

Molecular Diagnostic Study of Causes of Anaphylaxy During One Year of Follow-Up

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Abstract

Anaphylaxis is a severe and rapidly onset hypersensitivity reaction. It is triggered by the patient's contact, previously sensitized, with different external agents. The aim of the study has been to discover the causes of individualized anaphylaxis in patients to help prevent future anaphylactic reactions. Among the most important allergens as a cause of anaphylaxis in the area of our hospital, the pollens stood out: Poll1, Cynd1 and Alt1; among foods: Prup3, Cora8 and Walnut; and among the animals the cat turned out to be the animal that triggers the most anaphylaxis in our environment. Knowing which epitopes are causing the patient's allergy through faster and higher-throughput techniques such as microarrays makes more targeted therapy possible. Therefore, the molecular analysis by microarrays was more useful in the diagnosis and in the possible future treatment of the patients because it will allow a dietary elimination and a more targeted immunotherapy. In addition to being more efficient compared to other allergenic studies.

Keywords: Anaphylaxis, prevalence, allergy, microarray, prick, IgE.

Abbreviations

Poll *	: Pollen group 1.
Cynd1 *	: Group 1 of the Cynodondactylon (grass, Bermuda grass pollen).
Alt1 *	: Recombinant acid glycoprotein from <i>Alternaria alternata</i> (fungus).
Anis1 *	: Recombinant <i>Anisakis Simplex</i> serine protease inhibitor.
Prup3 *	: Peach lipid transport protein.
Cora8 *	: Hazelnut lipid transport protein.
PR_hazel *	: Hazelnut stress protein.
PR_peanut *	: Peanut stress protein.

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1. Introduction

Anaphylaxis is a severe, generalized, and rapidly onset hypersensitivity reaction. It is triggered by the patient's contact, previously sensitized, with different external agents. The most common causes of anaphylaxis are drugs (50%) and food (22%).

Symptoms develop quickly, usually within minutes or seconds. In the first contact with the allergen, a nonspecific immunoglobulin E will be generated, and in subsequent contacts we will find that the organism will react with an IgE already sensitized. This will present with cutaneous symptoms (angioedema, pruritus, urticaria), digestive (nausea, vomiting or diarrhea), cardiovascular (tachycardia, shock) or respiratory (dyspnea, laryngeal bronchospasm). It is a medical emergency that requires immediate attention and the drug of choice is intramuscular adrenaline [1].

The true prevalence of this disease is difficult to establish, since it is not a notifiable disease. Although anaphylaxis is underdiagnosed, it is estimated to have an incidence of 3–30 cases / 100,000 people / year and a mortality rate of around 1% [2].

Anaphylactic shock is a fatal disease that affects several organs at the same time and causes a failure of different vital systems. For this reason, it is essential to know the characteristic symptoms of this disease. It is necessary to carry out a study to objectify which causes are the most prevalent, in this case in Valladolid (for a year) and also to look for different associations with other hypersensitivity pathologies such as eosinophilic esophagitis, pollen allergy and celiac disease.

With all this, the patients most susceptible to suffering anaphylactic shock (higher risk groups) and which associations are more frequent in this pathology will be identified.

The purpose of this study is to find the most frequent causes of anaphylaxis and to know which molecules have caused it and, if possible, to try not to ingest them or not be in contact with them. Knowing these characteristics, a greater number of cases of anaphylaxis can be avoided by eliminating contact with different allergens in those patients susceptible to suffering from it.

The need to carry out this research is based on:

1. Data on the incidence of anaphylaxis and prevalence are scarce and often imprecise.
2. It is an underdiagnosed disease.
3. At present, molecular microarray diagnosis is proving useful to detect hidden allergens or not demonstrated by routine allergological tests (prick or IgE).
4. Your knowledge will provide a better development of prevention and treatment strategies for patients.
5. Understand more precisely the demographic characteristics of anaphylactic shock.

6. Facilitate the development of a more individualized approach to the management of patients with specific responses to specific allergens.
7. Provide data to improve management, clinical services and minimize adverse outcomes.
8. An evaluation with more precision techniques that allow estimating the real risk of anaphylaxis and mortality in allergic individuals.

2. Objectives

Discovering the causes of individualized anaphylaxis in patients would help prevent future anaphylactic reactions, preventing the individual sensitive to one or more allergens from coming into contact with any of them again. In addition, it is possible to find allergens that cause anaphylaxis more frequently in our health areas.

The most common symptoms of anaphylaxis are caused by food and drugs. Within the food allergy, there are vulnerable groups such as patients with celiac disease and esophagitis. The same person can suffer from celiac disease and also a food allergy to gluten due to IgE, both pathologies mediated by different immune responses. Immune diseases are not exclusive and the immune system can be involved in different types of cellular and humoral responses [3].

On the other hand, it is possible that there is a different molecular response to different food allergens depending on the sensitization pathway or the hypersensitivity mechanism involved, and that the causal epitopes can be investigated. By knowing these epitopes to which each type of patient responds, it is possible to achieve more targeted dietary avoidance and more specific and precise immunomodulatory therapy.

The main objective is to assess the causes of anaphylaxis in one year in Valladolid by performing allergological tests (routine skin prick and specific IgE), adding a molecular study by microarrays of a possible IgE-mediated hypersensitivity to different allergen molecules (native and recombinant) in a population more susceptible to food hypersensitivity (celiac disease, esophagitis) and environmental allergies also sensitized to food (pollen), to try to find the epitopes involved in the most prevalent causes of anaphylaxis.

Microarrays do not detect drug epitopes, only specific latex allergenic molecules, so this study includes causes of anaphylaxis due to environmental, food and latex allergens.

This research work aims to achieve:

- Know the epidemiological data of these patients and assess the possible causes to avoid new contacts with the allergen.

- Assess the incidence of IgE-mediated response in a large sample of patients with eosinophilic and celiac esophagitis and compare them with a sample of healthy allergy sufferers with respiratory symptoms (pollen asthmatics).

- Assess the usefulness of the measurement of molecular analysis by microarrays as an effective parameter in detecting a population hypersensitive to food proteins or other allergens by IgE mechanism, comparing the profitability and diagnostic efficiency of the different determinations.

- Assess the efficacy of skin tests and measurement of specific IgE to the possible causal food for the diagnosis and prevention of adverse reactions to it.

3. Materials and methods

Study design

An observational case-control study nested in a cohort has been carried out. This study consists of prospectively monitoring a cohort of subjects (from the target population of the study), and selecting the cases as they appear.

In the sample size, all the patients treated in the allergology unit who suffered anaphylactic shock during the year 2020 of the Valladolid Oeste health area have been included. Therefore, the selection of the sample is consecutive and not random.

Methodology

Allergic IgE-mediated hypersensitivity to different molecules has been assessed by microarray technique in patients who have suffered anaphylaxis for a year in Valladolid, comparing the results with those of other healthy patients with esophagitis, pollen, celiac disease. Patients distributed in groups:

- Patients with eosinophilic esophagitis (129 patients)
- Patients with celiac disease (53 patients)
- Pollen patients (50 patients)
- Patients who have suffered anaphylaxis (41 patients)
- Controls healthy population (50 patients)

Tests and studies carried out

The test variables are age (expressed in years), sex (male or female), and molecular variables. The different studies carried out are:

- **IgE:** with Immuno-CAP Thermofisher technique, Upssala, Sweden. Specific IgE (ImmunoCAP) is an objective measure of circulating IgE and of the patient's sensitization to a

specific allergen. It allows to obtain a quantitative measure of a wide range of individual allergens and their components.

- **Prick:** it is a micropuncture test performed on the forearm, done in Allergology, whose objective is to detect the presence of IgE on mast cells on the patient's skin. One or more allergens (allergic extracts) are placed on the skin and a very superficial puncture is made with a microlancer so that the proteins enter through the skin. If the mast cells in the patient's skin have IgE against any of these components, in that local area, they will activate and mediate inflammatory mediators such as histamine, in such a way that where the allergen is placed, a wheal will form and then After 20 minutes, it will be possible to read on the skin which were the allergens placed to which the patient reacted (which were positive and negative). Positive results indicate that the patient has IgE against that substance, that is, that the patient has an IgE-mediated allergy to that substance [4].

Routine allergological techniques (prick tests) and immunodetection of IgE have a poor predictive value for food allergens in esophagitis and celiac disease, for example [5]. On the other hand, food provocation techniques are very complicated and risky, especially if there is polysensitization, which could cause severe clinical symptoms in celiac patients. In addition, it would be very important to complete a molecular analysis of all the proteins involved in celiac patients and patients with esophagitis for a more targeted dietary restriction or, if this is not possible, a specific and safe hyposensitizing treatment. For this, the microarray technique will be used.

- **Microarray technique:** it is based on modern biochip technology. This technique makes it possible to identify the presence of specific IgE antibodies against multiple allergenic components by means of a semi-quantitative test that provides the sensitization profile of patients. It is a microimmunoassay platform that allows determining, in the current panel, up to 112 allergenic, native and recombinant components of aeroallergens and foods, being able to obtain a food sensitization profile of the allergic patient in a more specific and complete way [6]. In our center it is carried out by means of ImmunoCAP.



Figure 1. ImmunoCAP scanner and software

The allergenic components are immobilized on a solid substrate in microarray format (slides) and react with the specific IgE of the patient's serum sample.



Figure 2. Slide with four chips

After removing the non-specific IgE, those components that react with the serum are detected by a secondary antibody (Anti-human IgE) labeled with a fluorochrome. After incubation, the labeled anti-IgE antibodies that have not bound are removed by a new wash. The procedure is followed by fluorescence measurement using a microarray scanner. The intensity of the fluorescent signal ranges from blue (less intense) to red (more intense). The higher the response value, the more specific IgE there will be in the sample.

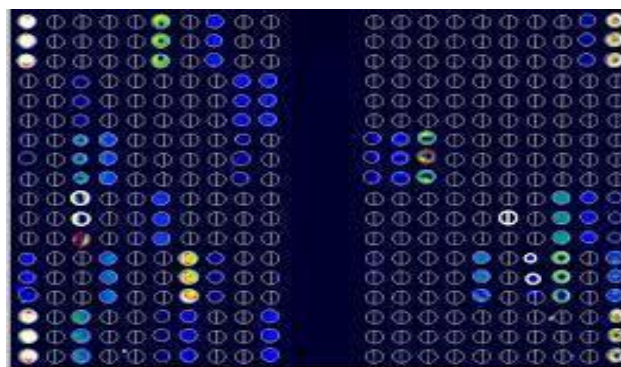


Figure 3. Chip reading

Test results are analyzed with Phadia Microarray Image Analysis (MIA) software and ISAC standardized units for specific IgE (ISU-E) are calculated.

The result is positive if the specific IgE binds to the allergenic component. If this join does not occur, the result is negative.

The results can be presented semi-quantitatively in 4 levels (0 = not detectable or very low, 1 = low, 2 = moderate to high, 3 = very high) (Figure 4)




ISAC unidades estandarizadas(ISU-E)	Nivel	
< 0.3	Indetectable	
0.3 - 0.9	Bajo	
1 - 14.9	Moderado /alto	
≥ 15	Muy alto	

Figure 4. Interpretation of results

An example of a microarray results sheet is shown in Figure 6.

The main advantages of this technique are:

- Simplicity
- Minimum sample volume
- High performance
- Automation

This procedure also facilitates component-based diagnosis, which offers greater security in establishing which allergen is recognized by a given patient. This helps to explain cross-reactions and solve puzzles such as that of patients with positivity to multiple pollens to whom they have never been exposed that with traditional methods would be practically impossible to analyze (the panel of natural and recombinant allergens that guarantee the presentation of a significant number of epitopes).

Statistical data analysis

Statistical analysis was performed with the SPSS version 15.0 program. To analyze the association between the study variables, Pearson's Chi-square test will be used. In the event that the number of cells with expected values less than 5 is greater than 20%, it will be calculated using the Likelihood Ratio Test. For the independent samples, the ANOVA analysis of variance technique will be used in the comparison of the mean values (for example: age variable).

The different analyzes used by descriptive statistics are classified as:

- Quantitative (for the age variable): mean \pm SD (standard deviation).
- Qualitative (N and percentages): they are described with the frequency table of their categories (they are all except age).

Table 1 is shown below with the variables age and sex, in relation to each study group. For all tests, a statistically significant difference is established for those with a p value less than 0.05. No significant differences were found in sex between disease groups, which were homogeneous and comparable. The only significant difference ($p < 0.005$) was that celiac patients were younger. The sensitization to different native and recombinant allergens in the groups studied by microarray technique can be seen in the following table 2. The following table 3 shows the positivity of each allergen by prick

	Healthy	Pollen	Celiac	Esophagitis	Anaphylaxis	P
Age	32 ± 11,1	25,8±10,3	5,9 ± 4,4	34,7 ± 16,2	29,4 ± 15,1	< 0,001
Sex (Males)	35 (70%)	27 (54%)	41 (77,4%)	83 (64,3%)	27 (62,8%)	0,142

Table 1. Relationship of groups with the variables age and sex

	Healthy	Pollen	Celiac	Esophagitis	Anaphylaxis	P
Prickloliium	2 (4%)	44 (88%)	3 (5,7%)	47 (36,4%)	26 (61,9%)	<0,0001
Prickcynodon	1 (2%)	20 (40%)	3 (5,7%)	35 (27,1%)	19 (45,2%)	<0,0001
Prickolea	0 (0%)	14 (28%)	0 (0%)	22 (17%)	12 (28,6%)	<0,0001
Prickartemisia	0 (0%)	7 (14%)	0 (0%)	6 (4,7%)	6 (14,6%)	<0,0001
Prickdog	1 (2%)	0 (0%)	1 (1,9%)	4 (3,1%)	6 (14,6%)	<0,012
Prickcat	0 (0%)	2 (4%)	1 (1,9%)	6 (4,7%)	9 (21,4)	<0,0001
Prickalternaria	0 (0%)	2 (4%)	0 (0%)	1 (0,8%)	10 (23,8%)	<0,0001
Prickwhiteegg	0 (0%)	0 (0%)	0 (0%)	3 (2,3%)	3 (7,1%)	<0,047
Prickmilk	0 (0%)	1 (2%)	0 (0%)	6 (4,7%)	0 (0%)	0,056
Prickpeanut	0 (0%)	2 (4%)	0 (0%)	12 (9,3%)	1 (2,4)	<0,004
Prickhazelnut	0 (0%)	3 (6%)	0 (0%)	12 (9,3%)	6 (14,3%)	<0,001
Prickpeach	0 (0%)	3 (6%)	0 (0%)	16 (12,4%)	6 (14,3%)	<0,0001
Prickanisakis	3 (6%)	2 (4%)	0 (0%)	6 (4,7%)	2 (4,8%)	0,290
Prickpistace	0 (0%)	0 (0%)	0 (0%)	3 (2,3%)	0 (0%)	0,234
Pricklatex	0 (0%)	0 (0%)	0 (0%)	6 (4,7%)	4 (9,5%)	0,006

Table 2. Most important molecules studied in the patients' microarrays.

	Healthy	Pollen	Celiac	Esophagitis	Anaphylaxis	P
Pol1*	1 (2%)	36 (72%)	13 (24,5%)	71 (55%)	26 (61,9%)	<0,001
Cynd1*	0 (0%)	17 (34%)	8 (15%)	50 (38,8%)	17 (40%)	<0,001
Alt1*	0 (0%)	2 (4%)	5 (9,4%)	10 (7,8%)	11 (26,2%)	<0,001
Anis1*	2 (4%)	3 (6%)	0 (0%)	16 (12,4%)	5 (11,9%)	0,007
Dog	0 (0%)	0 (0%)	2 (3,8%)	8 (6,2%)	12 (28,6%)	<0,0001
Cat	0 (0%)	0 (0%)	4 (7,5%)	16 (12,4%)	14 (33,3%)	<0,0001
Prup3*	0 (0%)	1 (2%)	3 (5,7%)	27 (20,9%)	15 (35,7%)	<0,001
Cora8*	0 (0%)	2 (4%)	4 (7,5%)	24 (18,6%)	14 (33,3%)	<0,001
PR_hazel*	0 (0%)	3 (6%)	0 (0%)	21 (16,3%)	7 (17,1%)	<0,001
PR_peanut*	0 (0%)	1 (2%)	0 (0%)	7 (5,4%)	1 (2,4%)	0,072
Nut*	0 (0%)	3 (6%)	3 (5,7%)	23 (17,8%)	11 (26,2%)	<0,001
Egg*	0 (0%)	0 (0%)	0 (0%)	7 (5,4%)	4 (9,5%)	0,004
Milk*	0 (0%)	1 (2%)	0 (0%)	9 (7%)	1 (2,4%)	0,021

Table 3. Table of results of the pricks performed on the study patients.

4. Results and Discussion

During 2020, a total of 5,265 patients were treated in the Allergology consultation of the Río Hortega University Hospital (HURH). Of these, 4182 associated their symptoms with aeroallergens and / or food and 1083 with medications (Figure 7).

During this same year, 41 patients with anaphylaxis required hospital admission to HURH, who were collected in the study database. Among the most important allergens as a cause of anaphylaxis, the pollens stood out: Pol1, Cynd1 and Alt1; among foods: Prup3, Cora8 and Walnut;

and among the animals the cat turned out to be the animal that triggers the most anaphylaxis in our environment.

This study with aeroallergens and food focused on 129 patients with eosinophilic esophagitis, 53 patients with celiac disease, 50 pollen patients and 50 healthy population controls. The youngest population was celiacs. It should be noted that the selected groups were homogeneous (healthy, pollen, esophagitis, anaphylaxis) except for the youngest age in celiac patients ($p < 0.0001$). The association between pollen and esophagitis and the association between anaphylaxis and esophagitis ($p < 0.025$) were also highly significant ($p < 0.0001$). In the rest of the groups, their associations were not significant ($p > 0.05$).

-Drugs

All drugs can cause allergy, however, there are certain drugs with a greater predisposition to cause it. The HURH Allergology Service obtained the following statistics (year 2020): within the drugs, hypersensitivity was demonstrated by skin tests, antibodies and / or specific provocation to amoxicillin in 47 patients, to other penicillins in 40 and to cephalosporins in 24. Within painkillers, of the 27 patients in whom hypersensitivity to them was found, ibuprofen was the cause in 9 patients, opioids in 8 and other NSAIDs in 10. Other drugs detected were codeine and ethylene oxide in two patients and tetanus toxoid in another 2. Among all these patients, 17 had suffered anaphylaxis: 6 from amoxicillin, 5 from analgesics (3 from ibuprofen and 2 from Dipyron) and 1 from tetanus toxoid.

-Aeroallergens

Aeroallergens are antigens, usually proteins of a very small size, transported by the air and capable of inducing the production of specific IgE antibodies in predisposed individuals. These antigens become allergens based on chemical, physical, or environmental factors. Although there are many substances in the environment that can cause allergic diseases, the most relevant are pollen, mites, animal allergens and fungi.

Pollen is the most important outdoor airborne allergen. Due to their small size, their ease of transport and their abundance in our environment, among others, they acquire great relevance in allergic pathology in the study area, specifically that of the Poaceae or grasses group. There are different groups of pollens, the most important are groups 1,4,5 and 6 of pollen.

In this research work, betaexpansins (pollen germination proteins) will be mentioned, especially Pol 1, as it is the most significant in our environment. Pol 1 was positive in 72% of the pollen in the sample, in 55% of the patients with esophagitis and in 61.9% of the patients who suffered anaphylaxis. Therefore, we can conclude that it is one of the most important causes of

allergy in our health area (Valladolid Oeste). Pol2, Pol3, Pol4, Pol5 and Pol 6 also had a significant relationship with pollen patients, but not with the other groups. This data is correlated with the data objectified in the atmospheric analysis in which group 1 of grass pollen is the predominant one in the atmosphere of Castilla y León (Figure 8) [7].

Il grasses are similar, but Cynd1 is different as it is evolutionarily a species that appeared prior to the ice age period. Cynd1 (cynodondactylon, bermudagrass or grass) is the pollen of the common grass. The herb known by the scientific name *Cynodondactylon* is by far the most widely used in both garden lawns and sports fields in the Mediterranean region. 40.5% of the patients with anaphylaxis in our study were positive for this allergen.

Despite the enormous number of fungal species present in the atmosphere, very few are clinically important as producers of allergic diseases. The main allergenic fungi are: *Alternaria*, *Aspergillus*, *Penicillium* and *Cladosporium*.

From an allergological point of view, the *Alternaria* genus is one of the most important and abundant species in our country. It is dominant in outdoor environments and the release of spores peaks on dry days in late summer and fall. It is very common and of universal distribution, it is saprophytic in plants, wood, fertilizers, food, tissues and different soil substrates. Its optimum growth temperature is about 25°C. The main allergen in *Alternaria alternata* is identified as Alt a 1, sensitizing 82% to 98% of *Alternaria* allergic patients. Alt 1 is an acid glycoprotein that normally affects grain farmers (which grow inside the stalks of grains and weeds) and people who work in vineyards and wineries, because it is more likely to appear in humid places, where it is found. spore [8]. In the statistical study carried out, 26.2% of the patients who suffered anaphylaxis have sensitivity to this protein. Therefore, it is an important cause of anaphylactic shock to consider, especially in workers at higher risk, such as farmers.

The measures to avoid pollens are limited, since they are floating in the air we breathe. The patient must know which plants he is allergic to and their pollination time, as well as avoid environmental exposure as much as possible. At that time, attention should be paid to the appearance of the first symptoms, starting in advance the preventive treatment indicated by the specialist. For this reason, the etiological diagnosis of the patient's allergy to different pollens is important, in order to avoid serious symptoms of their pathology and carry out a more personalized action plan.

-Domestic animals

Allergy to pets occurs when allergic symptoms appear on contact with animals or particles from them (hair, saliva, etc.). It can appear in people who live with pets, or in professionals who deal with animals on a daily basis, such as veterinarians or caregivers. The high number of people who

suffered anaphylaxis due to their own pets, be they dogs or, more frequently, cats, is striking. In 2019, in Spain a percentage of households that had at least one cat or one dog as a pet was reported of 26% for dogs and 11% for cats. Contrary to what is believed, the main problem of allergies towards these animals is not the hair, the real problem is the proteins of the cat's saliva, its urine and the dry dandruff impregnated by pheromones (lipocalins) that falls from the cats. Also, outdoor cats can carry pollens and other allergens on their skin [9]. That is why, regardless of the breed, hair length and how much it falls out, cats can trigger anaphylaxis in those who are more sensitive. Many people feel very attached to them, but their treatment and solution is the withdrawal of the animal from possible serious fatal complications. [10].

-Latex

Latex is a product obtained from the sap of the rubber tree (*Hevea brasiliensis*). The sap liquid undergoes multiple industrial processes, with heat and chemicals. After these processes the rubber is obtained. Its use is very widespread today due to its physical qualities such as its flexibility, impermeability or resistance, which is why it is found in many products that are used daily, and especially in the health field [11] [12].

Thanks to the molecular study, it was possible to objectify the molecules that caused more hypersensitivity to latex. 36 patients suffered symptoms with latex. Patients with anaphylaxis (20), 5 detected the rHevb6 molecule, 15 rHevb3 (suffered hives and asthma) and 16 also rHevb8 (latex profilin, suffered pharyngitis and oral syndrome, related to fruits). It might be necessary to create gloves with another material that avoids the allergens rHevb6 and rHevb3, advising their manufacturers. This could be useful in the medical field, for example, or even for food handling, since workers in this sector wear latex gloves [13].

-Food

PR peanut is the peanut allergen studied in this research work. Normally due to the influence of American television, it is common to think that peanuts are a very frequent causal allergen in anaphylactic shock, but in this study this belief has been denied in our environment, since there is no significant relationship between anaphylaxis and peanuts (p value = 0.072).

22.6% of the patients who suffered anaphylaxis and 17.8% of the patients with esophagitis in the study were positive for sensitivity to walnuts. The walnut is the most allergenic dried fruit in the Mediterranean area followed by the hazelnut. The allergy to nuts is one of the most frequent in Spain. According to the Spanish Association of People with Food and Latex Allergy (AEPNAA), it affects 1% of the population. It is not as frequent in infants as allergy to cow's milk or egg, but it is one of the most common food allergies in our country from 3 or 4 years of age, according to the

Spanish Society of Clinical Immunology, Allergology and Pediatric Asthma (SEICAP). In addition, walnuts are the nuts to which Spanish children are most frequently allergic (according to the Pronuts study, presented at the 2018 SEICAP congress). However, the prevalence varies depending on age and geographic area [14]. Normally, there is usually cross-reactivity with other nuts. This means that if you have a nut allergy, you may also have other nuts. It may also happen that, if you suffer from allergies to these, you also have pollens and / or latex, which can share substances with allergenic capacity.

Profilins and LTPs are panallergens. Theoretically, we could justify a large number of immunological cross-reactions as its association with anaphylaxis. This is due to the fact that in the LTP family we found that the Pru p3 allergen (LTP-peach) caused anaphylaxis in 35.7%, Artv3 (LTP- artemisa vulgaris) caused them in 33.3% [15]. These last two allergens share their sequence in 40%, which could lead to the conclusion of their possible crossing in terms of their reactivity. Profilins such as latex (ProfL) also showed a relevant percentage in anaphylaxis (14.3%) and in patients with esophagitis (14%).

Due to different factors such as globalization or immigration, there are numerous fish that are included in our diet and their allergy must be studied, since it is gaining importance over the years. A very important marine allergen is the Anis1 allergen, which caused anaphylaxis in 11.9% of our patients. Most of the fish that are consumed are usually infested by certain species of parasites that can cause parasites and / or allergic reactions. In case of sensitization to Anis1 (whose allergenicity partially disappears after correct freezing), it is recommended to freeze the fish at -20°C for 72 hours and avoid consuming raw, undercooked, salted and smoked fish. However, if the sensitization is to Anis3, it is a thermo stable protein and does not disappear with freezing or cooking, so it would be prudent to recommend the exclusion of fish and shellfish from the diet. Therefore, recommendations to patients allergic to a simplex should be made according to the protein they are sensitized [16].

-Comparison of allergen detection tests

Regarding the tests carried out in the study, significant differences were observed between them. The specific IgE test costs about 6 euros and the microarray costs 120 euros. The latter is more cost-effective, since it simultaneously analyzes 112 allergens consecutively with a minimal amount of sample (one drop of blood). If we created panels with the most prevalent and specific allergens, and studied them all at the same time, it would be more efficient than studying them 1 by 1 with Ig E (112 x 6 = 672 euros). In addition to the microarray technique presenting greater diagnostic precision, it allows us to interpret cross-reactivity phenomena, this means that a patient may be allergic to different allergenic sources (for example: pollens and plant foods or latex and fruits) and

this is due to the presence of antibodies against a single protein. With this technique we can achieve a more precise and targeted immunotherapy for each patient. Another advantage of the molecular study using microarrays is the prediction of a more or less severe clinical reaction depending on the type of proteins involved.

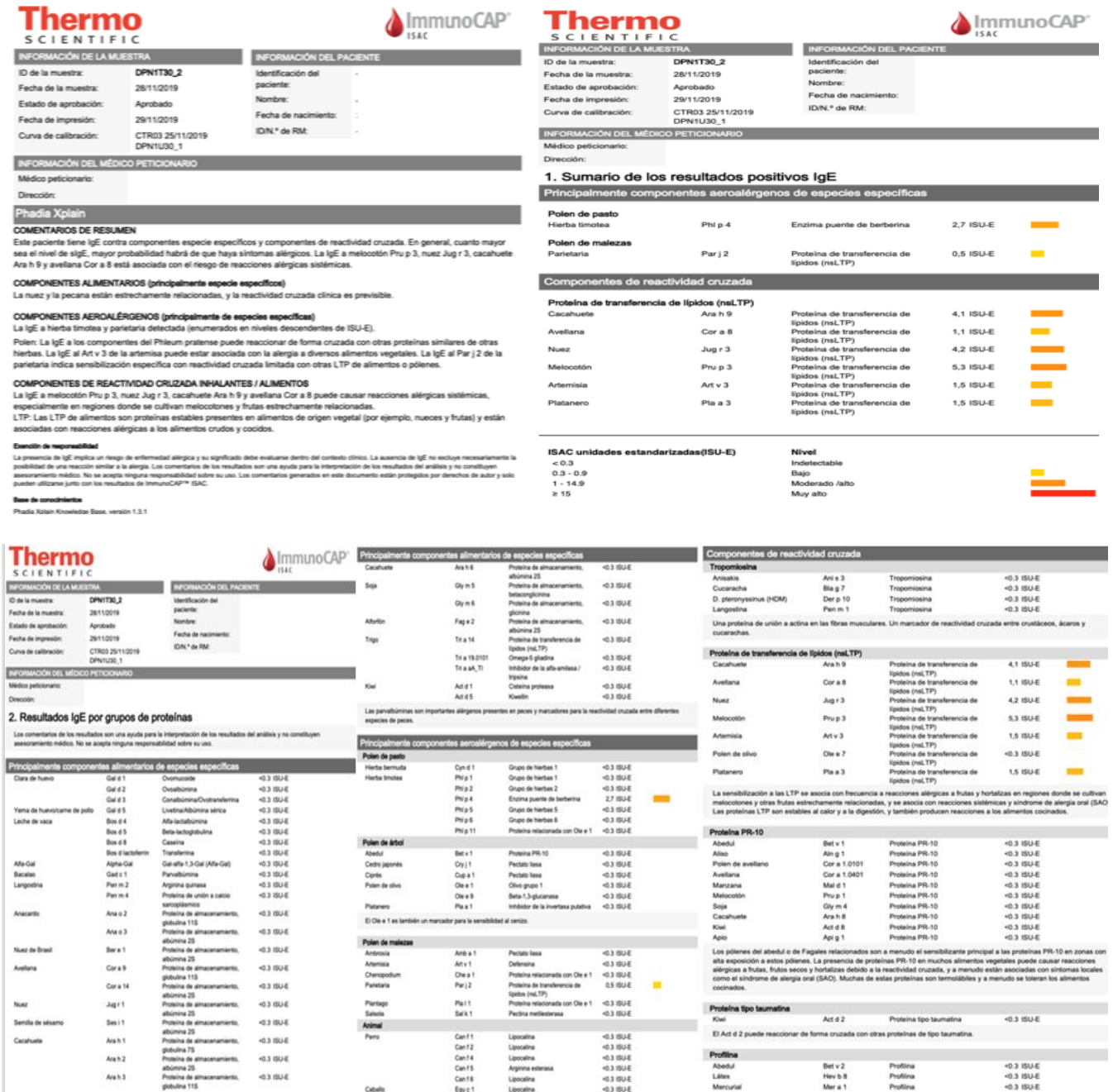


Figure 6. Example of a microarray results sheet.

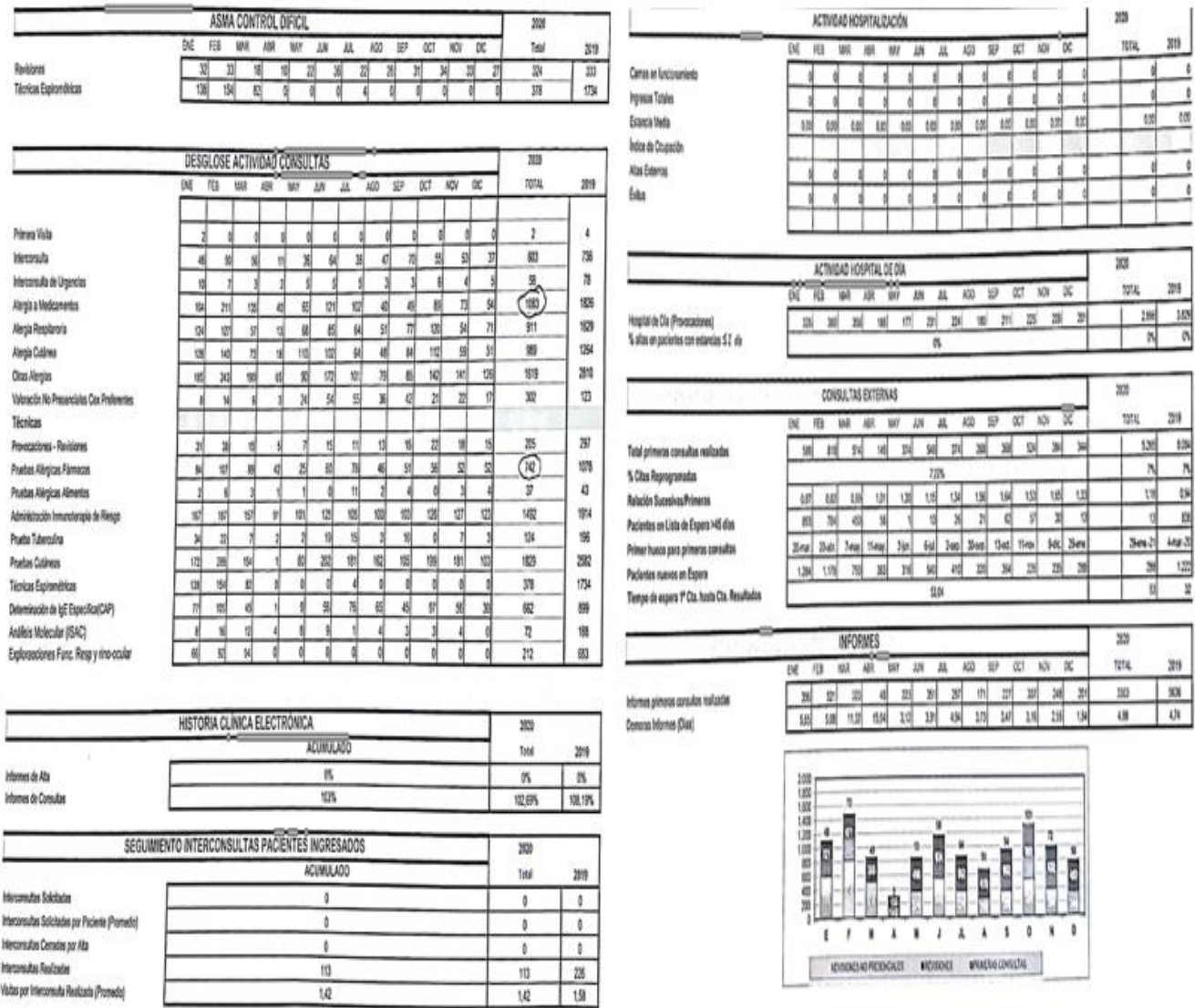


Figure 7. Statistical tables of patients treated in the Allergology Unit of the Río Hortega University Hospital, in 2020.

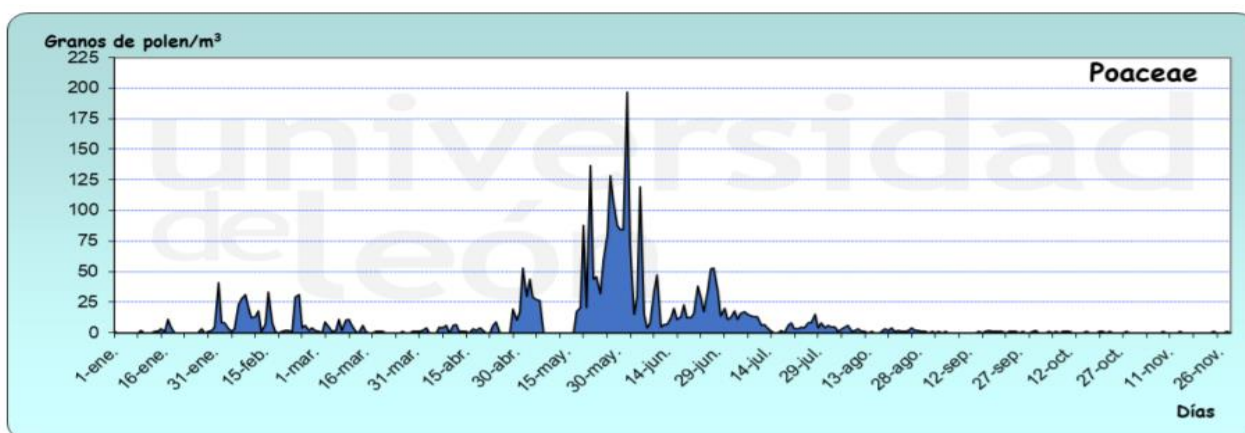


Figure 8. Atmospheric levels of Poaceae in Valladolid, Spain (2020).

5. Conclusions

The molecular analysis by microarrays was useful in the diagnosis and in the possible future treatment of the patients because it will allow a dietary elimination and a more targeted immunotherapy. Also, it is more efficient compared to other allergenic studies.

It might be necessary to create gloves with another material that avoids the allergens rHevb6 and rHevb3 by notifying their manufacturers, since they are the most frequent in our study.

The allergy to nuts is one of the most frequent in Spain and in turn, the walnut is the most allergenic dried fruit. Jug r 1 (2S albumin storage protein) is considered the best differentiating allergen in nut allergic patients and was positive in many of our anaphylactic patients.

Grass group 1 (Pol1) is the most significant pollen group in our environment. Pol1 was positive in 72% of the pollen in the sample, in 55% of the patients with esophagi is and in 61.9% of the patients who suffered anaphylaxis. Therefore, it can be concluded that it is one of the most important causes of allergy in our health area. Pol2, Pol3, Pol4, Pol5 and Pol6 also had a significant relationship with pollen patients, but not with the rest of the groups.

It is recommended to freeze the fish at -20°C for 72 hours and avoid consuming raw, undercooked, salted and smoked fish for the prevention of Anis1 in sensitized patients and the exclusion of these foods if it is sensitized to Anis3.

The most effective recommendations for allergies to pets are the removal of the pet from its home or in cases of profession a desensitization measure could be carried out. Other less effective measures are, not sleeping with them in bed, bathing your pets regularly, asking a family member if you can clean the pet's bed, meeting friends who have pets outside your home, always having the medication ready and wash your hands after handling your pet.

Faced with anaphylaxis, an etiological diagnosis must be made as soon as possible to avoid a cause of fatal outcome. The emergency treatment is adrenaline and the prognosis depends on the correct elimination of the trigger, its avoidance or specific immunotherapy to it.

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