were patients with one type of rash and patients with multiple types of rashes at the same time. The most common maculopapular rash was detected in the patients. In patients with a uniform rash, macules were 9 (27.3%), papules were 8 (24.2%), and bullae were 2 (6.1%). We did not have any patients with only nod-

ule and plaque-type lesions. In patients with more than one rash type, maculopapular rash was detected in 14 (42.4%) patients.
Darier's sign is pathognomonic for the diagnosis of CM. In the final classification of mastocytosis in the pediatric age group, it is included among the major criteria for the diagnosis of CM and is positive in almost all patients (19). Darier's sign: in the study (10) conducted by Akoğlu *et al.* in patients with a diagnosis of CM, 89.5%, Darier's sign was found to be positive between 90% and 100% in patients diagnosed with CM in different studies (6,14). In our research, Darier's sign was positive in 32 of 33 patients diagnosed with CM, and Darier's sign was negative in one (3%). In our study, Darier's sign positivity was 97%.
Evaluation of the examinations of patients diagnosed with CHM is necessary to understand whether there is systemic involvement. By evaluating the hemogram and peripheral smears of the patients, it is evaluated whether there is bone marrow infiltration of mast cells. Hemograms, liver function tests, and tryptase levels should be evaluated to exclude SM (20, 21). In their study, Carter *et al.* found anemia due to iron deficiency in 3 patients, lymphocytosis in 22 patients, monocytosis in 3 patients, and thrombocytosis in 12 patients, and evaluated these findings independently of the CM clinic (22). Kiszewski *et al.* evaluated the complete blood count of 28 of 71 patients and reported that six patients had anemia, three had eosinophilia, and one had thrombocytopenia (9). In this study, the examinations of the patients were reviewed retrospectively. Hemograms were obtained from 32 of 33 patients. Anemia in three patients (9.1%), neutropenia in one (3%), eosinophilia in six (18.2%), and thrombocytosis in two (6.1%) patients were detected. Atypia was not observed in the peripheral smears of the patients. Later hemograms taken during follow-up showed that these parameters were within normal limits.
The serum tryptase level indicates the mast cell load in the body, and sudden increases and persistent elevations in the serum tryptase level should be a warning for SM. It should be kept in mind that serum tryptase levels can be found to be high in diffuse CM and monomorphic MPCM in the pediatric age group (2). In children diagnosed with CM, high basal serum tryptase levels and skin findings carry the risk of developing serious symptoms due to mast cell activation (23). There was no effect of having a male or female gender on tryptase level (24). It has been observed that pediatric patients with smaller lesions diagnosed with CM have higher baseline tryptase levels and have a worse clinical picture than those with larger lesions (25). In a retrospective study involving 102 children with CM, high tryptase levels were significant in predicting anaphylaxis in children (26). In the study of Carter *et al.*, it was observed that the serum tryptase level in patients remained stable or decreased over time, and the increase in SM and tryptase levels was found to be related (22). In the study of Şahiner *et al.*, serum tryptase levels decreased with age; it was observed that there was no difference between tryptase levels in healthy boys and girls (26). This study measured serum tryptase levels in 16 (48.5%) patients. No significant difference was found between the mean of girls and boys in comparing tryptase values to gender in patients whose tryptase levels were checked. Since the patients were not tested for recurrent tryptase, no comment could be made about the decrease with age. Again, tryptase was tested in 17 of the 33 patients included in our study, and no significant correlation was found between the type of rash, age of onset of rash, age of regression, and age of diagnosis.
In addition to various system involvements, bone lesions that can be detected radiologically as osteoporotic or osteosclerotic can be seen in SM. Caksen *et al.* detected rickets in a 12-month-old patient diagnosed with CM. In the patient's examinations, calcium and phosphorus were within normal limits, and alkaline phosphatase was as high as 1401 U/L. The left wrist radiograph showed osteoporosis on the radius and distal end of the ulna. The patient was started on vitamin D therapy. The coexistence of CM and rickets in the patient was evaluated incidentally due to vitamin D deficiency in our country (27). In our study, vitamin D levels were checked in 15 of our patients. The patients' median vitamin D level was 14.9 (7.88-53.0 ng/mL). Vitamin D levels were below 12 ng/mL in 6 patients with vitamin D deficiency; a diagnosis of vitamin D insufficiency was made in 2 patients with a 12-20 ng/ml range. Vitamin D levels were within the normal range (20-100 ng/mL) in 7 patients. None of the patients had a history of bone-joint pain or spontaneous bone fracture. Clinical findings of osteoporosis/osteopenia were not detected in the patients.
Patients with a diagnosis of mastocytosis should avoid exposures that trigger or exacerbate their symptoms and complaints as much as possible. These exposures include sudden temperature changes (hot/cold), humidity, exercise, dry skin, emotional stress, alcohol, and lack of sleep. In infants and young children, skin rubbing, teething, fever, irritability (anger), and excessive excitement can trigger symptoms (23). Our study determined heat, stress, infection, food, and sun exposure as triggering factors (**table II**).

Table II - *Triggering factors in patients with cutaneous mastocytosis.*

Triggers	n	%
None	14	42.4
Hot (heat)	6	18.2
Heat and infection	5	13.2
Heat, stress	3	9.1
Stress	1	3
Sun	1	3
Heat, sun	1	3
Infection and stress	1	3
Heat, food	1	3

It has been reported that the risk of anaphylaxis is higher in patients followed up with a diagnosis of mastocytosis (16, 28). The prevalence of anaphylaxis in the community is thought to be 1-3/10,000 patients per year (29). In the study of Gonzalez *et al.*, anaphylaxis was observed in 22% of patients diagnosed with mastocytosis (16). Serum tryptase level was found to be higher in patients who had anaphylaxis (28). Vaccines, food, and sudden contact with cold are the cause of anaphylaxis in CHM patients (28). In the study of Lange *et al.*, ketamine, clindamycin, and radiocontrast material were shown to cause anaphylaxis (15). Anaphylaxis during operation can be the first presenting manifestation of mastocytosis. Cases with mastocytosis using chronic antimediator treatment and/or prophylactic medications before operation had an ordinary surgical progress (30). Since the risk of anaphylaxis increases in patients with mastocytosis with a high serum tryptase level, such as in a large mastocytoma or a diffuse CM, it is recommended that epinephrine autoinjectors be prescribed (15, 31, 32). In our study, 8 (24.2%) patients had an autoinjector prescription. Our patients had no history of anaphylaxis or angioedema. Eight (24.2%) of the patients were prescribed an adrenaline autoinjector to use in case of possible anaphylaxis, similar to other studies (**table III**). However, it was learned that none of the patients needed an adrenaline autoinjector prescription during their follow-up.

The use of antihistamines is recommended to prevent mediator release in patients with signs of mast cell degranulation (2, 23, 33). In our study, symptomatic medication was not prescribed for 7 (21.2%) patients. In the case of active disease, antihistamine was prescribed to 12 (36.4%) patients, local steroids were prescribed to 5 (15.2%), and antihistamines and local steroids were prescribed to 8 (24.2%) patients. Again, antihistamine, montelukast, and local steroids were prescribed to 1 patient for therapeutic purposes. Antihistamine was prescribed for 2 (6.1%) patients, and montelukast was prescribed for 2 (6.1%) patients for prophylaxis (**table III**).

CLDQI is an index that shows the effect of dermatological disease on quality of life in pediatric patients aged 4-16 years. It consists of 10 questions asked to the patients. In our study, this index could only be applied to 18 patients over the age of 4 years. Two (6.1%) patients had no impact on their lives, and sixteen (48.5%) had a slight/mild impact.

Since CHM is a rare disease, the number of cases is limited. Due to the retrospective nature of our study, prospective follow-up of patients with ongoing complaints in terms of SM is required, and our study period is insufficient for this.

In conclusion, childhood mastocytosis is a rare disease that may cause parents concern. Cutaneous mastocytosis has a benign course and is usually limited to the skin in the pediatric age group. However, it should be known that SM may develop years later in very few patients; therefore, there is a possibility of a fatal course (34, 35).

Table III *- Distribution of autoinjectors, prophylactic drugs and treatments in patients.*

		n	%
Autoinjector	None	25	75.8
	Yes	8	24.2
Treatment	None	7	21.2
	Used	26	78.8
	Antihistamine	12	36.4
	Local steroid	5	15.2
	Antihistamine, local steroid	8	24.2
	Antihistamine, montelukast, local steroid	1	3
Prophylaxis	None	29	87.8
	Used	4	12.2
	Antihistamine	2	6.1
	Montelukast	2	6.1

Fundings

None.

Contributions

AMD, ÖÖ: conceptualization, writing - original draft; ÖÖ: writing - review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Clinical severity of LTP syndrome is associated with an expanded IgE repertoire, FDEIA, FDHIH, and LTP mono reactivity

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Key words

Lipid transfer protein; profilin; PR10; macroarray; IgE; atopic dermatitis.

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Impact statement

This study shows that LTP syndrome severity associates with increased IgE repertoire and specific IgE to Ara h 9, Cor a 8, Mal d 3, and is modulated by exacerbating (FDEIA, FDNIH, LTP monoreactivity) or protective (atopic dermatitis, profilin/PR10) cofactors.

Summary

Background. *Lipid transfer proteins (LTP) allergy is often a challenge for clinicians. We evaluated a multiplex diagnostic approach with diverse cofactors to stratify LTP syndrome risk.* ***Methods***. *Of the 1,831 participants screened with 'Allergy Explorer-ALEX-2', 426 had reactions to at least one LTP. Data was gathered and recorded via an electronic database.* ***Results***. *Reactivity to peach Pru p 3 was found in 77% of individuals with LTP allergy. Higher levels of specific IgE and concurrent sensitization to more than 5 molecules (50% of all LTP-sensitised participants, 62% of symptomatic cases) were significantly associated with an increased risk of severe reactions (p = 0.001). Several cofactors, either alone or in combination, also influenced patients' clinical outcomes. Some cofactors increased the risk of severe reactions, such as mono reactivity to LTP in 44.6% of cases (p = 0.001), Food-Dependent Exercise-Induced Anaphylaxis (FDEIA) in 10.8% of patients (p = 0.001), and Food-dependent NSAID-induced hypersensitivity (FDNIH) in 11.5% (p = 0.005). On the other hand, reactivity to PR10 (24.2%; p = 0.001), profilin hypersensitivity (10.3%; p = 0.001), and/or atopic dermatitis (16.7%; p = 0.001) had a mitigating effect on symptom severity.* ***Conclusions***. *Clinical severity of LTP syndrome is associated with an expanded IgE repertoire in terms of the number of LTP components recognized and increased IgE levels in individual molecules. Ara h 9, Cor a 8, and Mal d 3 showed the strongest association with clinical severity. In addition, several cofactors may either exacerbate (FDEIA, FDHIH, and LTP monoreactivity) or ameliorate (atopic dermatitis and co-sensitization to profilin and/or PR10) individual patient outcomes. These factors may be utilized for the daily clinical management of LTP syndrome.*

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Introduction

The nonspecific lipid transfer proteins (LTPs), first described nearly 50 years ago (1), play a significant role as a food and environmental allergen, especially in regions with high consumption of plant foods. Initially, this problem was thought to be primarily limited to the Mediterranean region (2), but numerous studies carried out over the past two decades, including a recent one (3), have shown that LTP allergies are widespread (4) including in Northern Europe (5, 6), China (7), and North America (8). In addition, interesting differences in epitope recognition have been found in different populations, suggesting that individual, environmental, or dietary factors may influence the interaction with these molecules (9).

LTPs are small, heat-stable proteins, found in various plant foods such as fruits, vegetables, nuts, and seeds, that, although homologous, have a variable degree of structural identity influenced by plant relationships (10). Consequently, IgE antibody recognition to one LTP molecule does not necessarily imply reactivity to all other members of this protein family (11). There are three primary epitopes on the surface of LTPs, and Pru p 3, the peach LTP, is considered the molecule that identifies the most patients (12).

Evidence of the widespread occurrence of LTP IgE reactivity beyond the Mediterranean region has also led to interesting studies on the variability of clinical outcomes in LTP-hypersensitive patients, ranging from the complete absence of symptoms (13) to extremely severe reactions (14), and on the identification of the primary sensitizers. These may include mugwort pollen Art v 3, as observed in a subset of Chinese patients, Can s 3 from marijuana, even through passive smoke exposure, as hypothesized in Northern Europe (6), or Pru p 3 from peach via the oral sensitisation route in the Mediterranean basin (15).

The influence of different cofactors on the clinical outcomes of LTP allergic patients is the subject of an ongoing debate, and the exact mechanisms, either in a protective sense, where they may reduce or eliminate the risk of adverse reactions, or in an aggravating sense, leading to severe reactions including anaphylaxis, are not yet fully understood. To date, factors that have been shown to increase the severity of allergic reactions include physical exercise (Food-Dependent Exercise-Induced Anaphylaxis - FDEIA) or the use of Non-steroidal anti-inflammatory drugs (NSAIDs) (Food-dependent NSAID-induced hypersensitivity - FDNIH) in conjunction with the consumption of LTP-containing foods. FDEIA is a well-documented phenomenon in which physical exercise exacerbates the severity of allergic reactions to LTP-containing foods, particularly in the Mediterranean region (16). NSAIDs are another cofactor that may exacerbate the severity of allergic reactions in individuals with LTP allergy (17). It is generally believed that this effect is related to the increased absorption of allergenic molecules that are digestion-resistant and heat-stable. In a previous paper, by employing a completely distinct method for the assessment of specific IgE antibodies, we also identified another significant aggravating factor characterized by the presence of hypersensitivity to more than 5 LTPs among those tested (18), although this finding was not confirmed in a study performed in the United Kingdom (19). On the other hand, the presence of atopic dermatitis in LTP-sensitized patients is associated with a significantly lower frequency of severe reactive episodes than in the general allergic population (20). In addition, both singleplex (21) and multiplex approaches have shown that the detection of hypersensitivity to panallergens such as Profilins and/or PR10s is associated with a more favourable outcome than in individuals allergic to LTPs alone (18, 22).

Objectives

This study aimed to investigate an unselected population collected from a single Centre in central Italy characterized by IgE reactivity to at least one out of 15 LTPs for:

- Patient's individual LTP molecular recognition profile
- Clinical outcomes, ranging from tolerance to anaphylaxis
- External factors that may have influenced the observed reactivity, assessed individually or in combination.

Materials and methods

Study design

We conducted a cross-sectional clinical survey of lipid transfer protein allergy to evaluate the role of various cofactors that might have a mitigating or exacerbating effect on clinical outcomes. These cofactors included exercise after food intake, NSAID administration before LTP source intake, sensitization to panallergens other than LTP, and the presence of comorbidities such as atopic dermatitis, rhino-conjunctivitis, and bronchial asthma. We examined the individual role of each factor and their potential interactions. In addition, we assessed the factors that might be relevant in "tolerant" participants and examined the differences between responders with mild symptoms and those with severe reactions.

Setting

The study was conducted on 1,831 unselected participants born in central or southern Italy who presented to the allergy outpatient clinic of IDI-IRCCS in Rome with a history of adverse reactions to food, allergic rhinitis, bronchial asthma, and/or atopic eczema. The IDI-IRCCS is a national reference centre for skin diseases. The data collection period was from January 2021 to December 2022, and demographic information, as well as clinical data on food-related reactions, respiratory symptoms, and dermatological symptoms, were collected in a customized electronic database.

Participants

The main criterion for enrollment into the study was the detection of sensitization to at least one of the LTPs present on the IgE macroarray. The diagnosis of tolerance was based on a history of regular intake of LTP-containing foods without any problems.

Variables

The patient-reported allergic symptoms induced by LTPs encompassed a range of severity from mild manifestations, including isolated gastrointestinal reactions or oral allergy syndrome, to moderate to severe reactions, such as urticaria/angioedema or anaphylaxis. The occurrence of severe food reactions (anaphylaxis) was always documented by emergency department reports that recorded the use of epinephrine, H1 antihistamines, and steroids to treat symptoms. The group of participants who did not report adverse reactions despite following LTP ingestion was categorized as "tolerant".

Further categorizations were based on the presence of upper or lower airways symptoms, atopic dermatitis, monoreactivity to LTP, co-sensitization to other allergens (*i.e.*, PR -10, Profilin, Polcalcin, and seed storage protein [SSP]), food-dependent exercise-induced anaphylaxis (FDEIA) or food-dependent NSAID-induced hypersensitivity (FDNIH), and the number of LTP molecules detected in each patient.

Because of the observational nature of the study, no randomization procedure was performed at enrollment.

Data sources/measurement

Serum IgE reactivity was analysed using the Allergen Explorer-rALEX® (Macroarray Diagnostics, Vienna, Austria), where different allergens and extracts are spotted onto a nitrocellulose membrane in a cartridge chip, including a broad spectrum of LTP molecules: nAct d 10 - 9kD from kiwi (*Actinidia deliciosa*); rApi g 2 - 9kD from celery (*Apium graveolens*); rApi g 6 - 7kD from celery (*Apium graveolens*); rAra h 9 - 9kD from peanut (*Arachis hypogaea*); rArt v 3 - 9kD from mugwort (*Artemisia vulgaris*); rCan s 3 - 9kD from hemp (*Cannabis sativa*); rCor a 8 - 9kD from hazelnut (*Corylus avellana*); rJug r 3 - 9kD from walnut (*Juglans regia*); rMal d 3 - 9kD from apple (*Malus domestica*); rPla a 3 - 9kD from London plane tree (*Platanus acerifolia*); rPru p 3 - 9kD from peach (*Prunus persica*); nSola l 6 - 7kD from tomato (*Solanum lycopersicum*); rTri a 14 - 9kD from wheat (*Triticum aestivum*); nVit v 1 - 9kD from grape (*Vitis vinifera*); rZea m 14 - 9kD from corn (*Zea mays*). Fra a 1+3 and Ole e 7RUO were excluded from the evaluation. The former is a mixture of Fra a 1 and Fra a 3, making it unable to accurately distinguish Fra a 3 (LTP) reactive patients from Fra a 1 (PR10) allergic patients. The latter is still an experimental molecule in this array. The chip was incubated with 0.5 mL of a 1:5 dilution of the patient's serum, containing a CCD inhibitor under agitation. After two hours of incubation, the chip was washed three times, and a pre-titred dilution of anti-human IgE labelled with alkaline phosphatase was added and incubated for 30 min. After another cycle of extensive washing, the enzyme-substrate was added, and after eight minutes, the reaction was stopped by the addition of 100 µL of ALEX Stop Solution. The membranes were dried, and a charge-coupled device camera measured the intensity of the colour reaction for each allergen spot. The dedicated software digitalized the images and prepared a report listing the allergens and components and their score in kUA/L. Finally, systematic variations in signal levels between lots were normalized by heterologous calibration against an IgE reference curve. A curve fit was calculated, and the resulting equation was applied to transform arbitrary intensity units into quantitative units. Lot-specific calibration parameters are encoded in the barcode. The measuring range of ALEX-specific IgE is 0.3-50kUA/L.

Bias

The diagnosis of LTP allergy was not confirmed through blinded or open oral food challenges.

Quantitative variables

Quantitative measurements of specific IgE towards the single LTPs under investigation were compared among the various clinical subsets of patients, between males and females, and across different age groups.

Statistical methods

All data were analyzed using the SPSS/PC + statistical package for statistical evaluation (IBM SPSS, version 29, Chicago, IL). The TD-Synergy Laboratory Information System was used to search and collect demographic (age and gender), clinical, and laboratory data for patients who attended the outpatient Allergy clinic and underwent specific IgE testing. In univariate analysis, the non-parametric Mann-Whitney U-test (two groups) was first used to compare continuous IgE values in males, females, and subjects with or without a given clinical involvement. Subsequently, each variable of interest was dichotomized as negative or positive to examine the proportion of subjects with symptoms in the two resulting groups.

Pearson's χ^2 test or Fisher's exact test (used for two-by-two contingency tables with less than 50 cases) were used to assess if paired observations on two variables expressed in a contingency table, were independent of each other.

Receiver operating characteristic (ROC) analysis was performed to test results concerning Pru p 3 reactivity or severity of symptoms and was presented as the area under the curve with a 95% confidence interval (95%CI).

We performed multiple logistic regression for the clinical variables with dichotomous scores (present, absent) to see whether the association between clinical symptoms and different allergens reactivity was present after simultaneously adjusting for the other variables of interest.

The degree of relationship between the quantitative variables studied was analyzed using Spearman's non-parametric correlation (ρ) test, given the skewed distribution of the observed values. A very high positive correlation was defined as a Spearman's correlation coefficient of 0.90 to 1.00, 0.70 to 0.90 for high, 0.50 to 0.70

for moderate, 0.30 to 0.50 for low, and 0.00 to 0.30 for negligible. A value of $p < 0.05$ was considered statistically significant. To provide a visual representation of the distribution of the different molecules in panallergen families, we have produced Venn diagrams using the VennMaster 0.38 package (23).

Ethical issues

The study was approved by the Ethical Committee of IDI-IRCCS (IDI-IRCCS CE | 495-17). Data collection was conducted anonymously, utilizing only information obtained from routine specialist surveys. Recruited patients provided written informed consent for the utilization of their clinical data in an anonymous format. The research was conducted ethically following the World Medical Association Declaration of Helsinki.

Results

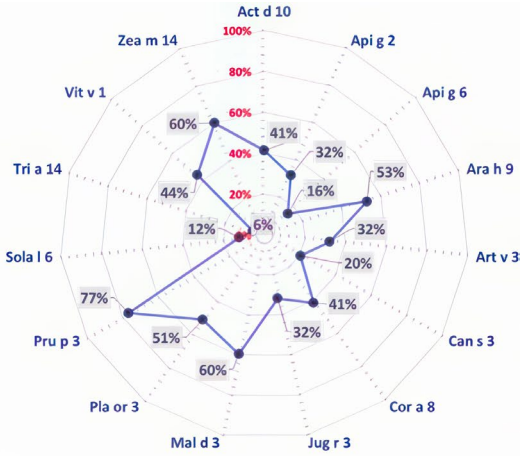
Participants

A total of 426 individuals reactive to nsLTPs (239 females and 187 males; mean age 34±16 years, range 2-74 years) were included in the study. Among the selected patients, 144 (34%) had a history of local reactions (oral allergy syndrome), while 202 reported moderate (65.8%) to severe (34.2%) reactions. Eighty participants (half of whom were females) reported tolerance to LTP-containing foods.

Sequence identity and IgE recognition

Reactivity to Pru p 3 was detected in most patients (77%), followed by Mal d 3 and Zea m 14, which tested positive in 60% of

Figure 1 – (A) *The radar chart shows the prevalence of positive outcomes for the assessed LTPs in the study;* ***(B)*** *Bivariate analysis of the reciprocal relationships among the 15 LTPs studied.*



	Act d 10	Api g 2	Api g 6	Ara h 9	Art v 3	Can s 3	Cor a 8	Jug r 3	Mal d 3	Pla a 3	Pru p 3	Sola l 6	Tri a 14	Vit v 1	Zea m 14
Act d 10	176														
Api g 2	0,576 102	136													
Api g 6	0,439 52	0,447 47	66												
Ara h 9	0,595 139	0,612 117	*0,37* 52	225											
Art v 3	*0,493* 99	0,569 87	*0,427* 46	0,605 118	137										
Can s 3	0,413 64	0,602 72	0,449 42	0,522 76	0,591 68	87									
Cor a 8	0,518 117	0,585 103	*0,365* 46	0,647 143	0,603 110	0,561 76	175								
Jug r 3	0,526 98	*0,642* 93	*0,427* 45	*0,68* 121	0,607 92	*0,67* 75	0,688 115	135							
Mal d 3	0,566 148	0,575 123	0,349 55	0,758 195	0,618 127	0,509 79	0,727 162	0,713 129	254						
Pla a 3	0,544 136	0,615 117	0,38 54	0,63 167	0,563 114	0,55 81	0,621 146	0,692 123	0,715 187	218					
Pru p 3	0,487 161	0,509 129	0,3 58	0,659 208	0,531 126	0,449 79	0,613 164	0,631 132	0,859 243	0,638 197	326				
Sola l 6	0,21 32	0,246 28	0,544 34	0,171 33	0,249 30	0,326 28	0,211 33	0,247 28	0,171 35	0,244 38	0,136 38	53			
Tri a 14	0,148 16	0,271 17	0,212 10	0,176 16	0,278 17	0,41 16	0,187 16	0,288 18	0,142 17	0,221 17	0,086 17	0,303 10	25		
Vit v 1	0,621 127	0,732 120	0,49 57	0,703 156	0,647 113	0,607 80	0,692 135	0,727 115	0,678 163	0,693 156	0,564 170	0,273 39	0,27 18	187	
Zea m 14	0,611 148	0,663 125	0,475 61	0,668 191	0,523 117	0,533 80	0,562 145	0,601 118	0,623 195	0,568 168	0,496 212	0,285 41	0,224 19	0,674 159	255

Spearman coefficient values and the absolute number of IgE-positive subjects are given for the paired allergens. A correlation coefficient between 0.7 and 1.0 indicates a strong positive relationship (dark yellow), whereas a coefficient between 0.3 and 0.7 indicates a moderate positive relationship (light yellow).

Table I - *The table provides a quantitative comparison of specific IgE levels (kU/L) directed against the different LTPs assessed in different subgroups.*

A	Male	Female	B	Tolerant	Not Tolerant	C	OAS	Moderate Severe	D	AD Absent	AD Present	E	LTP < 5 mol	LTP > 5 mol	F	LTP Mono	LTP Poly
Act d 10	1.8 ± 3.9	1.7 ± 3.9		0.9 ± 3.4	1.9 ± 4.0 *		1.4 ± 4.0	2.3 ± 4.0 *		1.7 ± 4.0	1.9 ± 3.6		0.2 ± 0.4	3.2 ± 5.0 *		1.7 ± 4.2	1.8 ± 3.5
Api g 2	1.3 ± 3.3	1.0 ± 3.1		0.6 ± 4.0	1.3 ± 3.0		0.9 ± 3.1	1.5 ± 2.9		0.9 ± 2.8	1.8 ± 4.4 *		0.0 ± 0.2	2.2 ± 4.2 *		0.9 ± 2.4	1.5 ± 4.0 *
Api g 6	0.8 ± 3.5	0.4 ± 2.0		0.1 ± 0.5	0.7 ± 3.0		0.6 ± 2.5	0.8 ± 3.3		0.5 ± 2.3	1.0 ± 3.9		0.0 ± 0.2	1.2 ± 3.8 *		0.6 ± 3.3	0.6 ± 1.9
Ara h 9	2.8 ± 5.8	2.0 ± 4.2		0.8 ± 3.7	2.7 ± 5.2 *		1.7 ± 4.8	3.5 ± 5.3 *		2.3 ± 4.7	2.6 ± 5.9		0.3 ± 1.0	4.3 ± 6.2 *		2.3 ± 4.3	2.5 ± 5.7
Art v 3	1.4 ± 3.8	1.3 ± 3.9		0.7 ± 3.2	1.5 ± 3.9		0.7 ± 1.7	2.1 ± 4.9 *		1.3 ± 4.0	1.4 ± 3.0		0.2 ± 0.9	2.4 ± 5.0 *		1.4 ± 4.3	1.2 ± 3.2
Can s 3	0.9 ± 3.3	0.5 ± 2.5		0.1 ± 0,5	0.8 ± 3.2 *		0,6 ± 2,8	1.0 ± 3.4		0.5 ± 2.1	1.4 ± 4.7 *		0.1 ± 0.1	1.2 ± 3.8 *		1.1 ± 3.7	0.5 ± 2.3 *
Cor a 8	1.8 ± 4.7	1.5 ± 3.5		0.5 ± 3.0	1.9 ± 4.2 *		1.2 ± 3.8	2.4 ± 4.4 *		1.5 ± 3.5	2.1 ± 5.4		0.1 ± 0.4	3.1 ± 5.2 *		1.5 ± 2.8	1.9 ± 5.1
Jug r 3	1.5 ± 4.8	0.7 ± 2.1 *		0.1 ± 0.8	1.3 ± 3.9 *		0.5 ± 1.3	1.9 ± 4.8 *		0.8 ± 2.3	1.8 ± 6.2 *		0.02 ± 0.1	1.9 ± 4.7 *		1.1 ± 2.7	1.1 ± 3.9
Mal d 3	3.6 ± 7.0	2.7 ± 4.6		0.9 ± 4.4	3.6 ± 6.0 *		2.0 ± 4.1	4.7 ± 6.8 *		3.0 ± 5.2	3.4 ± 7.4		0.4 ± 1.5	5.6 ± 7.1 *		3.2 ± 6.0	2.9 ± 5.5
Pla a 3	2.2 ± 4.5	1.6 ± 3.4		0.8 ± 3.5	2.1 ± 4.0 *		1.4 ± 2.6	2.6 ± 4.6 *		1.7 ± 3.5	2.4 ± 4.9		0.3 ± 0.9	3.4 ± 4.9 *		1.8 ± 4.0	1.9 ± 3.8
Pru p 3	5.6 ± 9.3	4.9 ± 7.0		1.5 ± 4.9	6.0 ± 8.4 *		4.1 ± 7.1	7.4 ± 9.0 *		5.0 ± 7.4	5.7 ± 10.0		1.6 ± 3.9	8.5 ± 9.5 *		5.4 ± 8.2	4.9 ± 7.9
Sola l 6	0.8 ± 3.4	0.5 ± 2.0		0.1 ± 0.4	0.7 ± 3.0		0.6 ± 2.2	0.9 ± 3.4		0.6 ± 2.8	0.8 ± 2.3		0.2 ± 1.5	1.0 ± 3.4 *		0.5 ± 2.9	0.7 ± 2.4
Tri a 14	0.2 ± 0.7	0.1 ± 0.9		0.0 ± 0.2	0.2 ± 0.9		0.1 ± 0.6	0.2 ± 1.1		0.1 ± 0.7	0.2 ± 1.0		0.1 ± 0.7	0.2 ± 0.9		0.1 ± 0.8	0.2 ± 0.8
Vit v 1	2.6 ± 6.5	1.5 ± 3.7 *		0.9 ± 4.9	2.2 ± 5.2 *		1.4 ± 3.7	2.8 ± 5.9 *		1.8 ± 4.8	2.6 ± 6.0		0.1 ± 0.4	3.7 ± 6.7 *		1.9 ± 5.4	2.0 ± 4.8
Zea m 14	4.1 ± 8.1	3.3 ± 7.1		1.8 ± 6.6	4.1 ± 7.7 *		3.2 ± 7.8	4.6 ± 7.6		3.2 ± 6.4	5.2 ± 10.4 *		0.5 ± 1.4	6.6 ± 9.5 *		3.4 ± 7.4	4.0 ± 7.7

The subgroups include **(A)** men and women, **(B)** individuals who tolerate LTP-containing foods and those who do not, **(C)** patients with mild symptoms and those with severe symptoms, **(D)** individuals with atopic dermatitis, **(E)** patients with fewer or more than 5 nsLTP sensitizations, and **(F)** LTP monoreactors and LTP reactors to PR10 and/or Profilin. The asterisk indicates a significant difference ($p < 0.05$) between the two subgroups studied.

cases (**figure 1A**). Ara h 9 and Pla a 3 reactivity was also detected in over 50% of cases, while the two LTP2s included in the chip (Api g 6 and Sola l 6) scored positive in only a small percentage of patients. Reactivity to Tri a 14 from wheat was found in about 6% of the study participants.

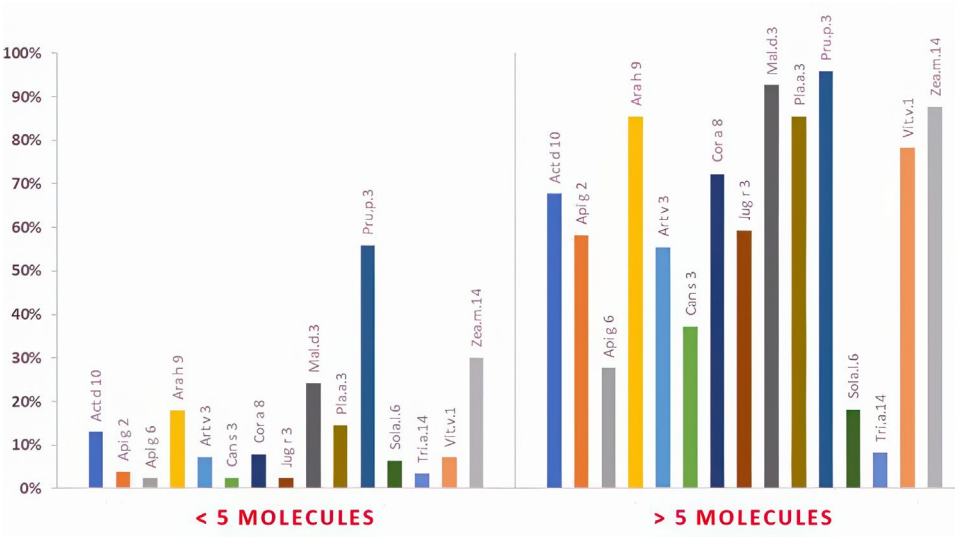
We then cross-referenced the sequences of the 15 studied LTPs with all the LTPs currently listed on the World Health Organization and International Union of Immunological Societies (WHO/IUIS) website (http://www.allergen.org/index.php). It is well-established that cross-reactivity between homologous molecules is primarily determined by the percentage of amino acid identity observed by structural comparisons. IgE cross-reactivity is highly unlikely when the structural identity is below 50%, while it is highly probable when the identity exceeds 70%. (24). Accordingly, Act d 10 from kiwi fruit exhibits significant structural similarity only with Act c 10 but not with any other LTP (**figure 1S**). This suggests that the simultaneous recognition of Act d 10 and, for example, Pru p 3 in patients who are reactive to both molecules might be the result of distinct molecular detections. As expected, Pru p 3 demonstrated high sequence identity primarily with taxonomically related molecules, such as Pyr c 3 from pear, Pru d 3 from European plum, Pru av 3 from sweet cherry, Pru ar 3 from apricot, Mor n 3 from mulberry, and Mal d 3 from apple. Interestingly, the two LTP2s on the chip (Api g 6 and Sola l 6) also displayed significant sequence identity with the other available LTP2 for comparison, such as Ara h 16 from peanut (**figure 1S**).

When comparing the percentage of molecules detected in each patient subset (*e.g.*, the 326 Pru p 3 reactors, **figure 2S**) with the percentage of structural identity between that molecule and all other tested LTPs, we observed significant heterogeneity in recognition. In some cases, the two percentages were in complete agreement, but in several instances, discrepancies were noted. For instance, while Mal d 3 and Pru p 3 exhibited a similar profile of molecular recognition as predicted, the IgE detection of Api g 6, Sola l 6, and Tri a 14 was much higher than expected based on structural identity. This discrepancy may be attributed to the influence of the large number of Pru p 3 reactors present in these patient subsets. This is supported by the observation that in several cases (*e.g.*, Can s 3, Jug r 3, Vit v 1, Ara h 9, Act d 10), approximately 90% of the reactors were Pru p 3 positive. In line with the structural relationships, the bivariate analysis of the reciprocal relationships of IgE mutual co-recognition confirmed that the strongest positive association was observed among molecules with the highest amino acid sequence identities. For example, strong associations were found between Mal d 3 and Ara h 9, Cor a 8, Jug r 3, Pla a 3, and Pru p 3. A moderate correlation was observed in approximately half of the cases (**figure 1B**).

Descriptive data

Females exhibited significantly lower IgE levels towards Jug r 3 from walnut and Vit v 1 from grapes compared to males (**table I**). Interestingly, patients with moderate to severe symptoms had significantly higher IgE levels specific for Ara h 9 ($p < 0.001$), Cor a

Figure 2 *- LTP-IgE detection profiles and the distribution of reactivity in patients with fewer or more than 5 LTP. Percentages of each molecule indicate the proportion of patients positive for that molecule within the reactivity category.*



Significant differences ($p < 0.05$) were observed in all cases except Tri a 14.

8 ($p < 0.01$), Jug r 3 ($p < 0.001$), Mal d 3 ($p < 0.0001$), Pru p 3 ($p < 0.0001$), and Vit v 1 ($p < 0.01$) than patients with mild symptoms, and accordingly, participants who tolerated LTP sources showed significantly lower IgE values towards these molecules than "non-tolerant" patients (**table I**). Likewise, individuals who tested positive for more than 5 molecules (50% of all LTP-sensitized participants, 62% of symptomatic cases) exhibited significantly higher IgE levels for the respective LTPs than patients with fewer than 5 molecules.

Seventy-six participants (17.8%) showed reactivity to a single molecule, with 46.1% reacting to Pru p 3, while 193 participants (45.3%) showed sensitization to up to 4 molecules. The cut-off of 4.5 molecules was determined based on an ROC analysis, which demonstrated the optimal combination of sensitivity and specificity for a severe reaction. Notably, considering the archetypal LTP, Pru p 3, as a reference, 55.8% of cases with reactivity to fewer than 5 molecules were positive for Pru p 3. In contrast, the pattern of molecular recognition in participants with more than 5 reactivities was more diverse (**figure 2**).

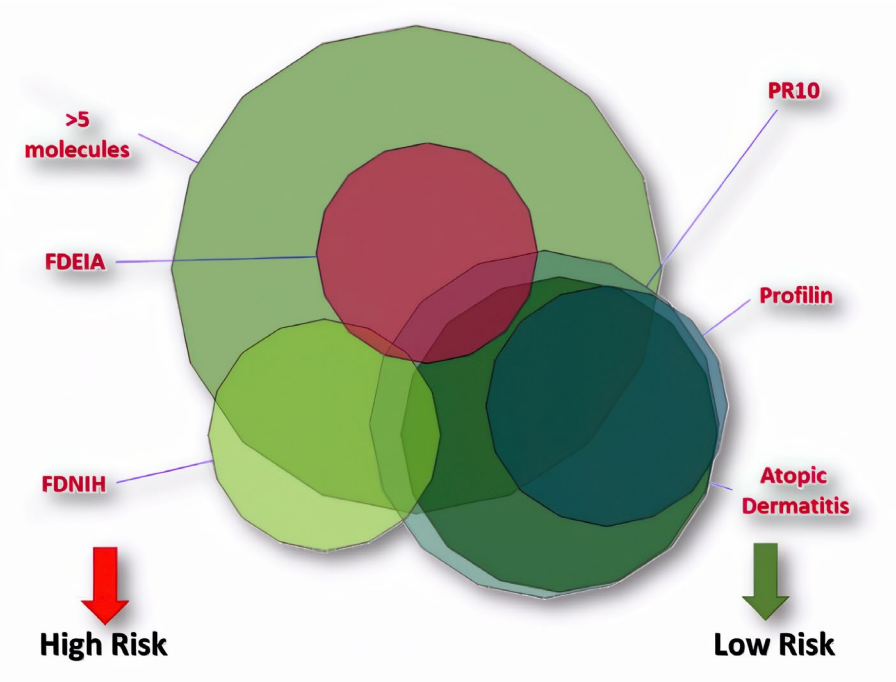
Eighty individuals (18.8%) who exhibited reactivity to LTPs did not report clinical symptoms when consuming LTP sources, while 202 participants reported severe reactions. Interestingly, although there were no significant differences in anaphylactic events between males and females, the number of females who reported generalized urticaria after consuming LTP-containing foods was significantly higher (85 *vs* 48, respectively, $p < 0.01$). One hundred ninety-three participants (58.9%) reported respiratory symptoms and 88 (20.7%) of them reported bronchial asthma.

Cofactors

Forty-six participants (10.8%) had a history of FDEIA, 53 patients (12.4%) had taken an anti-inflammatory drug in combination with the ingestion of LTP-containing food (FDNIH), and 98 individuals (23%) had atopic dermatitis. When considering FDNIH and AD, females were less prevalent than males in the AD group (19.1% *vs* 27.5%, $p = 0.039$), but more prevalent in the FDNIH group (16.2% *vs* 7.4%, $p = 0.006$).

Notably, while subjects with FDEIA and/or FDNIH were mostly not concurrently reactive to PR10 or profilins, nearly all subjects with AD in our LTP-reactive cohort were sensitized to PR10 and/or SSP (**figure 3**). Almost all participants associated with FDEIA or FDNIH exhibited reactivity to more than 5 LTP molecules.

Figure 3 *- The Venn diagram illustrates the relationships between various cofactors that may influence the clinical outcome of patients with LTP allergy, indicating mitigating factors (co-sensitization to PR10 and/or Profilin and presence of atopic dermatitis) and aggravating factors (reactivity to more than 5 LTPs simultaneously, exercise, and/or intake of NSAIDs after consumption of LTP-containing sources).*



The figure provides an algorithm that can be used in daily clinical practice for the management of patients with LTP syndrome.

Cosensitization

By examining the molecular sensitization profile of the study population, we found that 234 participants (54.9%) were solely sensitized to LTPs, while the remaining individuals were also sensitized to other panallergens: 118 (27.7%) to PR10, 56 (13.1%) to profilin, and 23 (5.4%) to polcalcin.

LTP mono-reactors outnumbered patients sensitized to other panallergens in women (148 *vs* 91), while the opposite trend was observed in men (86 *vs* 101). As a result, the frequency of LTP mono-reactivity in females was significantly higher (61.9% *vs* 45.8%, respectively, $p < 0.05$).

One hundred sixty-two patients (38%) did not report any cofactor associated with adverse reactions to LTPs. However, no differences were observed in sex, age, symptoms severity, and molecular recognition profile of the individual LTPs studied among these patients.

Clinical outcome data

Clinical symptoms and molecular profile

When examining individual LTPs, we observed an increased frequency of reactivity with each step indicating a worsening in clinical severity upon ingestion of LTP-containing food. As shown in **table II**, the linear-by-linear association was highly statistically significant, except for the LTP_2s (Api g 6 and Sola l 6) and Tri a 14. The prevalence of every single LTP reactivity was particularly pronounced in patients with urticaria/angioedema or anaphylaxis compared to those with oral allergy syndrome (*i.e.*, the reactivity to Mal d 3 was observed in 88% of patients experiencing anaphylaxis, whereas it was present in only 46% of those diagnosed with OAS). When considering molecules associated with a high risk of severe reactions, it is important to note that the absence of specific IgE reactivity is highly indicative of tolerance to that specific source. Specifically, Ara h 9, Cor a 8, and Mal d 3, which showed the strongest linear-by-linear association, were rarely positive among tolerant subjects (15%, 9%, and 20%, respectively, **table II**).

Clinical symptoms and cofactors

We then examined the association of several cofactors of interest with the clinical outcome in the 346 symptomatic patients, categorized as "mild" (oral allergic syndrome) and "severe" (urticaria/angioedema and anaphylaxis). As presented in **table III**, the recognition of more than 5 LTPs was significantly associated

Table II *- The distribution of patients tolerating LTP-containing foods or showing adverse reactions are divided by gender and molecular reactivity.*

	Tolerant	Oral Allergy Syndrome	Urticaria Angioedema	Anaphylaxis	χ^2 linear-by-linear	P-value
No	80 (19%)	144 (34%)	133 (31%)	69 (16%)		
Male	40 (9.40%)	65 (15.30%)	48 (11.30%)	34 (8.00%)		
Female	40 (9.40%)	79 (18.50%)	85 (20.00%)	35 (8.20%)		
Act d 10	18%	32%	54%	64%	45.91	< 0.0001
Api g 2	10%	21%	40%	65%	62.36	< 0.0001
Api g 6	5%	15%	20%	20%	8.66	< 0.005
Ara h 9	15%	40%	72%	87%	105.54	< 0.0001
Art v 3	14%	22%	41%	57%	41.6	< 0.0001
Can s 3	5%	15%	26%	38%	29.45	< 0.0001
Cor a 8	9%	26%	58%	78%	101.24	< 0.0001
Jug r 3	6%	22%	43%	59%	61.68	< 0.0001
Mal d 3	20%	46%	83%	88%	109.25	< 0.0001
Pla a 3	19%	44%	62%	81%	66.98	< 0.0001
Pru p 3	46%	72%	91%	93%	59.68	< 0.0001
Sola l 6	8%	12%	13%	19%	3.98	< 0.05
Tri a 14	3%	6%	6%	9%	ns	
Vit v 1	13%	33%	58%	77%	79.83	< 0.0001
Zea m 14	33%	49%	74%	87%	62.1	< 0.0001

Within each clinical group (tolerant, OAS, urticaria/angioedema, or anaphylaxis), the percentage of patients reacting to each LTP studied is given. The linear relationship between all LTPs studied in alphabetical order and the clinical outcome is given molecule by molecule with chi-square values and significance.

Table III - *The demographic and clinical characteristics of the study population are presented.*

		LTP symptomatic patients											
				Severe		Mild							
Variable	**Level**	**n**	**%**	**n**	**%**	**n**	**%**	**ORc**	**95%CI**	**P-value**	**ORadj**	**95%CI**	**P-value**
Overall		346	100%	202	58%	144	42%						
> 5 LTP molecules	Yes	213	62%	160	79%	53	37%	1			1		
	No	133	38%	42	21%	91	63%	6.541	4.0-10.5	< 0.001	17.512	8.8-34.5	< 0.001
LTP Mono reactivity	Yes	190	55%	135	67%	55	38%	1					
	No	156	45%	67	33%	89	62%	3.261	2.1-5.1	< 0.001			
PR10 Cosensitization	Yes	103	30%	38	19%	65	45%	1					
	No	243	70%	164	81%	79	55%	0.282	0.2-0.5	< 0.001			
Profilin Cosensitization	Yes	44	13%	18	9%	26	18%	1					
	No	302	87%	184	91%	118	82%	0.444	0.2-0.8	< 0.001			
FDNIH	Yes	49	14%	38	19%	11	8%	1			1		
	No	297	86%	164	81%	133	92%	2.802	1.4-5.7	< 0.005	4.1118	1.6-10.6	< 0.005
FDEIA	Yes	46	13%	44	22%	2	1%	1			1		
	No	300	87%	158	78%	142	99%	19.77	4.7-83.0	< 0.001	71.398	13.9-366.9	< 0.001
Atopic Dermatitis	Yes	71	21%	24	12%	47	33%	1			1		
	No	275	79%	178	88%	97	67%	0.278	0.2-0.5	< 0.001	0.3006	0.1-0.6	< 0.003
Bronchial Asthma	Yes	67	19%	32	16%	35	24%	1					
	No	279	81%	170	84%	109	76%	0.586	0.3-1.0	< 0.05			

Multiple logistic regression analysis including age, sex, and all cofactors shows a significant association between the presence of more than 5 nsLTPs, FDEIA, and FDNIH and a significantly higher risk of severe LTP-related food allergy. On the other hand, the presence of atopic dermatitis is associated with a less severe course.

with an increased risk of severe reactions, as well as mono-reactivity to LTP or the presence of other cofactors such as exercise or NSAID intake. If the 5 positive molecules were Ara h 9, Cor a 8, Zea m 14, Mal d 3, and Pru p 3, the risk of severe and/or anaphylactic reactions was even higher (χ^2= 19.196, p < 0.0001; OR = 7.272 (2.4-21.9)). Conversely, sensitization to PR10, Profilin, or the presence of atopic dermatitis (both moderate and severe) showed an inverse association and may therefore be considered a protective factor in LTP allergy.

Multiple logistic regression analysis, when incorporating all the cofactors together with age and sex into the model, confirmed the individual findings observed for the presence of more than 5 molecules simultaneously, FDEIA, FDNIH, and atopic dermatitis (see adjusted odds ratios, ORadj, and respective significances in **table III**). It is worth noting that PR10 reactivity, which is strongly associated with atopic dermatitis, did not reach statistical significance in the final model.

Discussion and conclusions

Key results

Although we studied a large number of LTP molecules, including both LTP1 and LTP2, Pru p 3 remains the molecule most commonly associated with LTP allergy, as it yielded a positive result in 78% of patients, making it the most suitable molecule of all those tested to detect LTP sensitization.

However, due to its broad reactivity, Pru p 3 seems to be less effective in detecting patients at higher risk for severe reactions to LTP than specific sensitizations such as Cor a 8, Mal d 3, or Ara h 9, at least considering the multiplex system used in this study. At the same time, all these molecules as well as Pru p 3 showed significantly higher IgE levels in patients with severe adverse reactions (25, 26). This observation suggests that a comprehensive evaluation of the LTP reactivity profile is useful for distinguishing the different clinical phenotypes observed in LTP syndrome.

LTPs are homologous molecules with a structured identity that largely depends on the taxonomic relationships between the sources of origin. Therefore, botanically related molecules will have a higher identity and a higher likelihood of IgE cross-reactivity compared to taxonomically distant molecules. Interestingly, proportions greater than 60 per cent are rarely recorded, indicating that LTPs are homologous molecules with low structural identity, unlike other panallergens (*i.e.*, profilins) (27). Upon comparing the prevalence of individual LTPs as determined by the macroarray with the structural identity information documented in the IUIS database, we encountered instances of complete concordance as well as complete discordance between the two measurements. The total disagreement may be due to the hook effect of the corecognition of other molecules, even if taxonomically unrelated, but widely detected in our LTP cohort. For instance, Pru p 3 or Mal d 3 exhibited positive reactivity in up to 90% of Can s 3 or Act d 10 reactors, despite exhibiting low sequence identity with these molecules. This leads us to hypothesize that some sensitizations to homologous molecules belonging to taxonomically distant biological sources may result from independent sensitizations (for example, Act d 10 of kiwi and Pru p 3 of peach). Therefore, the prevalence of molecular recognition within a specific group of homologous proteins is not always determined solely by sequence identity and may be influenced by the recognition of other molecules, even those not currently available for *in vitro* evaluation, which can result in unexpected recognition rates.

One of the main goals of this study was to comprehensively evaluate the mutual relationship of several known cofactors in the same population of patients with LTP sensitization. Among all the known cofactors, the 5-molecule cut-off proves to have the highest combination of sensitivity and specificity, both considering reactivity to Pru p 3 and the clinical outcome of patients. It proves to be a useful tool for the rapid classification of LTP reactors. Once again, Pru p 3 hypersensitivity is widely expressed in patients with both more and fewer than 5 molecular recognitions, so it does not allow discrimination between the two groups. However, some molecules that are scarcely detected in subjects showing IgE reactivity to less than 5 LTPs are widely represented in > 5 LTPs reactors and may represent a further useful tool for the immediate discrimination of patient's subsets (*i.e.*, Ara h 9, Mal d 3, Zea m 14, or Vit v 1). It is noteworthy that most subjects with FDEIA and/or FDNIH fall into the group with more than 5 molecules, and it is possible to hypothesize that the two factors have an additive effect, as shown by the multiple logistic regression analysis.

Another crucial point is the importance of conducting a comprehensive evaluation of these patients, which should also involve testing for reactivity to other plant panallergens, such as profilins or PR10. This is because the presence of such reactivities allows the identification of patients with a more favorable prognosis (18, 21). Notably, this pattern of poly-reactivity is specific to patients observed in northern European territories (3, 14).

LTP sensitization is a characteristic of the more severe form of atopic dermatitis, as previously described (20). However, it is typically not associated with anaphylactic reactions in these patients. The discovery of this reactivity in patients with generalized and severe forms of atopic dermatitis suggests that LTP sensitization may contribute to a delayed re-exacerbation of T cell-driven dermatitis rather than immediate IgE-mediated symptoms, particularly in patients from the Mediterranean area where dietary habits often involve high consumption of plant foods. Probably, in these patients, sensitization occurs via a disrupted skin barrier, as demonstrated for peanut allergy in the U.S.A. (28), rather than via the gastrointestinal tract. It is well known that sensitization does not always correlate with clinical symptoms.

Limitations

The clinical data of the patients were primarily based on self-reported history, and no food challenge was conducted. This selective criterion may not have affected the subset of patients who consistently tolerate LTP-containing foods. However, it is important to emphasize that the occurrence of severe food reactions (anaphylaxis) was always confirmed by emergency room records documenting the administration of adrenaline and steroids for symptom management.

The simultaneous presence of reactivity to PR10 and atopic dermatitis does not enable discrimination regarding which of the two factors plays a central role in mitigating the adverse effects of LTP reactivity in certain patients.

Finally, in this study, we did not establish causality but solely reported the association between cofactors (exercise, intake of NSAIDs, sensitization to other panallergens, presence of atopic dermatitis, *etc.*) and the consumption of LTP-containing foods.

Generalisability

Simultaneous proteomic evaluation of multiple LTPs that are homologous but not identical molecules is useful to reveal the presence of sensitization profiles associated with different clinical subgroups. The clinical severity of the LTP syndrome is associated with an expanded IgE repertoire characterized by increased numbers of recognized LTP components and elevated levels of specific IgE. Ara h 9, Cor a 8, and Mal d 3 showed the strongest association with clinical severity, and they were rarely detected in 'tolerant' individuals.

In addition, we investigated the potentially attenuating or exacerbating role of different cofactors within the same patient groups. We found that higher LTP detection capacity (5 molecules), even in combination with exercise and/or NSAID use, strongly correlated with a higher frequency of severe episodes.

In contrast, the concomitant presence of atopic dermatitis and hypersensitivity to other panallergens had a mitigating effect

on the incidence of systemic or anaphylactic reactions in our patient cohort.

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Contributions

ES, EC, VV, MG: investigation, data collection. ES, VV, MG: reosurces. ES, DA: formal analysis. ES, DV, LC, VP, RA: conceptualization, data curation.

Conflict of interests

ES has received consultant arrangements and speakers' bureau participation from DASIT and Thermo Fisher Scientific. The rest of the authors declare that they have no conflict of interests.

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LETTER TO THE EDITOR

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Rita Brás[1], Rita Limão[1], Elisa Pedro[1], Anabela Lopes[1,2]

Pruritus burden assessment in severe atopic dermatitis patients under dupilumab: response predictor?

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Key words

Anti-IL-4/IL-13; atopic dermatitis; dupilumab; pruritus.

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To the Editor,

pruritus burden in atopic dermatitis (AD) is underestimated (1). Dupilumab significantly reduced pruritus in clinical trials (2-5). We aimed to characterize pruritus burden in severe AD patients and to analyze dupilumab's effect on skin pruritus.

Prospective study including AD patients with 52 weeks (w) of dupilumab of a Portuguese tertiary hospital. The study was approved by the hospital's ethics committee and a written informed consent was obtained for every patient. Itch Severity Scale (ISS) and Pruritus Numerical Rating Scale (NRS-P) were applied at 0, 2, 4, 16, 24 and 52 w of treatment to assess pruritus severity. Correlations with SCORAD/EASI, DLQI and NRS for sleep disturbance (NRS-SD) at the same time-points, were investigated. Subgroup analyses of ISS and NRS-P in patients reaching < 50% or ≥ 50% improvement in EASI at 4 w, and patients reaching < 75% or ≥ 75% improvement in EASI at 16 w, were also performed. Statistical analyses were performed with IBM SPSS software (v25.0). P-values < 0.05 were considered statistically significant.

Total of 16 patients, 69% female, mean age 36.5 ± 12.2years [17-60], mean AD duration 31.5 ± 10.2 years [14-48], 94% had allergic rhinitis, 63% asthma, 31% allergic conjunctivitis and 25% food allergy. Median TIgE 7863U/mL [254-27,365] and median Eos 570/mm^3 [60-2,020].

Considering baseline pruritus characterization, the majority of patients (n = 15; 94%) reported worsening by night, describing their itch mostly as "annoying" (n = 15; 94%), "unbearable" (n = 14; 88%) and "worrisome" (n = 13; 81%). All body surface areas were implicated. When evaluating pruritus in its average state, 10 (63%) patients considered it "strong", while in its worst state 15 (94%) patients gave a classification of "very strong". Disturbances of mood (n = 15; 94%), particularly agitation and trouble in concentration (n = 10; 63%), were reported. Sexual activity was also impacted, with 56% (n = 9) complaining of lower sexual desire and 38% (n = 6) impaired sexual function. All patients considered pruritus led to difficulty in sleeping, with 81% (n = 13) also reporting awakenings and 69% (n = 11) needing medication to sleep (considering sleep disturbance related to pruritus).

Analyses of both pruritus scales (ISS/NRS-P) evolution during dupilumab treatment revealed a significant reduction since 2 w (**figure 1A**), with ISS improving from 15.3 points (median) at baseline, to 10.8 points at 2 w and 3.4 points after 52 w, and NRS-P changing from 7.5 points (median) at baseline, to 6 points at 2 w and 2 points after 52 w. Considering ISS, all items improved significantly (**figure**

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1B), with all patients reporting no mood affection or sleep disturbance related to pruritus at 52 w. Subgroup analyses of patients reaching < 50% or ≥ 50% improvement in EASI at 4 w, and patients reaching < 75% or ≥ 75% improvement in EASI at 16 w, revealed a significant reduction in ISS (**figure 1C.1,2**) and NRS-P (**figure 1C.3,4**) for both subgroups in each timepoint (4 w and 16 w), including those patients with lower severity indexes improvement. ISS showed correlations with NRS-P (r = 0.555, p = 0.026) and DLQI (r = 0.655, p = 0.045). NRS-P also correlated with DLQI (r = 0.550, p = 0.042). Correlation of both pruritus scales with NRS-SD did not reach statistical significance, as well as with severity indexes.

AD has one of the highest pruritus prevalence and intensity (6), although studies addressing its burden, particularly in severe AD patients, are scarce. Clinical trials (2, 3, 5), as well as real-life studies (7, 8) reported pruritus improvement in severe AD patients treated with dupilumab using only NRS-P. We also applied ISS to assess pruritus severity as this seven-item questionnaire (9) complements pruritus qualitative evaluation, covering other domains (body extension, effects on mood, sexual activity and sleep) (10). Although further studies with larger samples are needed to allow extrapolations of results, both scales showed good correlation with each other and with QoL, highlighting pruritus rapid and significant improvement in severe AD patients treated with dupilumab. The authors emphasize the importance of pruritus burden assessment as it may contribute to identify early treatment responders, especially in patients with lower severity indexes' improvement, as well as to improve patients' motivation to therapeutic adherence.

Fundings

None.

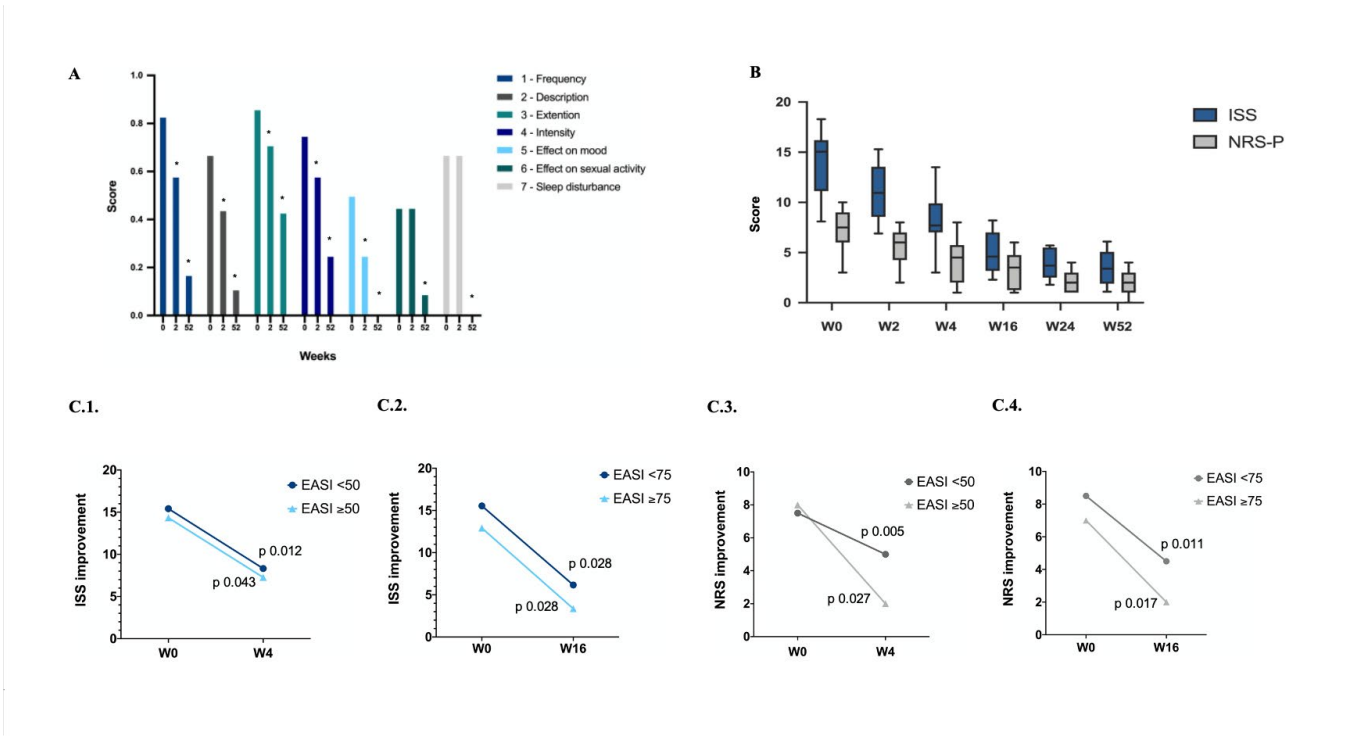
Contributions

RB, AL: conceptualization. RB, RL: data curation. RB, RL: formal analysis. RB: writing – original draft. RL, EP, AL: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

Figure 1 *- Pruritus improvement using ISS (Itch Severity Scale) and NRS-P (Pruritus Numerical Rating Scale).*



(A) ISS items' scores evolution – weeks 0, 2 and 52 of Dupilumab treatment. * p < 0.05; **(B)** ISS and NRS-P scores evolution during treatment with Dupilumab. All values were statistically significant (p < 0.05); **(C)** ISS (C.1-2) and NRS-P (C.3-4) improvement in patients reaching < 50% or ≥ 50% improvement in EASI at 4 w, and patients reaching < 75% or ≥ 75% improvement in EASI at 16 w, in these time courses.

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LETTER TO THE EDITOR

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Probable apixaban-induced purpura

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Key words

Apixaban; purpura; cutaneous leukocytoclastic vasculitis; direct oral anticoagulants.

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To the Editor,

the factor X inhibitor apixaban is used in atrial fibrillation and venous thromboembolism with an acceptable safety profile.
A 59-year-old woman was started on apixaban (10 mg bid) because of pulmonary embolism.
The patient, who signed an informed consent, reported a history of hypertension, vocal cord paralysis, chronic respiratory failure, and hypothyroidism for which ramipril, amlodipine, and levothyroxine had been prescribed. She had no known drug allergies and there was no family history notable for autoimmune, allergic, and cutaneous diseases.
After 2 days of treatment with apixaban, she complained of painful, swollen, and warm knees and we noted palpable and painful purpura involving both the lower limbs below the knees. The patient had no other symptoms, vital signs were normal, and physical examination was otherwise unremarkable.
Laboratory investigations, including complement levels, an autoimmunity screening, cryoglobulins, and search for hepatitis B and C viruses, revealed no atypical findings. C-reactive protein was elevated to 19.7 mg/dl. A duplex ultrasound showed no thrombosis or obstruction.
The patient denied permission for a skin biopsy. Apixaban was discontinued and enoxaparin and prednisone were initiated. Arthritis quickly resolved and the purpura progressively faded and disappeared on the 24th day. Prednisone was tapered off and the anticoagulant treatment was continued with the factor II inhibitor dabigatran due to the ongoing embolic risk. At the time, the patient had no relapse of arthritis or purpura and did not experience any other adverse events. At follow-up 2 months later, she was doing well while still on dabigatran. The cause of pulmonary embolism remained undetermined.
The clinical diagnosis was apixaban-induced purpura. Taken together, however, findings in this case were most consistent with cutaneous leukocytoclastic vasculitis (CLV) even though this was not confirmed by pathology examination because the patient refused to undergo a skin biopsy (1). Presentation, clinical features, laboratory findings, and the resolution of purpura with glucocorticoids support our view that leukocytoclastic vasculitis was the underlying immunopathologic process.

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The temporal clustering and scoring on the Naranjo Scale, that standardizes causality assessment for adverse drug reactions, indicated a probable causal relationship between apixaban and the adverse event (2). We ruled out other causes upon history, clinical examination, laboratory and imaging investigations. The patient had no systemic manifestations and did not relapse during treatment with dabigatran.

There are reports of purpura and CLV associated with the direct oral anticoagulants (DOACs) apixaban, rivaroxaban, and dabigatran (3-6). The key dimensions of the problem are unknown, and findings of the post-marketing surveillance suggest that rivaroxaban might be associated with a greater risk of CLV as compared to other DOACs (7). Available data do not support the hypothesis of CLV as a class-specific side-effect of DOACs (8). It is unclear if older patients with comorbidities could have a worse clinical outcome with prolonged disease course and complications. The pathogenesis of apixaban-induced CLV is poorly understood. Molecular mimicry between apixaban and vascular endothelium antigens, endothelial injury due to immune activation and release of proinflammatory cytokines triggered by apixaban, and hypersensitivity to circulating immune complexes that contain apixaban on the vessel wall may be at play.

Our patient had no flare-up of purpura during treatment with dabigatran. This suggests that patients with apixaban-induced CLV may be safely treated with an alternative DOAC. However, cross-reactivity between DOACs has been reported based upon clinical findings and results of patch testing (9, 10).

Clinicians should be aware of the potential risk of purpura and CLV in patients treated with apixaban.

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Contributions

GF: conceptualization, data curation, writing - original draft, writing - review & editing. FC: investigation. GF, FC: project administration.

Conflict of interests

The authors declare that they have no conflict of interests.

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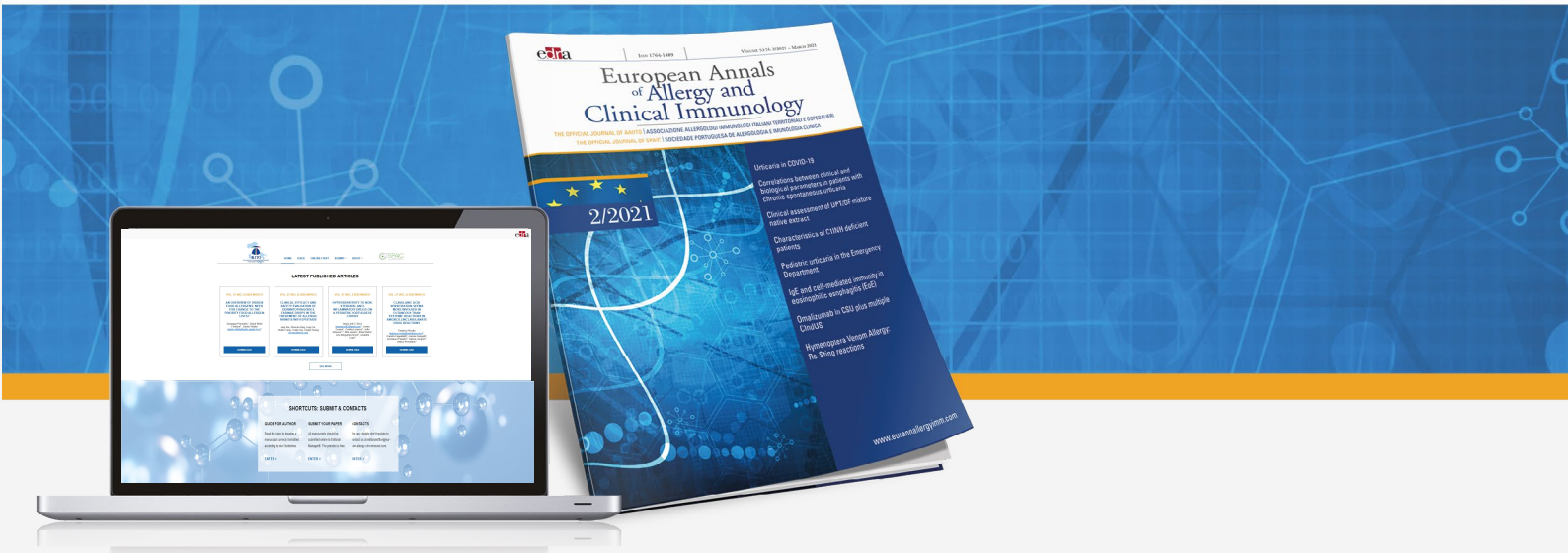

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