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# Lipid transfer protein syndrome: How to save a life through careful education

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## ABSTRACT

**Introduction:** Lipid transfer proteins (nsLTPs) are ubiquitous allergens. Patients affected by nsLTP syndrome experience symptoms to various plant-derived foods, ranging from local manifestations to anaphylaxis, the critical treatment of which is represented by self-administration of adrenaline. The principle aim of this study is to assess how dietary recommendations influence the occurrence of new and severe cases and if poly-sensitization to different nsLTPs may play a role. We also investigated about the appropriate use of adrenaline auto-injector during the episodes of anaphylaxis. Moreover, we examinated how other features (ie, co-sensitization to profilin and PR-10 and the presence of risk co-factors) affect these events.

**Materials and methods:** We evaluated 78 patients allergic to nsLTPs, investigating adherence to diet and ability to use the adrenaline auto-injector. Number of sensitization to nsLTPs, co-sensitization to other panallergens, and presence of risk factors for new reactions were also assessed. Diagnosis was based on clinical history and positivity to *in vivo* and *in vitro* tests. During the follow-up, compliance, diet modifications, and new reactions were noted, and re-training for the use of epinephrine auto-injector was performed. At the last visit we evaluated the patients' ability to use the self-injector.

**Results:** The whole of fruits belonging to the *Rosaceae* family emerged as the most frequent culprit foods (28%), followed by walnut (17%), peanut (17%), and hazelnut (10%). At the baseline visit 23% of the patients described the presence of a risk factor during the allergic reaction (mainly nonsteroidal anti-inflammatory drugs [NSAIDs] and exercise). Forty-five percent of the patients reported anaphylactic reactions; no association between the type of food and the severity of the reactions was found. The presence of sensitization to 4 or more nsLTPs was associated to more severe reactions ($p < .05$; OR 1.67). During the follow-up 38% of the patients experienced at least 1 new allergic reaction: in 79% of them the culprit food was previously tolerated, and in 69% the reaction was an anaphylaxis. Only 47% of the patients showed a proper use of adrenaline auto-injector during the final evaluation, but a significant correlation between periodic education and reduction of the probability of mistakes in the use was reported ($p < .05$; OR 0.34). Furthermore, an association between co-sensitization to PR-10 (in particular Bet v1) and profilin and less severe symptoms was found, but without a significant odds ratio.

**Conclusion:** A careful education aimed to the prevention of new reactions, through dietary restrictions and avoidance of risk co-factors, and to the management of anaphylaxis, through the

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training for the correct use of adrenaline auto-injector, should be a routine practice in nsLTP syndrome.

**Keywords:** Food allergy, LTP syndrome, Co-factors, Anaphylaxis, Adrenaline self-injector

## INTRODUCTION

Lipid transfer proteins (nsLTPs) were recognised for the first time by *in vitro* experiments in 1985, as ubiquitous allergens occurring in various plant species.[1] They are currently known as the primary cause of IgE-mediated food allergy and food-induced anaphylaxis in adults living in the Mediterranean area.[2,3] Thanks to their resistance to heat and pepsin digestion, nsLTPs are able to reach the bowel without undergoing modifications, with the peach nsLTP (Pru p 3) playing an important role as a sensitizer.[4] Due to their widespread distribution in the plant kingdom and to the high homology between nsLTPs of taxonomically unrelated plant foods or pollen,[5] patients sensitized to nsLTPs may experience allergic symptoms to a wide range of different vegetable foods. The so-called "nsLTP syndrome" may range from local manifestations, such as mild contact urticaria, oral allergy syndrome (OAS), or gastrointestinal issues, up to anaphylaxis and even anaphylactic shock.[6–8] In particular, anaphylaxis is a life-threatening reaction caused by hypersensitivity to an allergen, which onsets in minutes or hours and involves at least 2 districts (ie, skin and mucosae, airways, gastrointestinal tract, and cardiovascular system) after the exposure to a probable allergen, or it results in a sudden pressure decrease after the exposure to a known culprit allergen.[9] More than one-third of anaphylactic episodes in Italy is related to nsLTPs, likely to occur at a younger age than in other food allergies.[2] The main culprit responsible for nsLTP-related food induced anaphylaxis (nsLTP-FIA) is represented by *Rosaceae* family, especially peach, but also apricot, apple, pear, and many other stone fruits, followed by nuts (walnut, hazelnut, cashew nut, and peanut) and other vegetables (wheat, tomato, lettuce, maize, green bean, goji berry, eggplant, sunflower seed, and flaxseed).[10,11] Moreover, many co-factors, ie, exercise and non-steroidal anti-inflammatory drugs (NSAIDs), have proved to be involved in nsLTP-FIA.[10]

Such features make nsLTP syndrome a challenge for the allergist: while the diagnosis, based on clinical presentation, timing of the reaction, and analysis of the possible culprit food is somewhat easy, the long-term management of these patients is rather complex. In order to prevent new allergic reactions, particularly anaphylaxis, the allergist has to decide on looser or stricter dietary restrictions, taking also into account that even new reactions to previously tolerated foods may arise over time. As self-administered adrenaline is the main out-of-hospital treatment for anaphylaxis, patients who experienced an episode of nsLTP-FIA even just once in a lifetime should be provided with adrenaline auto-injector.[9]

The principal purpose of this study was to investigate, in the long term, how specific educational methods and tools provided at the baseline visit and in the subsequent ones, such as dietary restrictions and avoidance of risk co-factors, may influence the occurrence of new reactions in nsLTPs allergic patients. Particularly, we tried to understand whether severe reactions are related to the type of food allergen or to multiple sensitization to nsLTPs. Another main aim was to assess the ability of the patients to properly use the adequate treatment for anaphylaxis, epinephrine self-injector, after a training. Other aims of the study were to verify the outbreak of novel plant food allergies resulting in new anaphylactic episodes and to analyse the impact of the concomitant sensitization to other panallergens and of some co-factors which may increase the risk for new or more severe reactions.

## MATERIALS AND METHODS

### Type of the study

We performed a retrospective observational study on 78 patients (51 females, 27 males) with a mean age of 54 years, who presented in eleven

years (2010–2021) at the Allergy Unit of the Hospital of Parma (Azienda Ospedaliero-Universitaria di Parma). Follow-up visits were performed 20–24 months after the former evaluation, with a follow-up's average of 71 months. The study was approved by the ethics committee of the Hospital of Parma (793/2020), and all patients signed an informed consent form.

## Selection of the patients

The patients were selected on the basis of the diagnosis of nsLTP syndrome characterized by the presence of all of the following features: a) a suggestive clinical history (onset of local or systemic symptoms after ingestion of nsLTP containing food); b) positive response to *in vivo* tests, such as skin prick tests (SPTs) executed with commercial extracts of peach-LTP, peanut, walnut, almond, and wheat, or prick-by-prick tests carried out with genuine suspected foods, when that specific extract was not available on commerce; c) *in vitro* confirmation, defined by a value of over 0.1 kU/L of specific IgE for at least 1 of the 5 LTPs tested (peach - rPru p3, peanut - rAra h9, walnut - rJug r3, almond - rCor a8, wheat - rTri a14) measured by ImmunoCAP™.

Criteria of exclusion were concomitant sensitization to other heat-stable and gastric-stable proteins (ie, seed storage proteins and tropomyosin), minor age, and pregnancy.

### Evaluation of the clinical history

At the first visit, patients underwent an interview to define the clinical presentation of the allergic reaction, and to identify the possible culprit food. Reported symptoms were classified as local manifestations, such as contact urticaria, oral allergy syndrome (OAS) and isolated gastrointestinal manifestations (vomiting, nausea, and/or diarrhea), and systemic manifestations, comprising urticaria/angioedema, and anaphylaxis or anaphylactic shock. The reactions were considered food-induced if they occurred within 2 hours after the ingestion of the potential culprit food.

Given that in subjects sensitized to other panallergens of plant-foods, such as profilin and/or PR-10 (ie, Bet v1), symptoms occur especially when they eat raw vegetables, in the cases of concomitant sensitization to these panallergens and nsLTPs we focused only on the reactions occurring after the ingestion of cooked or processed foods.

Additionally, the therapies administered after the onset of anaphylaxis and any access to the emergency department were noted.

The presence of risk co-factors, such as NSAIDs, exercise, alcohol, fasting, and menstrual cycle has also been investigated. Moreover, the presence of comorbidity, as asthma and cardiovascular diseases, and concomitant therapies (ie, betablockers or ACE-inhibitors) was noted.

### In vivo tests: skin prick tests (SPTs) and prick-by-prick tests (PTPs)

During the first visit we performed skin prick tests (SPTs) to a standard panel of food allergens (egg, milk, shrimp, cod, soy, and tomato) and to nsLTP-containing foods (peach-LTP, walnut, almond, hazelnut, and wheat). Such tests were carried out with commercial extracts (ALK). Moreover, profilin and birch extracts were used to evaluate eventual co-sensitization to other panallergens (profilin and/or Bet v1/PR-10). In selected patients, when specific foods had to be investigated, if available, SPTs with that specific commercial extract (ALK) were performed; when it was not available, prick-by-prick tests (PTPs) with fresh food were carried out. SPTs were performed on the volar side of the forearm with a different sterile 1 mm-tip lancet (ALK) for every type of extract, pricking through the drop of each one. PTPs were carried out by pricking in a first moment the fresh food and then the skin of the patients. In both cases a SPT with histamine 10 mg/ml as reference as positive control and a SPT with physiological solution as negative control were taken. Readings were taken after 20 minutes, and results were considered positive when a wheal of at least 3 mm appeared.[12,13]

### In vitro tests

Serum specific IgE levels to rPru p3 (peach-LTP), rAra h9 (peanut-LTP), rJug r3 (walnut-LTP), rCor a8 (almond-LTP), rTri a14 (wheat-LTP), rBet v1 (the major birch pollen allergen, as representative of the PR-10 allergens family), and rPhl p12 (profilin of grass, as representative of profilins) were measured by ImmunoCAP™ (ThermoFisher Scientific) by following manufacturer recommendations. Values

were expressed in kU/l and when > 0.1 kU/l they were considered as positive.

### Educational tools proposed

At the end of the baseline visit, patients were given dietary recommendations: they were told to avoid the food responsible for the reaction and also fruits or nuts of the same family (ie, avoiding *Rosaceae* if the patient experienced reaction to peach or apricot). They were also provided of an information sheet reporting an extended list of the main vegetable foods containing nsLTPs and were informed of the risk of future reactions to other foods containing nsLTPs.

The role of co-factors (exercise, NSAIDs, alcohol, menstrual cycle, and fasting) facilitating the outbreak of a nsLTP-FIA was also explained to the patients, highlighting the importance to avoid the association with possible sensitizing foods.

At the end of the visit, patients were prescribed adrenaline auto-injector (Jext 300 mcg, ALK) and educated on the indications for its use. A particular focus was put on the recognition of the onset of an anaphylactic reaction, requiring epinephrine as treatment, in comparison to less severe forms of allergic reaction, which can be treated through the administration of anti-histamines and corticosteroids. In order to facilitate the understanding of the concept of anaphylaxis, verbally explanations and an action plan conceived on the basis of the World Allergy Organization (WAO) guidelines published at the time were provided.[14] Finally, patients were trained for the use of the auto-injector through a practical demonstration performed by 1 of the 2 dedicated physicians, with a trainer injector supplied by manufacturers (Jext Trainer, ALK), identical to the original but without needles and medication. Then, every patient was invited to repeat that sequence of acts themselves. Also in this case, an information sheet was released and online references to videos about the use of epinephrine auto-injector were suggested.

### Follow-up

In the follow-up years (from 2010 to 2021, with an average of 71 months), patients were re-evaluated every 20–24 months, by analysing verbal questionnaires regarding the compliance to the prescribed dietary regimen and possible spontaneous changes in their dietary habits. Moreover, they were re-trained in the use of the self-injector at every follow-up visit. Reactions (local and/or systemic) that had occurred between the visits were noted, focusing again on the culprit food (same or new), the presence of co-factors, and the patient's behaviour, mainly evaluating the eventual use of the adrenaline auto-injector. In the last follow-up visit we verified the patients' capacity to properly use the device by means of the same trainer auto-injector used at the baseline visit. The examination was based on 4 steps: 1) correct removal of the safety cap; 2) proper handling of the auto-injector; 3) location if the mid-antero-lateral thigh as the site of injection; and 4) holding of the auto-injector in place at least for 10 seconds.[15]

### Statistical analysis

Statistics proportions were compared by chi-square test with Yates' correction.

Specific IgE levels were compared by two-tailed Student's t-test. Probability values $< 0.05$ were considered statistically significant. Whenever a significant association was assessed, binomial and logistic regressions were further used to assess the relationship between a categorical dependent variable and independent categorical or continuous variable respectively.

## RESULTS

Baseline clinical features of the patients, the offending foods, clinical characteristics of allergic reactions, and eventual co-factors, are summarized in Table 1.

At the first visit, the whole of peach and other fruits of the *Rosaceae* family emerged as the most frequent cause of symptoms (22/78; 28%), tying for second place were walnut (13/78; 17%) and peanut (13/78; 17%), followed by hazelnut (8/78; 10%) and wheat (2/78; 3%); 20/78 reactions (25%) were caused by other types of foods, mainly corn, grapes, fennel, sunflower seeds, mango, kiwi, and pomegranate. Following the consumption of nsLTPs containing foods, 35/78 patients (45%) reported a history of anaphylaxis (4 of which experienced anaphylactic shock), 37/78 (47%) patients reported muco-cutaneous symptoms (urticaria/

**Sex**

| **Male** | **Female** |
|---|---|
| 27 | 51 |

| **Mean age** |
|---|
| 54 years |

| **Comorbidities** | | | |
|---|---|---|---|
| **Asthma** | | **Cardiovascular diseases** | |
| Number of patients 18 | 23% | Number of patients 6 | 8% |
| | | ACE-inhibitors 2 | 2,5% |
| | | Beta-blockers 4 | 5% |

**Number of sensitizations to nsLTPs**

| **1 nsLTP** | **2 nsLTPs** | **3 nsLTPs** | **4 nsLTPs** | **5 nsLTPs** |
|---|---|---|---|---|
| 11 (14%) | 12 (15%) | 25 (32%) | 23 (30%) | 7 (9%) |

| **Culprit food at the first reaction** | | |
|---|---|---|
| | Number of patients | |
| *Rosaceae* | 22 | 28% |
| Walnut | 13 | 17% |
| Peanut | 13 | 17% |
| Hazelnut | 8 | 10% |
| Wheat | 2 | 3% |
| Other foods | 20 | 25% |

(continued)

| Symptoms of the first reaction | | | | | |
|---|---|---|---|---|---|
| **Local symptoms** | | | **Systemic symptoms** | | |
| | Number of patients | | | Number of patients | |
| OAS | 4 | 6% | Urticaria/angioedema | 37 | 47% |
| Contact urticaria | 1 | 1% | Anaphylaxis | 31 | 40% |
| Isolated gastrointestinal symptoms | 1 | 1% | Anaphylactic shock | 4 | 5% |
| **Defined co-factors** | | | | | |
| | Number of patients | | | | |
| Exercise | 8 | 10% | | | |
| NSAIDs[a] | 6 | 8% | | | |
| Alcohol | 4 | 5% | | | |
| Menstrual cycle[a] | 1 | 1% | | | |
| Fasting | 0 | 0% | | | |

**Table 1. (Continued)** Demographic and clinical characteristic of the patients. *Clinical presentation of the first reaction, culprit food and eventual co-factors. Abbreviations: nsLTPs, non-specific lipid transfer proteins; OAS, oral-allergic syndrome; NSAIDs, non-steroidal anti-inflammatory drugs. [a]One patient reported the co-occurrence of two co-factors: menstrual cycle and use of NSAID.*

angioedema), and 6/78 patients (8%) experienced only local symptoms (4 OAS, 1 contact urticaria, 1 gastrointestinal symptoms). Specific IgE to rPru p3 confirmed peach-LTP sensitization in 62/78 (79%) of the patients.

Only 11/78 of the patients (14%) emerged as mono-sensitized to nsLTPs at the baseline visit: 8 to peach-LTP, 2 to peanut-LTP, 1 to walnut-LTP. Remaining patients (67/78; 86%) emerged instead as poly-sensitized to nsLTPs, whose 12 sensitized to 2 nsLTPs (15%), 25 to 3 nsLTPs (32%), 23 to 4 nsLTPs (30%), and 7 to 5 nsLTPs (9%). The association between the type of food ingested, particularly peach, walnut, and peanuts, and the anaphylaxis at the first episode has been calculated, but no significant evidence has been reported (peach: $p$ = NS; walnut: $p$ = NS; peanuts: $p$ = NS). The IgE titles for nsLTPs showed no association to the severity of the reaction ($p$ = NS). The presence of 4 or more nsLTPs' sensitizations, instead, was associated to more severe reactions (19/35 vs 11/43; 54% vs 26%; $p < .05$). With a logistic regression, we estimated that probability of severe reactions was increased [odds ratio (OR) 1.67, 95% confidence interval (CI) 1.1–2.5, $p < .05$] for every nsLTP sensitization.

Concerning of the occurrence of new episodes despite dietary recommendations, 29/78 patients (37%) experienced during the follow-up years at least 1 new allergic reaction (Table 2), which consisted in anaphylaxis in 20/78 cases (26%), 4 of them being anaphylactic shock (5%). 3/78 patients (4%) experienced more than 1 episode of anaphylaxis. 24/29 patients (83%) experienced a new reaction with a previously tolerated food: in 19/29 cases (66%) the offending food was botanically unrelated to the food that caused the first reaction, in 4/29 (14%) it belonged to the same family, and 5/29 (17%) experienced reactions after the ingestion of the same original allergen; 1/29 patient (3%) reported 2 different reactions, 1 to an unrelated food and 1 with the original offending food. Considering only the subset of the new anaphylactic episodes, 16/20 of the patients (80%) manifested at least an anaphylactic episode to a different food from the original offending one. In detail, for 4/20 patients (20%) the culprit food was the same food of the first reaction; for 2/20 (10%) it was a different food belonging to the same family; in 13/20 cases (65%) it was a different food of a different family; and 1/20 (5%) experienced anaphylaxis caused by both the original culprit food and a food of a different family.

During the follow-up years a higher title of specific IgE did not show association to new reactions in the long term.

As for adherence to the diet, most of the patients (72/78, 92%) reported that over the years they maintained the avoidance of the culprit food, while 65/78 (83%) also maintained the avoidance of the fruits or nuts of the same family. A small group of patients (6/78, 8%) reported even avoidance of traces. From our data, the indication to avoid peach proved to be the one with the major adherence rate (73/78; 94%), even though we observed novel reactions to cherry and plums, as not all patients were aware of their membership in the *Rosaceae* family. Of the 6 patients who experienced new reactions after the ingestion of the original culprit food, during the interview 3 clarified that the ingestion was accidental (traces of the food in cakes or cream); 2 explained that sometimes they consumed the culprit food in small quantity (walnut and hazelnut). The last one experienced a reaction after the ingestion of unpeeled apple in fruit salad made by a friend; since he never experienced new reactions before with peeled apple, he did not ponder to ask the friend about the preparation of the food before eating it.

Among the 26/78 patients (33%) who experienced systemic symptoms, only 2 used the auto-injector while 3 other patients were administered adrenaline: 1 during the transport to the hospital, the other 2 in the emergency department. No deaths were recorded during the follow-up period.

Regarding the competence of the patients in the use of the auto-injector of adrenaline evaluated in the follow-up visits, 85% of the patients answered that they believed they knew the correct use of the auto-injector, while only the 48% of them (37/78) showed all 4 steps of auto-injector use correctly. The majority committed at least 1 error and the 4% failed in all 4 steps. Number and type of errors of the patients are listed in Table 3. The most common error (30/78; 38%) was not holding the auto-injector for at least 10 seconds in place; 19% of the patients (15/78) did not correctly pull off the safety cap; 21% (17/78) did not handle the

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| | Symptoms | Number of reactions | Culprit food | Use of self-injector | Emergency department? | Co-factor | Same food or not? | Positive IgEs title [kU/l] |
|---|---|---|---|---|---|---|---|---|
| 1 | OAS | 1 | Cherry | No | No | No | No, but the same family | Ara h9 0.12<br>Jug r3 0.16<br>Pru p3 1.06 |
| 2 | Anaphylaxis | 1 | Cherry | No | No | No | No, but the same family. | Ara h9 3.76<br>Cor a8 4.15<br>Jug r3 2.59<br>Pru p3 4.28<br>Tri a14 2.71 |
| 3 | Urticaria/ Angioedema | 1 | Chocolate with hazelnut | No | No | **NSAID** | No | Ara h9 1.63<br>Cor a8 0.90<br>Jug r3 1.14<br>Pru p3 4.34 |
| 4 | Anaphylaxis | 2 | Walnut, carrot | No | **Yes (adrenaline**) | **Fasting** | Yes (walnut)<br>No (carrot) | Ara h9 1.52<br>Pru p3 1.79 |
| 5 | Anaphylaxis | 1 | Hazelnut | No | No | No | No, but the same family | Ara h9 0.16<br>Cor a8 0.12<br>Jug r3 2.46<br>Pru p3 2.13 |
| 6 | Urticaria/ Angioedema | 2 | Persimmon<br>Sunflower seeds | No | **Yes (after consumption of sunflower seeds)** | **Fasting** | No | Ara h9 0.11<br>Pru p3 0.15 |
| 7 | Anaphylaxis | 1 | Melon | No | No | No | No | Ara h9 1.42<br>Cor a8 0.42<br>Jug r3 1.68<br>Pru p3 1.96 |
| 8 | Anaphylaxis | Multiple | Rosaceae, berries, grapes | No | No | No | No | Ara h9 1.80<br>Jug r3 1.30<br>Pru p3 11.90 |
| 9 | Anaphylaxis | 1 | Lupin bean | No | Yes | No | No | Ara h9 0.53<br>Cor a8 0.12<br>Jug r3 0.72<br>Pru p3 1.46 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 10 | Nausea, vomiting | 1 | Hazelnut | No | No | No | No, but the same family | Cor a8 1.96<br>Jug r3 4.59<br>Tri a14 0.47 |
| 11 | Urticaria/ Angioedema | 3 | Kiwi, plum, Strawberry | No | No | **Excercise** | No | Pru p3 11.90 |
| 12 | Anaphylaxis | 1 | Corn | No | **Yes (adrenaline)** | No | No | Ara h9 5.24<br>Jug r3 4.47<br>Pru p3 5.49<br>Tri a14 4.47 |
| 13 | Anaphylactic shock | 1 | Wheat | No, ma praticata dal MET | Yes | No | No | Ara h9 14.71<br>Cor a8 4.15<br>Pru p3 15.6 |
| 14 | Anaphylaxis | 1 | Cherry | No | No | No | No | Ara h9 3.09<br>Jug r3 2.44<br>Pru p3 4.39<br>Tri a14 0.70 |
| 15 | Anaphylaxis, OAS | 2 | Peach, fennel | No | No | No | No | Ara h9 4.01<br>Jug r3 3.04<br>Pru p3 2.23 |
| 16 | Anaphylaxis | Multiple | Grapes, apple, hazelnut, almond | **Only once** | No | No | No | Ara h9 5.02<br>Cor a8 2.07<br>Jug r3 4.09<br>Pru p3 8.11 |
| 17 | Anaphylaxis | 1 | Wheat | **Yes** | Yes | No | No | Ara h9 0.57<br>Cor a8 0.48<br>Jug r3 0.53<br>Pru p3 4.11 |
| 18 | Anaphylaxis | 1 | Lettuce | No | No | No | No | Jug r3 1.21<br>Pru p3 1.52 |
| 19 | Anaphylaxis | 1 | Almond | No | No | No | No | Ara h9 6.12<br>Cor a8 5.51<br>Jug r3 5.23<br>Pru p3 10.91 |

(continued)

| | Symptoms | Number of reactions | Culprit food | Use of self-injector | Emergency department? | Co-factor | Same food or not? | Positive IgEs title [kU/l] |
|---|---|---|---|---|---|---|---|---|
| 20 | Anaphylactic shock | 1 | Hazelnut | No | Yes | No | Yes | Ara h9 7.02<br>Cor a8 4.42<br>Jug r3 2.14<br>Pru p3 9.02 |
| 21 | Anaphylaxis | 1 | Tree nuts mix | No | No | **Excercise** | Yes | Ara h9 0.81<br>Jug r3 0.90<br>Pru p3 1.90 |
| 22 | Anaphylaxis | 1 | Walnut | No | Yes | No | Yes | Ara h9 1.23<br>Jug r3 1.12<br>Pru p3 2.90 |
| 23 | Anaphylaxis | 1 | Fig | No | Yes | No | No | Ara h9 0.8<br>Cor a8 0.7<br>Jug r3 1.22<br>Pru p3 1.09 |
| 24 | Anaphylaxis | 1 | Corn | No | Yes | No | No | Ara h9 3.01<br>Cor a8 2.56<br>Jug r3 2.34<br>Pru p3 7.08<br>Tri a14 1.95 |
| 25 | Anaphylaxis | 1 | Apple | No | No | No | Yes | Jug r3 2.32<br>Pru p3 1.45 |
| 26 | Urticaria/ angioedema | 2 | Hazelnut | No | Yes | No | No | Ara h9 1.09<br>Cor a8 1.45<br>Jug r3 2.93<br>Pru p3 4.01 |
| 27 | OAS | 1 | Walnut | No | No | No | Yes | Jug r3 3.01<br>Pru p3 2.05 |
| 28 | Urticaria/ angioedema | 1 | Grapes | No | No | **Alcohol** | No | Ara h9 1.09<br>Cor a8 1.72<br>Jug r3 1.95<br>Pru p3 3.01 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 29 | Urticaria/ angioedema | 1 | Corn | No | No | **Excercise** | No | Ara h9 2.05<br>Cor a8 1.94<br>Jug r3 3.01<br>Pru p3 3.86<br>Tri a14 0.91 |

**Table 2. (Continued)** Characteristics of the 29 patients who experienced new reactions during the follow-up period: clinical symptoms, culprit food, number of new reactions, co-factors, emergency department access and title of the positive IgEs (negative ones were not reported). *Abbreviations: Ara h9, peanut-LTP; Jug r3, walnut-LTP; Pru p3, peach-LTP; Cor a8, almond-LTP; Tri a 14, wheat-LTP*

injector correctly; in 34% of the patients (27/78) the site of injection was wrong. A prior history of anaphylaxis was not associated with fewer errors, as 52% of the patients who reported anaphylaxis at the first visit made at least 1 mistake using the self-injector during the trial. The factor most associated to the proper use of the device was the periodic education of the patient (25/37 vs 17/41; 68% vs 41%; $p < .05$), while age, the time from first prescription, and the severity of the first reaction did not affect the ability to properly use the auto-injector. With a binomial regression, we estimated that the probability of mistakes in the use of the device was significantly decreased [odds ratio (OR) 0.34, 95% confidence interval (CI) 0.13–0.86, $p < .05$] by periodic education.

Furthermore, we tried to assess the effect of other elements on the occurrence of allergic reactions and their severity in patients affected by nsLTP syndrome. As regards co-sensitization to other panallergens over nsLTPs, 24/78 patients (31%) showed PR-10 (in particular rBet v1) co-sensitization, 9/78 (12%) profilin co-sensitization, and 9/78 (12%) showed both rBet v1 and profilin co-sensitization. In our group of patients, the double sensitization to rBet v1 and profilin was associated with less severe symptoms (8/9 vs 35/69, 89% vs 51%, $p < .05$). Binomial regression showed no significant relationship between co-sensitization and severity of symptoms (OR 0.13, 95% CI 0.015–1.085, $p$ = NS). Moreover, we examined the impact of risk co-factors both at the baseline visit and during the follow-up. At the first episode the presence of at least 1 co-factor was reported in 18 cases (23%): 8 for exercise (10% of all patients; 5 Food-Dependent Exercise-Induced Anaphylaxis, 3 urticaria/angioedema), 6 for NSAIDs (8%; 4 food-dependent NSAID-induced anaphylaxis, 2 urticaria/angioedema), 4 for alcohol (5%; 3 anaphylaxis, 1 urticaria/angioedema), and 1 for menstrual cycle (1%; this patient had also taken NSAID for pain control and experienced anaphylaxis). Presence of co-factors was not significantly associated to more severe reactions (10/30 vs 8/48; 33% vs 17%, $p$ = NS). During the follow-up the effect of contingent co-factors appeared similar in comparison to the baseline visit (18/78 vs 7/29, 23% vs 24%; $p$ = NS). Taking into account the reactions subsequent to the first one, a co-factor was reported in 7/29 cases (24%): exercise in 3 cases

| Number of errors | | |
|---|---|---|
| | Number of patients | |
| No error | 37 | 47% |
| One to three errors | 38 | 49% |
| Four errors | 3 | 4% |
| **Types of error** | | |
| | Number of patients | |
| Did not pull the safety cap | 15 | 19% |
| Incorrect handle of the device | 17 | 22% |
| Wrong site of injection | 27 | 35% |
| Holding the auto-injector in site for less than 10 s | 30 | 38% |

**Table 3.** Evaluation of patients' ability to use the auto-injector

(10%), fasting in 2 cases (7%), alcohol in 1 case (3.5%) and NSAIDs in 1 case (3.5%).

Neither comorbidities (asthma and cardiovascular diseases) nor concomitant medications (beta-blockers and ACE-inhibitors) proved to be associated with more severe reactions.

## DISCUSSION

From the data we collected it emerges that nsLTP syndrome is a heterogeneous condition: patients may experience from local symptoms to anaphylaxis straight away, and many others can experience new reactions in the long-term, even more severe than the first one. No significant correlation between the severity of the first reaction and the type of food (peach, walnut, and peanut) has been found; neither a significant correlation between a subsequent episode of anaphylaxis and the ingestion of the baseline offending food in comparison to other foods containing nsLTP has been reported.

As, according to other studies,[4,10] *Rosaceae* emerged as the most common culprit even for new reactions, dietetic rules involving the avoidance of them in all patients with nsLTP syndrome and the avoidance of nuts in selected ones are mandatory to prevent new episodes in most cases. However, as new sensitizations tend to appear over time and previously tolerated foods may become new causes of reactions, a periodic follow-up is crucial. As nsLTPs can be found in various and different types of food, it is important to highlight that sometimes the culprit does not belong to the most common groups (*Rosaceae* and nuts), so suspecting nsLTP syndrome in every case of severe reaction to fruits, plants, or seeds is advisable.

If on the one hand prevention plays a pivotal role, on the other hand the prescription of adrenaline auto-injector in patients who experienced a case of anaphylaxis and their ability to use it correctly represent vital elements. There are many studies describing the effectiveness of education training in the management of food induced anaphylaxis and in particular in the use of the epinephrine auto-injector. However, most of them are referred to childhood and assess trainings conducted not directly on the patients but on supportive categories, ie, school nurses, teachers, or childcare personnel.[16-18] On the other side, a recent study conducted on parents of allergic children highlighted a certain reluctance to the use of adrenaline auto-injector, probably suggesting that both educational techniques and emotional involvement are relevant.[19] Unfortunately, even if our data showed that periodic education reduces the number of errors in the use of adrenaline auto-injector, it is likewise true that patients are insecure about the

appropriate circumstance of use of adrenaline and most of them still remain uncertain about the correct technique of self-administration. This makes follow-up visits of the utmost importance, not only to detect the development of new sensitizations, but also to re-educate the patients about the indications and the correct use of the epinephrine auto-injector, which remains the first line of treatment for anaphylaxis.

A lot of studies tried to define the risk factors for severe reactions in patients sensitized to nsLTPs: some studies showed a higher risk of systemic reactions in patients mono-sensitized to LTP from peach (*Prunus persica*), Pru p3, while others have demonstrated a higher risk of anaphylaxis in patients poly-sensitized to nsLTPs, especially in those sensitized to 5 or more different nsLTPs.[20,21] Our study confirms this last hypothesis: more severe reactions and a higher number of them were experienced by poly-sensitized subjects, in particular by those sensitized to 4 or more nsLTPs. At the same time positive specific IgE to rPru p3 were reported in most of the patients, so, as already seen by Casas-Saucedo et al,[22] the confirmation by SPT for peach-LTP and/or measurement of rPru p3 specific IgE may be considered as good markers to define the diagnosis of nsLTP syndrome, especially when mild symptomatic (as occur in contact urticaria or OAS).

In the matter of co-sensitization to other pan-allergens, as PR-10 and profilin, our study confirms what already reported in literature,[10] that is, an association to less severe symptoms in the reactions experienced by patients who show co-sensitization to nsLTP and profilin and/or PR-10. Unfortunately, as long debated, we found no significant odds ratio proving a clear causative relationship. Further studies in this subject are necessary.

A further typical feature of nsLTP allergy is its relevant link to the presence of some specific co-factors: according to our study, where we defined the presence of a risk factor at the baseline visit in 23% of the cases, the estimated incidence of a rection associated to a co-factor is 25–40%.[3] The most important ones are NSAIDs, that can increase basophil activation following allergen exposure[23] and physical exercise, which is thought to increase the absorption of partially-digested food proteins, including allergens, into the circulation, from where they migrate into the perivascular and tissue spaces, where allergen-specific mast cells reside.[24] It must be noted that while NSAIDs were the most common in the first reactions, they were involved just in one reaction during the follow-up, proving a good adherence rate of the patients in avoiding them in association to nsLTP containing foods. Unfortunately, exercise and fasting were reported by patients as more difficult to avoid.

## CONCLUSIONS

Dietary restrictions, involving the avoidance of fruits of the *Rosaceae* family always and of nuts in selected cases are mandatory in patients affected by nsLTP syndrome. As nsLTPs can be found in different types of vegetable foods, it is important to suspect nsLTP syndrome in every case of severe reaction to fruits, plants or seeds. Moreover, taking into account that new sensitizations may occur over time to previously tolerated foods, periodic follow-ups are very important, also to educate and to periodically train the patient in the use of adrenaline auto-injector, which represent the main out-of-hospital treatment in case of anaphylaxis.

As regards other features that may influence the severity of the allergic response, patients presenting 4 or more different sensitizations to nsLTPs are more likely to perform more severe reactions. In the matter of an eventual protective effect of the concomitant sensitization to nsLTPs and PR-10 and/or profilin, more studies are needed. Furthermore, our data confirm the negative role of risk co-factors, such as exercise, NSAIDs, alcohol, menstrual cycle, and fasting, in the occurrence of anaphylaxis, suggesting a good adherence rate during the follow-up in the avoidance of NSAIDs but not as good in the avoidance of exercise and fasting.

Given the above considerations, it becomes evident that careful education aimed at the prevention of new reactions and the management of anaphylaxis should be a routine practice in nsLTP syndrome.

### ABBREVIATIONS

nsLTP, non specific Lipid Transfer Protein; nsLTP-FIA, non specific Lipid Transfer Protein's Food-induced Anaphylaxis; SPT, Skin Prick Test; OAS, Oral Allergy Syndrome; NSAIDs,

non-steirodal anti-inflammatory drugs; ARBs, Angiotensin II Receptor Blockers; MET, Medical Emergency Team

### Ethics
The study was approved by the ethics committee of the Hospital of Parma (793/2020). All patients signed informed consent forms.

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### Author contribution
Erminia Ridolo involved in conceptualization, project administration, presentation to Ethics Committee, resources, investigation, data collection, writing original draft, review; Francesco Pucciarini involved in investigation, data collection, writing the original draft, editing and elaboration of the tables; Francesca Nicoletta involved in review, writing and editing of the final draft and tables; Paola Kihlgren involved in conceptualization, project administration, data collection, writing the original draft; Alessandro Barone involved in investigation and data collection and elaboration; Silvia Peveri involved in investigation, review and editing; Marcello Montagni involved in investigation, review and editing; Cristoforo Incorvaia involved in investigation and in the final review and editing of the paper.

### Authors' consent for publication
All authors have read and agreed to the published version of the manuscript.

### Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Declaration of competing interest
The authors declare no conflict of interest.

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## REFERENCES

1. Abbey M, Bastiras S, Calvert GD. Immunoprecipitation of lipid transfer protein activity by an antibody against human plasma lipid transfer protein-I. *Biochim Biophys Acta*. 1985;833(1):25–33. https://doi.org/10.1016/0005-2760(85)90249-8.
2. Asero R, Antonicelli L, Arena A, et al. Causes of food induced anaphylaxis in Italian adults: a multi-centre study. *Int Arch Allergy Immunol*. 2009;150:271–277. https://doi.org/10.1159/000222679.
3. Skypala IJ, Asero R, Barber D, et al. European academy of allergy; clinical immunology - EAACI task force: non- specific lipid transfer protein allergy across europe. Non-Specific lipid transfer proteins: allergen structure and function, cross-reactivity, sensitization, and epidemiology. *Clin Transl Allergy*. 2021;11:e12010. https://doi.org/10.1002/clt2.12010.
4. Asero R, Piantanida M, Pinter E, Pravettoni V. The clinical relevance of lipi[d] transfer protein. *Clin Exp Allergy*. 2018;48(1):6–12. https://doi.org/10.1111/cea.13053.
5. Egger M, Hauser M, Mari A, Ferreira F, Gadermaier G. The role of lipid transfer proteins in allergic diseases. *Curr Allergy Asthma Rep*. 2010;10(5):326–335. https://doi.org/10.1007/s11882-010-0128-9.
6. Asero R, Mistrello G, Roncarolo D, et al. Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. *Allergy*. 2002;57(10):900–906. https://doi.org/10.1034/j.1398-9995.2002.t01-1-23541.x.
7. Pascal M, Muñoz-Cano R, Reina Z, et al. Lipid transfer protein syndrome: clinical pattern, cofactor effect and profile of molecular sensitization to plant-foods and pollens. *Clin Exp Allergy*. 2012;42:1529–1539. https://doi.org/10.1111/j.1365-2222.2012.04071.x.
8. Deng S, Yin J. Mugwort Pollen-related food allergy: lipid transfer protein sensitization and correlation with the severity of allergic reactions in a Chinese population. *Allergy Asthma Immunol Res*. 2019;11:116–128. https://doi.org/10.4168/aair.2019.11.1.116.
9. Bilò MB, Martini M, Tontini C, Corsi A, Antonicelli A. *Anaphylaxis. Eur Ann Allergy Clin Immunol*. 2021;53(N.1):4–17. https://doi.org/10.23822/EurAnnACI.1764-1489.158.
10. Mota I, Gaspar A, Benito-Garcia F, et al. Anaphylaxis caused by lipid transfer proteins: an unpredictable clinical syndrome. *Allergol Immunopathol*. 2018;46(6):565–570. https://doi.org/10.1016/j.aller.2018.04.002.
11. Ukleja-Sokołowska N, Gawrońska-Ukleja E, Żbikowska-Gotz M, Sokołowski Ł, Bartuzi Z. Recurrent anaphylaxis in patient allergic to eggplant - a Lipid transfer protein (LTP) syndrome. *Asian Pac J Allergy Immunol*. 2018 Jun;36(2):109–112. https://doi.org/10.12932/AP0846.
12. Dreborg S, Frew A. Position paper: allergen standardization and skin tests. *The European Academy of Allergology and Clinical Immunology Allergy*. 1993;48:49–75.
13. Heinzerling L, Mari L, Bergmann KC, et al. The skin prick test – European standards. *Clin Transl Allergy*. 2013 Feb 1;3(1):3. https://doi.org/10.1186/2045-7022-3-3.
14. Simons FER, Ardusso LRF, Bilò MB, et al. World allergy organization guidelines for the assessment and management of anaphylaxis. *World Allergy Organ J*. 2011;4:13–37.
15. Ridolo E, Montagni M, Bonzano L, et al. How far from correct is the use of adrenaline auto-injectors? A survey in Italian patients. *Intern Emerg Med*. 2015;10:937–941. https://doi.org/10.1007/s11739-015-1255-z.
16. Shiraishi Y, Ishikawa Y, Shiraishi Y, Matsumura M. Effectiveness of epinephrine auto-injector skill training for school nurses. *J. Gen. Fam. Med.* 2019;21(1):30–31. https://doi.org/10.1002/jgf2.282.
17. Ferran LR, Tornero NG, Álvarez NC, Piedra FT. Anaphylaxis at school. Are we prepared? Could we improve? *Allergol*

*Immunopathol*. 2020;48(4):384–389. https://doi.org/10.1016/j.aller.2019.10.006.

18. Sasaki K, Sugiura S, Matsui T, et al. A workshop with practical training for anaphylaxis management improves the self-efficacy of school personnel. *Allergol Int*. 2015 Apr;64(2):156–160. https://doi.org/10.1016/j.alit.2014.10.005.
19. Leszkowicz J, Pieńkowska A, Nazar W, et al. Does informal education training increase awareness of anaphylaxis among students of medicine? Before-after survey study. *Int J Environ Res Publ Health*. 2021 Aug 1;18(15):8150. https://doi.org/10.3390/ijerph18158150.
20. Basagaña M, Elduque C, Teniente-Serra A, Casas I, Roger A. Clinical profile of lipid transfer protein syndrome in a Mediterranean area. *J Investig Allergol Clin Immunol*. 2018;28:58-60. https://doi.org/10.18176/jiaci.0209.
21. Scala E, Till SJ, Asero R, et al. Lipid transfer protein sensitization: reactivity profiles and clinical risk assessment in an Italian cohort. *Allergy*. 2015;70:933–943. https://doi.org/10.1111/all.12635.
22. Casas-Saucedo R, de la Cruz C, Araujo-Sánchez G, et al. Risk Factors in severe anaphylaxis: which matters the most, food or cofactors? *J Investig Allergol Clin Immunol*. 2022;32(4). https://doi.org/10.18176/jiaci.0698.
23. Pascal M, Muñoz-Cano R, Milà J, et al. Nonsteroidal anti-inflammatory drugs enhance IgE-mediated activation of human basophils in patients with food anaphylaxis dependent on and independent of nonsteroidal anti-inflammatory drugs. *Clin Exp Allergy: journal of the British Society for Allergy and Clinical Immunology*. 2016;46(8):1111–1119. https://doi.org/10.1111/cea.12735.
24. Morita E, Kunie K, Matsuo H. Food dependent exercise-induced anaphylaxis. *J Dermatol Sci*. 2007;47:109–117. https://doi.org/10.1097/ACI.0000000000000531.