Sensitization to Alternaria in patients with respiratory allergy

Joan Bartra¹, Jordina Belmonte², Josep Maria Torres-Rodriguez³, Anna Cistero-Bahima⁴

¹Allergy Unit, Department of Pneumology, Hospital Clinic, University of Barcelona, Spain, ²Botany Unit, Autonomous University of Barcelona, Spain, ³Clinical and Experimental Mycology Research Group, IMIM, Autonomous University of Barcelona, Barcelona, Spain, ⁴Allergy Department, Institut Universitari Dexeus, Autonomous University of Barcelona, Spain

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Material and Methods
   3.1. Clinical study
   3.2. Aerobiological study
   3.3. Data analysis
4. Results
   4.1. Clinical study
   4.2. Aerobiological study
5. Discussion
6. Acknowledgments
7. Appendix
8. References

1. ABSTRACT

Alternaria alternata (AA) is an important mould in respiratory allergic diseases. The objective is to determine the prevalence of AA sensitization in respiratory allergic patients and to examine the annual variation of Alternaria spores. 824 patients between 5 and 65 years old with allergic rhinitis and/or asthma were enrolled. Prick tests were performed with two different standardized AA extracts with quantification of Alt a 1. Alternaria spores concentrations were provided. 151 patients (18.3%) were sensitized to AA. The patients sensitized to AA were affected more frequently by asthma when compared to the patients not sensitized to AA (chi-square test = 7.34; p = 0.003). The prevalence of AA sensitization in paediatric population was statistically significantly higher than in patients older than 13. Atmospheric levels of Alternaria spores showed two periods of maximum concentration: July/August and October/November. The sensitization prevalence of AA in patients with respiratory allergy is meaningful, fundamentally in paediatric patients and/or allergic asthma patients. There is a significance spore concentration of Alternaria in the studied area, with a seasonal behaviour.

2. INTRODUCTION

Fungal allergy is a worldwide problem (1-3). Exposure to certain fungal spores can cause human illness such as asthma. Alternaria alternata (AA) is considered to be one of the most frequently involved molds in reaginic-based allergic diseases. AA is a cosmopolitan organism that can be isolated in outdoor environments, where it grows on dead vegetation and can be recovered from soil samples. Nonetheless, exposure to Alternaria allergens can also occur indoors (4). Exposure to the airborne spores of AA can initially result in sensitization that later on may lead to the development of a fungal allergic disease.

The diagnosis of IgE-mediated allergy to AA is usually based on clinical history and skin test. Diagnosis can be supported by in vitro allergen-specific IgE determination. In vivo and in vitro AA allergy diagnosis is usually performed using an Alternaria extract containing a complex mixture of proteins, glycoproteins, polysaccharides, and other substances. The AA extracts frequently show a considerable variability due to inter-strain genomic differences, differences in culture conditions and the use of different extraction procedures.
Sensitization to Alternaria

Figure 1. Geographic situation of the patients area studied with the places of centers participants. Extension of the area studied: 32.196 km².

Currently, there are no standardized allergen products available in the United States (7), and the quality of commercial fungal extracts used in Europe is often low (8). More than 9 allergens have been described in AA extracts, although only 2 of them are considered major allergens (Alt a 1 and Alt a 2). Alt a 1, the most relevant allergen found in AA extract, reacts with serum IgE in 82 to 100% of AA-sensitized patients (9,10).

The real prevalence of AA sensitization in the general population or in patients with respiratory allergy is difficult to establish due to the use of un-standardized extracts in epidemiological studies also carried out with different methodologies. Two epidemiological studies based on general population reported a prevalence of sensitization to AA varying between 4.4 and 12.9% (11,12). Prevalence studies of AA sensitization in patients with respiratory allergy living in areas with similar climatic characteristics have shown great disparity with percentages ranging from 3 to 24%. (13,14). Recently published GA2LEN network results also shows marked differences in the sensitization to AA between 1.7 and 20.5% (15). The review of the epidemiological studies on the prevalence of AA sensitization so far reported reveals that they have been performed in mixed populations: people with suspected allergy from a population sample, patients tested in hospital, patients with rhinitis and/or asthma, adult population, pediatric population.

Several studies have clearly established the role of fungal sensitization in the development of symptomatic asthma. An accumulated body of evidence suggests that fungal sensitization, particularly to *Alternaria*, is associated with asthma (1,16,17) and mostly with severe and near-fatal asthma (18-20). There also reports that show that AA sensitization is more frequent in asthmatic children than in adults (1, 21-23).

The aims of our study were: 1) To determine the prevalence of AA sensitization by means of the skin prick testing using two different standardized extracts with quantification of Alt a 1 in pediatric and adult patients with suspected respiratory allergy. The results of the skin test and in vitro determination of the specific IgE were also compared to assess the concordance between the two tests and 2) to examine the annual variation of *Alternaria* spores in the atmosphere of Catalonia (Spain).

3. MATERIALS AND METHODS

3.1. Clinical study

Fourteen Allergy Units geographically representative of Catalonia (Spain) were selected to participate in this multicenter study (Figure 1). Each center carried out at least 74 evaluations according to a common experimental protocol in patients affected by rhinitis and/or asthma of suspected allergic etiology. Patients of both sexes aged 5 to 65 years, visited in the selected centers in consecutive order (7 patients per month) from January 2002 to December 2002 were enrolled. Patients on specific allergen immunotherapy and those affected by severe infections, malignant disorders or metabolic disease were excluded. Subjects with acute skin symptoms, with negative skin reactions to a histamine test or under treatment with drugs whose activity could interfere with skin prick test responsiveness were also excluded.

A questionnaire including demographic and disease data was completed by each patient included in the study. In all centers the correct use of the questionnaire as well as the consistency of clinical data and prick testing results was assessed by an allergologist. All patients or their caregivers were informed about the design and aim of the study and consented to participate. The study was
that had been kept frozen. ImmunoCAP, Phadia (Uppsala, Sweden). All in vitro IgE quantifications were performed at the end of the trial in the same laboratory using the same reagents on sera samples.

To determine specific serum IgE, 10 mL of blood (to obtain 5 mL of serum that was frozen and maintained at –20ºC) was collected only from those patients who performed the in vivo tests outlined the profile of each allergen tested. The drop of the extract was then wiped off one minute after the prick. Histamine (10 mg/mL) and glycerol-saline solution were used as positive controls respectively. The assessment was done 15-20 minutes after the prick: a wheal of > 7 mm² and negative controls respectively. The assessment was done 15-20 minutes after the prick: a wheal of < 7 mm² 2 and negative controls respectively. The assessment was done 15-20 minutes after the prick: a wheal of < 7 mm² as cut-off to establish the limit of positive and negative result. Correlation between the wheal area and specific IgE serum levels was calculated using the linear correlation test. The association between both AA sensitization and the presence of asthma and AA sensitization and pediatric patients was evaluated using the Chi-square test.

4. RESULTS

4.1. Clinical study

One thousand fifty six patients were included in the study, 232 of whom were excluded because they were skin prick testing negative. Demographic and clinical characteristics of the 824 atopic patients are summarized in Table 2. One hundred fifty one patients (18.3%) were sensitive to at least one of the two AA extracts. Demographic and clinical characteristics of this group are summarized in Table 3.
Table 2. Demographic and clinical characteristics of allergic patients. All data are expressed as number of subjects and percentage (in parenthesis).

<table>
<thead>
<tr>
<th>Total</th>
<th>151 (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>87 (58)</td>
</tr>
<tr>
<td>Men</td>
<td>64 (42)</td>
</tr>
<tr>
<td>≥ 14 years old</td>
<td>102 (68)</td>
</tr>
<tr>
<td>&lt; 14 years old</td>
<td>59 (32)</td>
</tr>
<tr>
<td>Age range</td>
<td>5-58 years old</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>80 (53)</td>
</tr>
<tr>
<td>Rhinitis and asthma</td>
<td>71 (47)</td>
</tr>
<tr>
<td>Sensitization to House dust mite</td>
<td>49 (32)</td>
</tr>
<tr>
<td>Sensitization to House dust mite and Pollen</td>
<td>9 (6)</td>
</tr>
<tr>
<td>Sensitization to House dust mite and Epithelium</td>
<td>17 (11)</td>
</tr>
<tr>
<td>Sensitization to House dust mite and moulds</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Sensitization to House dust mite, epithelium and pollens</td>
<td>15 (10)</td>
</tr>
<tr>
<td>Sensitization to House dust mite, epithelium and moulds</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Sensitization to House dust mite, epithelium, pollens and moulds</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Sensitization to Pollens</td>
<td>26 (17)</td>
</tr>
<tr>
<td>Sensitization to Pollens and epithelium</td>
<td>14 (9)</td>
</tr>
<tr>
<td>Sensitization to Epithelium</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Sensitization to only Alternaria</td>
<td>3 (2)</td>
</tr>
</tbody>
</table>

All data are expressed as number of subjects and percentage (in parenthesis). House dust mite: Dermatophagoides pteronyssinus, Dermatophagoides farinae; Pollen: grass mix, communis wall pellitory (Parietaria judaica), mugworth (Artemisia vulgaris), olive pollen (Olea europea), plane tree pollen (Platanus hispanica), Cypress (Cupressus arizonica); Epithelium: cat, dog. Other moulds: Cladosporium herbarum, Aspergillus fumigatus.

The patients sensitized to AA were affected more frequently by asthma respect to the patients not sensitized to AA (chi-square test = 7.34; p = 0.003).

The prevalence of AA sensitization in patients between 5 and 13 years was statistically significantly higher than in patients older than 13 years (chi-square test = 31.33; p = 0.001).

Of the 151 patients sensitized to AA, 147 patients were positive to the two extracts AA A and B. From among of the 151 patients, one had a skin prick test positive to extract of AA B and negative to extract A; 4 patients had a skin prick test positive to extract AA A and negative to extract B. There were no statistically significant differences ( p = 0.37) between AA extract A and AA extract B in the percentage of positive tests. Nor were significant differences in the wheal areas of the positive reactions with the AA extract A and AA extract B (p = 0.575).

Prick tests with the AA extracts and the level of specific Ig E were compared in 103 of the 151 patients sensitized to AA. Of these 103 patients, 67 had a specific Ig E more than 0.35 kU/L (range between 0.36 and > 100 kU/L) and 63 more than 0.70 kU/L. The range of specific Ig E was between 0.36 kU/L and more than 100 kU/L. In 36 patients (35%) the in vitro test did not confirm the in vivo test positivity. There was neither correlation between the wheal area of AA extract A and the levels of specific Ig E, nor between the wheal area of AA extract B and levels of specific Ig E.

4.2. Aerobiological study

The main results obtained in the aerobiological study are shown in Table 4. Higher levels in spore scores (annual index and mean daily spore concentrations) were reached in inland (Girona) and rural areas (Bellaterra, LLeida), with respect to coastal and urban areas (Barcelona, Tarragona). The inland and rural area of LLeida showed the highest annual levels of Alternaria, followed by Barcelona and Girona. Similar levels and tendencies were found in Bellaterra and Tarragona, where levels accounted for less than one third of those detected in Lleida. Figure 2 represents the annual evolution of Alternaria spores in the atmosphere depicted as mean weekly spore concentrations. Atmospheric levels of Alternaria spores show two periods of maximum concentration, in July/August and in October/November.

5. DISCUSSION

This epidemiological study evaluates the prevalence of AA sensitization in a geographical area with different climatic conditions in patients with respiratory allergy, using a biologically standardized extract of the major allergen Alt a 1. Outdoor counts of Alternaria spores was also studied.

In our study the AA sensitization prevalence was 18.3% with a major percentage in patients with allergic asthma and in those younger than 14 years of age. Cutaneous tests were more sensitive to establish AA sensitization than serum specific IgE determinations. The aerobiological study shows the presence of Alternaria spores throughout the study period with seasonal increases in Spring and Autumn when daily peaks of spores could reach levels close to 800 spores/m3.

AA sensitization prevalence in the atopic population varies considerably according to different studies. Discrepancies in AA sensitization could be partly related to: 1. lack of standardized extracts, 2. Differences in
Sensitization to Alternaria

Table 4. Annual indexes (Sum of mean daily spore concentrations) and maximum mean daily total spores and Alternaria spores in the studied localities during 2002

<table>
<thead>
<tr>
<th>Sampling locality</th>
<th>Total spores</th>
<th>Alternaria spores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Annual Index (Spores)</td>
<td>Maximum mean daily concentration (Spores/m³)</td>
</tr>
<tr>
<td>Barcelona</td>
<td>284,253</td>
<td>5,606</td>
</tr>
<tr>
<td>Bellaterra</td>
<td>404,939</td>
<td>6,118</td>
</tr>
<tr>
<td>Girona</td>
<td>985,639</td>
<td>18,374</td>
</tr>
<tr>
<td>Lleida</td>
<td>388,038</td>
<td>6,572</td>
</tr>
<tr>
<td>Tarragona</td>
<td>290,727</td>
<td>8,641</td>
</tr>
</tbody>
</table>

The studied population is exposed to high levels of atmospheric AA spores especially in Summer and at the end of Autumn, when medium weekly concentrations are higher and maximum daily peaks are found. Although we have no knowledge of published studies showing the exposure threshold of AA spores able to sensitize predisposed subjects, these conditions are propitious to sensitize the patients. This seasonality also agrees with aerobiological studies done in other locations, where maximum Alternaria peaks are obtained from Spring until the end of autumn (1,29,30).

The correlation between prick test results and IgE values agrees with other studies in which skin test response and seric values were compared (8,14,27). As with other allergens, the skin test is more sensitive than specific IgE determination. Mold extracts and mold allergen molecules are rich in carbohydrates. These compounds are known to interfere with the linking of allergen molecules to solid supports employed for IgE in vitro analysis.

The association between AA sensitization and asthma observed in our study is in consonance with other epidemiological studies (16,17,21,28) in which fungal allergy (concretely AA) is considered a major risk factor for asthma, including severe asthma. This association is more frequently found in the paediatric population, as confirmed in our study. Currently the mechanism through the AA is a risk factor for the development of asthma are unknown but, the Alternaria proteases can damage the bronchi and the allergens can be more aggressive, children being more vulnerable and especially when presenting genetic condition.

Although Alternaria exposure may occur outside as well as indoors, aerosampling data conducted at a time when Alternaria spores are prevalent in the outdoor environment indicate that spores can also infiltrate the indoor environment (1). A relationship exists between indoor fungal accumulations and outdoor weather. Higher indoor levels are noted when outdoor fungal levels increase (31). Some studies of Alternaria sensitization prevalence demonstrate, in geographic areas with low atmospheric Alternaria spore levels, that a low prevalence of Alternaria sensitization also exists (32,33). The number of Alternaria spores varies extensively depending on the climatic and geographic conditions. Another fact that demonstrates the importance of fungal atmospheric concentration are the studies that show a parallelism between asthma attack exacerbations with high fungal spore peak (3,34,35). Therefore, it is important to have a spore exposure map to give information to sensitized patients about their exposure, even though we cannot conclude data from this study about the clinical relevance of this sensitization. In function with this seasonality of Alternaria spores checked in former studies, patients should be enrolled in a randomized way.

3376
Sensitization to Alternaria

Figure 2. Seasonal variations of mean weekly Alternaria spore concentrations. A: Barcelona; B: Bellaterra; C: Girona; D: Lleida; E: Tarragona.

In conclusion, data in this study reveals that the sensitization prevalence of AA in patients with respiratory allergy is meaningful, fundamentally in paediatric patients or allergic asthma patients. AA should be considered in their diagnostic procedure using biological standardized extracts with allergen quantification of at least major allergens. There is a significant spore concentration of Alternaria in the studied area, with a seasonal behaviour. Further studies have to be directed in the clinical relevance of this sensitization, even in severe asthma patients. The elaboration of fungal maps with fungal spore concentration prediction are needed to make the prophylaxis and control of allergic pathology easier due to fungal spores exposure. The atmospheric concentration of AA spores does not always correlate with the concentration of Alt a 1, as the Alt a 1 concentration varies as a function of spore activity, and is highest during the germination phase. For this reason, in addition to fungal spore maps, the performance of studies on the atmospheric concentration of Alt a 1 should be recommended in order to correlate these concentrations more closely with the patients’ symptoms.

6. ACKNOWLEDGEMENTS

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7. APPENDIX

Mould Allergy Study Group of the Societat Catalana d’Al·lèrgia i Immunologia Clínica (SCAIC): T. Alfaya (Hospital Santa Maria de Lleida), P. Amat (Alergocentre. Barcelona), M. Baltasar (Hospital Universitari Germans Trias i Pujol. Badalona), I. Bartra (Hospital Universitari de Girona Dr. Josep Trueta), J. Belmonte (Unitat Botànica, Universitat Autònoma de Barcelona), M. Bosque (Corporació Sanitària Parc Taulí. Sabadell), V. Cardona (Hospital Universitari Vall d’Hebró. Barcelona), M.J. Castillo (Consorci Hospitalari Terrassa), M.T. Cerdà (Hospital Universitari de Girona Dr. Josep Trueta), A. Cisterò-Bahima (Institut Universitari Dexeus. Barcelona), M. Corominas (Ciutat Sanitària i Universitària de Bellvitge. Hospital de Llobregat), E. Gabarra (Aerobiological Network of Catalonia), P. Gaig (Hospital Universitari de Tarragona Joan XXIII), P. García-Ortega (Ciutat Sanitària i Universitària de Bellvitge. Hospital de Llobregat), M. Guilarte (Hospital Universitari Vall d’Hebró. Barcelona), M. Ibero (Consorci Hospitalari de Terrassa), R. López-Abad (Hospital Universitari de Tarragona Joan XXIII), R. Llatser (Hospital Sant Pau i Santa Tecla. Tarragona), R. Lleonart (Fundació Altaia. Manresa), A. Malet (Alergocentre. Barcelona), Ll. Marqués
Sensitization to Alternaria


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Sensitization to Alternaria

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**Abbreviations:** AA: Alternaria alternata; IgE: Immunoglobulin E

**Key Words:** Alternaria alternata, Alt a 1, Asthma, Rhinitis, standardized extract

**Send correspondence to:** Joan Bartra, Allergy Unit. Department of Pneumology. Hospital Clinic. University of Barcelona. Spain, Villarroel 170. 08036 Barcelona. Spain, Tel: 34-93-227-55-40, Fax: 34-93-227-54-55, E-mail: jbartra@clinic.ub.es