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ORIGINAL ARTICLE

Respiratory allergy to fungi in Barcelona, Spain: Clinical aspects, diagnosis and specific treatment in a general allergy unit

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Abstract
Background: The importance of hypersensitivity to fungal allergens is a relatively unknown and somewhat controversial subject.
Methods: An open prospective study was carried out in just one centre to determine the clinical and epidemiological characteristics as well as the diagnostic usefulness of skin prick and conjunctiva provocation tests, associated with total and specific IgE determination in two groups of patients, one of which was monosensitised to fungi and the other of which had multiple sensitisations, including fungi.
Results: Rhinitis, exclusive or associated with asthma, was the main consultation cause (88% in monosensitised patients). Severe asthma was rarely found. In the polysensitised group, 64% were simultaneously allergic to moulds and mites.

Alternaria alternata was the most common sensitising fungus, although a considerable number of cases were associated with other species such as *Cladosporium*, *Penicillium* and/or *Aspergillus*. The skin prick test gave the highest sensitivity and specificity. In 67% of the cases, the specific IgE was found between classes 3 and 4. The conjunctival provocation test was an innocuous and highly useful method for verifying the diagnosis and determining the degree of clinical sensitisation. A large number of patients exclusively allergic to fungi received specific immunotherapy, and it was generally well tolerated.
Conclusions: This protocolised study shows the importance of *Alternaria* and other fungi sensitisations in rhinitis alone or associated with asthma. Combined diagnosis of prick test, specific IgE and conjunctiva provocation test is very useful for deciding specific immunotherapy.

Introduction

There is growing interest in understanding the role that airborne fungi play in respiratory allergies. Numerous publications refer to the increasing severity of diseases caused by hypersensitisation to moulds such as severe asthma,[1,2] allergic bronchopulmonary aspergillosis[3] and hypersensi-

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tivity pneumonitis,[4] which are all fortunately infrequent clinical manifestations. Less severe ailments such as rhinitis or mild, intermittent asthma are largely the reasons for consultations in allergological services.

Due to their biological characteristics, fungi can develop outdoors, principally on decaying vegetal material, but they also grow and disperse their allergenic spores inside buildings.[5,6] Climatological conditions, temperature, humidity, wind, as well as the availability of organic substrates on which they can develop are the factors that determine the presence of fungal species in the atmosphere outdoors, and this can vary depending on the geographical region studied. Thus, variable levels of fungal sensitisation have been described. Cold zones have low levels,[7] while warm or hot areas have high levels.[8,9] In Mediterranean geographical zones, there are high levels of sensitivity to fungi, as shown in several published studies.[8,10]

Study methodology, with few exceptions, has not been standardised, and the criteria for defining and evaluating fungal allergy are quite diverse. Furthermore, in many publications the number of subjects studied is too low to draw definitive conclusions.

The fact that most patients are also sensitised to other pneumoallergens makes it difficult to clearly understand the importance of fungal allergy. In order to determine if there are differences between polysensitised patients who also are allergic to fungi, and those patients only sensitised to fungi, it is important to have a large enough pool of similarly studied patients.

This open prospective study was performed using standardised methodology, carried out by the same group of professionals, in patients from a single clinical centre, thus allowing for a detailed analysis of different aspects of clinical manifestations, the relationship between sensitisation to fungi and other allergens, as well as routine diagnostic procedures, and to consider some aspects of therapeutic procedures.

Patients and methods

(1) Between September 2005 and September 2010, a total of 12,000 patients were seen for the first time, 73% of which were for respiratory manifestations. Approximately 30% of them were not allergic, the remainder showed positive test for dust mites and for different type of pollen or epithelia. We excluded patients with negative test to fungi.

(2) After a detailed medical record and a spirometry were carried out, diagnostic tests for pneumoallergens were performed.

The first test was the skin prick test, based on the GA^{2}LEN[11] proposals, expanded to include four moulds.

All persons whose tests presented a wheal $\geq$3 mm ($\geq$9 mm^2) with adequate positive and negative controls, to one or more fungal allergens were included in a questionnaire regarding age, where they lived, the clinical manifestations and degree of severity as well as seasonality of their symptoms.

The other systematic routine diagnostic tests were carried out and the results were recorded on the same questionnaire.

(3) Diagnostic tests:

Standard skin prick test: 36 glycerinated extracts from plant and tree pollens prevalent in the zone, cat and dog epithelia, feathers, house dust mites, as well as four moulds (*Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus* and *Penicillium crhysogenum*) were used. A reduced battery of 16 allergens was used for children under 7 years of age. Fungal extracts were provided by Bial-Aristegui Laboratories (Bilbao, Spain); a positive control was used (histamine 10 mg/mL) as well as a negative control (glycerinated saline solution). When deemed appropriate, skin prick test against other fungi were also performed (*Candida albicans*, *Fusarium* sp., *Mucor* sp., *Helminthosporium* sp., *Botrytis* sp., *Stemphylium* sp., *Ustilago* sp.).

Tests were read 20 min after the puncture, and the diameter of the wheal was recorded on a special form.

Analytical determinations: A count of blood eosinophils and total serum IgE was performed in all cases. Specific IgE level was determined using the ImmunoCap test (Phadia AB, Uppsala, Sweden) for all allergens which induced clinically significant skin prick test results.

Conjunctival provocation test: Depending on the analytical results, and considering the clinical data, a provocation test was performed. One drop of aqueous extract of the corresponding allergen (Bial Aristegui Laboratories) was placed into the conjunctival sac, beginning with the smallest concentration, (1/10 which corresponds to 3.027 BEU*/ml) (*BEU = Biological Equivalent Units, that is: activity units from the allergen manufacturer used in the study), and the concentration was increased to 25%, 50% or 100% (63,280 BEU), until redness, itching and in some cases, epiphora, sneezing and nasal secretions were produced. At this point the reaction was stopped using saline eye washes, and if necessary, using eye drops with azelastine 0.5 mg/ml or dexamethasone 1 mg/ml. This semiquantitative test is positive considering the lowest extract dilution which provokes the reaction.

As needed, other diagnostic tests were performed (study of food or drug allergies). If deemed necessary, X-rays of the thorax or sinus scans were requested.

Once the study of each patient was complete, the specialist indicates the specific immunotherapy to the implicated fungus, and recorded this treatment on the questionnaire form.

(4) Statistical evaluation.

A descriptive analysis of the samples was performed using demographic variables and the medical history of the patients. The average, *SD* and range were determined for the quantitative variables, and a frequency analysis was performed on the qualitative variables.

Afterwards, an analysis was performed to establish the comparability between monosensitised and polysensitised patients. The non-parametric Mann–Whitney test was used for continuous quantitative variables and chi-square analysis was used for the qualitative variables.

The data were analysed using the PASW Statistics v18.0 statistical package (SPSS Inc, Chicago, Illinois).

Table 1 Demographic data for 180 polysensitised patients and 55 sensitised exclusively to fungi. Expressed in percentages.

Group	Males	Females	Age range in years	2–7	3–12	13–19	20–30	30–40	>40
Monosensitised	49	51	3–65	21.8	23.6	10.9	20	9	12.7
Polysensitised	54.7	45.3	2–66	8.8	19.8	8.8	34.6	22	6

Table 2 Distribution of clinical manifestations in 180 polysensitised patients and 55 exclusively sensitised to fungi. Expressed in percentages.

Group	Only asthma	Only rhinitis	Asthma and rhinitis	Coughing	Urticaria	Eczema
Monosensitised	7.2	46.4	41.8	29	9	18
Polysensitised	7.7	37.5	54.7	20.4	29.3	21.5

Results

During the 5-year study period, 235 patients with positive skin prick tests to one or more fungal allergens were included.

Of these, 55 (23.4%) presented positive skin tests only to fungi, and were negative to the other pneumoallergens. The remaining 180 patients were sensitised to dust mites, pollens and or epithelia, but also to one or more fungal allergen.

There were no statistically significant differences ($p = 0.587$) in distribution by gender, although there was a slight predominance of males in the polysensitised group. Distribution by age showed statistically significant differences ($p = 0.042$): a greater number of patients 7 years of age or younger were monosensitised, as were patients older than 40, which included two patients older than 60 (Table 1).

Clinical symptoms are shown in Table 2. In both groups, the principal manifestations were rhinitis, and rhinitis associated with asthma. No differences were found between the two groups regarding bronchial asthma alone, without associated rhinitis.

Conjunctivitis was present in more than half of the patients, and it was associated with rhinitis in the vast majority of cases.

Persistent non-productive coughing, with or without asthma, was found in a large portion of the monosensitised patients. In three (5.4%) of these patients, who were eight, 37 and 46 years old, sinusitis was diagnosed, and in six patients (10.9%) there was an antecedent to foods or drugs allergy, although no symptoms had been attributed to these allergies in the past year.

In the group of polysensitised patients, 6.7% presented sinusitis with nasal polyposis diagnosed by scanner or during otorhinolaryngological exploration.

Urticaria and/or angio-oedema were more common in polysensitised patients, although the difference was not statistically significant ($p = 0.191$). In this group, 5% presented food allergies, and 3.4% were allergic to drugs.

In monosensitised patients, asthma was classified as severe persistent in three patients (5.4%), all of whom were under 12 years of age, while it was mild persistent or intermittent in the rest. Rhinitis was considered severe persistent in two patients (3.6%). Severe eczema associated to severe persistent rhinitis was found exclusively in the only patient hypersensitive to *Candida albicans*.

With regard to seasonality, 61% of the polysensitised patients had perennial symptoms, and the rest were highly variable, according to the other coexisting allergens.

Among the monosensitised, 66% of patients' symptoms were also perennial but then worsening during the summer and autumn months in half of the cases. In 22.6%, symptoms were exclusively at the end of spring to the beginning of autumn, and in 9.4%, symptoms were only present in autumn and winter.

Among the polysensitised patients, the skin prick test for the different pneumoallergens is shown in Table 3. Moulds were associated with mites in 68.2%, exclusively or shared with other pneumoallergens (Table 3).

Apart from grass pollens, a large number of patients presented positive skin tests to *Olea europaea*, *Platanus* sp., *Parietaria* sp., *Artemisia* sp., and *Chaenopodium* sp. and other trees or plants typical of the zone.

Among the group of patients sensitised exclusively to fungi, 76% were only sensitised to *Alternaria*, 5.4% to *Penicillium*, 1.8% to *Aspergillus* and *Candida*. The rest (15%) presented sensitivity to more than one fungal allergen, mainly *Alternaria* and *Cladosporium*, or *Alternaria* and *Aspergillus*. Skin prick tests presented a maximum

Table 3 Distribution of the skin prick test to pneumoallergens in 180 polysensitised patients allergic to fungi.

	House dust mites	Animal epithelia	Grass pollen	Other pollens	3 or more allergenic groups	Only *Alternaria*[a]	Other fungi[a]	*Alternaria* and others[a]
N	115	81	80	107	40	113	4	63
%	63.9	45	44.4	59.4	22.2	62.7	2.3	35

[a] Fungal allergens (includes *Aspergillus*, *Penicillium*, *Cladosporium* and *Candida*).

Table 4 Distribution of specific IgE values in patients sensitised exclusively to fungi and patients with other associated sensitisations. Expressed in kU/L (percentages in parenthesis).

	<0.35	0.36–0.69	0.7–3.49	3.5–17.9	18–49.9	50–100	>100
Monosensitised (*N* = 55)	3 (5.4)	3 (5.4)	13 (23.6)	24 (43.7)	9 (16.4)	3 (5.4)	0
Polysensitised (*N* = 169)	11 (6.5)	5 (2.95)	48 (28.4)	75 (44.4)	23 (13.6)	6 (3.55)	1 (0.6)

wheal size of 10 mm with an average diameter of 5.8 mm (33.6 mm^2).

In 54.4% of the polysensitised patients, and 53% of the monosensitised patients, an increase in eosinophils (≥5%) was detected.

Total serum IgE in the polysensitised group was found to be in the range of 7–4.217 kU/L, and 71.6% presented values ≥100 kU/L. In the monosensitised group, IgE was distributed between 4 and 3.000 kU/L. Values ≥100 kU/L were detected in 48% of the patients.

The specific IgE distributed in classes is shown in Table 4, in the monosensitised group, 43.7% presented values between 3.5 and 17.9 kU/L. Among the three patients with negative specific IgE, two were sensitive to *Alternaria* and one to *Penicillium*, but all three presented positive to the conjunctival provocation test and the total IgE total was lower than 40 kU/L.

The conjunctiva provocation test was carried out in 43 monosensitised patients (78%).

Positive test at any concentration was found in 98% of patients, among them 45.2%, reacting with low or very low allergen concentrations: 3.027–6.054 BEU/mL, 31% to concentrations between 12.108 and 24.216 BEU/mL and 23% to high concentrations ≥48.432 BEU/mL.

The same conjunctiva provocation tests against *Alternaria* or other fungi were performed on 157 polysensitised patients (87%) and in 95.5% of the cases, the result was positive for fungi. The majority (54.8%) were sensitive to low concentrations, 17% to medium concentrations, and 23.5% to high concentrations of the allergen.

After evaluation, in 36 patients diagnosed with exclusive allergy to fungi (66%), specific immunotherapy was prescribed. In 23 patients, (43%) it was exclusively for *Alternaria*, in one case it was for *Aspergillus* (2%), one for *Penicillium* (2%) and for the rest it was for two or more fungal allergens, mainly *Alternaria* and *Cladosporium* (18%).

Allergy vaccines were administered by sublingual route in 20% and subcutaneously in the other 80%. In no case was it necessary to suppress immunotherapy due to adverse effects during the initiation phase or within the first 12 months of treatment.

Discussion

The vast majority of publications about respiratory allergy to fungi are multicentre studies and do not differentiate between patients exclusively sensitive to fungi and those which present also sensitisation to others allergens, and thus it is difficult to determine which clinical manifestations are attributable to fungal allergens and which to other allergens.

This study was carried out in a single centre using identical methodology with standardised extracts of *Alternaria alternata* as well as three other fungal species.

Systematic information obtained homogeneously has shown that 23.4% of patients were exclusively sensitised to fungi, with a clear predominance of *Alternaria* (76%), or this fungus associated with another species. Sensitisation was due to other species in only 7.2% of the cases.

Separation of the patients into two groups, polysensitised and monosensitised, permitted the comparison of clinical and diagnostic aspects.

Among the different age groups the greatest predominance was observed in the population under 13 years old, where specifically 45.5% of the monosensitised patients belonged to the 2–12-year-old age group against 28.6% of polysensitised, presenting a significant statistical difference.[12]

It is noteworthy that rhinitis, exclusively or associated to asthma, was the most frequent disease in both groups. Among the patients monosensitised to fungi, it was the most common single manifestation (46.4% versus 37.5%), although this difference was not statistically significant ($p = 0.310$).

Although there is evidence of an association between rhinitis and sensitivity to fungi, this topic is still controversial. Orlandi et al.[13] consider fungi not to be relevant to chronic rhinosinusitis, while others[14] have found an evident relationship, in paediatric populations, between allergic rhinitis and sensitivity to *Alternaria*, independently of asthma and compatible with the inhalation of this fungus into the upper respiratory system;[15] this same publication from Singapore describes the percentage of patients monosensitised to fungi as 12.2%.

In our experience, asthma as an exclusive manifestation, turned out to be much less frequent, and was reported to a similar degree in both groups. Obviously patients with severe asthma are attended at emergency services of pneumology units, but we assume that after recovering, allergy studies should be done in specialised services.

In the monosensitised group, persistent severe asthma was only found in 5.5%, which means that in most cases the asthma was intermittent, mildly persistent or moderate, which is quite different from the more severe cases described in other publications, based on data obtained from pneumology or hospitalisation services.[1,16] Asthma associated with rhinitis was most common in the polysensitised group, 54.6% versus 43.6%, (not statistically significant, $p = 0.152$). However, persistent cough was more frequently observed in the monosensitised group (29.1% vs. 18.7%; $p = 0.174$), but there is no evidence attributed to their fungi sensitisation.

Other non-respiratory clinical manifestations such as urticaria and angio-oedema were observed more frequently in the polysensitised group, probably because of the high

number of patients allergic to food and also to pollens and epithelia. Eczema was slightly more common in the monosensitised group.

Symptom seasonality among patients allergic to *Alternaria* has principally been described between May and September in southern Europe, coinciding with increased concentrations of fungal spores of this species outdoors.[17] This data coincides with increased prevalence of symptoms among monosensitised patients during this period, even though the majority had perennial symptoms, a fact which could be related to the presence of *Alternaria* and other moulds inside the home, as we have documented in this environment.[6]

Skin prick tests were useful in detecting patients allergic to fungi, showing high specificity. In 74% of the patients, the wheal was equal to or greater than histamine, and the average diameter was 5.8 mm (33.6 mm^2).

Peripheral eosinophilia was found to be increased in more than half of the patients without any other differences between the groups. High levels of total serum IgE were more frequently found in polysensitised patients, mostly in those who presented reactivity to multiple allergens.[18] The highest values were found in patients with severe eczema associated with rhinitis. Extrinsic atopic dermatitis has been associated with the highest levels of IgE.[19]

Ninety-five percent of monosensitised patients presented specific IgE for one or more fungi, close to the 93.5% found in polysensitised patients. These data do not correlate with the lower sensitivity described by others.[9,12] Specific IgE distribution by classes was not different between the two groups. Among the monosensitised patients, high or very high levels of specific fungal IgE were found, 64.4% of them were distributed among classes 3–5 (3.5–99.9 kU/L).

The provocation test is considered to be the one that best correlates sensitivity demonstrated by cutaneous or specific IgE tests with the clinical repercussion of this test.[20,21] Conjunctival provocation is one of the most used in allergology because it is safe and easy to perform,[22] specificity is also high or very high according to our experience, which concurs with several publications.[23,24] However, there is little information about its utility in the study of allergy to *Alternaria* and other fungi. In our experience, this test is very efficient in confirming the degree of clinical sensitisation. Following the strict protocol this test was applied on almost 80% of the patients, 40.5% of monosensitised patients and 30.1% of the polysensitised ($p = 0.203$) reacted with low or very low concentrations of the allergen. Only 6.5% did not react; all of the monosensitised – except one case – presented positive provocation test, showing the high degree of clinical sensitisation in this group.

After diagnosis of a fungal allergy most practitioners recommended specific immunotherapy. Among the 55 monosensitised patients, 66% of them received this treatment, mostly subcutaneously (80%). No severe adverse effects were shown and it was not necessary to discontinue the treatment either during the induction phase or during the first 12 months. Immunotherapy with fungal vaccines has been a controversial topic due to initial results published during the 1980s[25] which indicated frequent and important adverse side effects, mostly in children vaccinated with *Cladosporium herbarum*. In more recent publications, severe secondary reactions to vaccines with *Alternaria* are rare, principally due to advances in the availability of well-standardised vaccines for this fungus.[26–29] In our experience we have not detected systemic nor local severe reactions in the few patients who received vaccines against *Penicillium chrysogenum* or *Aspergillus fumigatus*.

Clinical results of immunotherapy should be considered after more than 1 year of treatment and they are difficult to assess, in any case this topic was not included in the main objectives of this study.

This protocolised study with a large number of patients allergic to *Alternaria* or less frequently to *Cladosporium*, *Aspergillus*, *Penicillium* or *Candida albicans*, shows the importance of these sensitisations, in many cases associated with other pneumoallergens. However, analysis of patients who only show sensitisation to fungi has demonstrated that the most common clinical presentation was rhinitis or rhinoconjunctivitis alone or associated with asthma.

Applying a rigorous diagnostic methodology: skin tests and serum specific-IgE associated with a conjunctiva provocation test, is very useful for confirming the clinical relevance of fungal sensitisation and allows for more rigorous prescription of specific immunotherapy. Subcutaneous or sublingual immunotherapy has proven to be safe as long as the patient was adequately monitored.

Conflict of interest

The authors have no conflict of interest to declare.

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