

Epidemiology of *alternaria alternata* allergy: a prospective study in 6840 italian asthmatic children

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Abstract. – *Background:* The prevalence of *Alternaria Alternata* (AA), a mold causing in children severe asthma is scarcely known, also due to the underdiagnosis of AA allergy, frequently due to multiple sensitizations to molds.

Objective: To analyze this issue we prospectively studied all children attending our Division between January 4, 1990 and December 31, 1997.

Methods: A total of 6840 children with asthma or allergic rhinitis were evaluated. Diagnosis was established by family and personal history, physical examination, skin prick tests (SPTs) and RAST (Radio Allergo-Sorbent Test) for inhalants including AA. We further evaluated: (1) sensitization only to AA allergens without positivity for additional inhalants; (2) prevalence of AA positivity among children with asthma or allergic rhinitis; (3) concordance between SPTs and RAST for allergy to molds, (4) proportion of children treated with specific immunotherapy (SIT).

Results: Among the 6840 children 213 were positive to AA (3.3%), only 89/6840 children (1.3%) had AA monosensitization ($p = 0.0001$), a concordance between SPTs and RAST was present in 21/89 (23.6%) children ($p = 0.0009$), and only 9 children out of 89 were SIT treated. Concerning the clinical manifestations, 83 had asthma or allergic rhinitis, and 6 had asthma associated with atopic dermatitis. Family history was positive in 82.9% of children. The mean onset of AA sensitivity was at age 4 for males, and at age 5 for females.

Conclusions: In childhood, AA allergy is a genetic affection. The SPT concordance with history and clinical examination appears to be operative. Due to life-threatening reactions in children with AA allergy, we suggest that those with suspected inhalant allergy be tested with AA allergens, and treated with SIT if positive.

Key words:

Alternaria Alternata, Prospective study, Epidemiology, Family history, Children, Asthma, Allergic rhinitis.

Introduction

AA is a well-known agent of asthma and/or rhinitis in children, belonging to the class Deuteromycetes, very common in worldwide as an indoor allergen. AA spores are diffused according to the geographic place, the season, the prevalent atmospheric conditions, and the time of the day¹⁻³. AA grows above all in humid places with $T = 20-30^{\circ} C$, such as kitchens, bathrooms, garage, etc. in all season of the year, but above all in summer. Rainy days and relative humidity help the growth of molds also evident after spread in dry days. The spores are very small in diameter ($<5 \mu m$), cigar-shaped and long even $70 \mu m$, and diffuse easily in the air in the windy days¹⁻³. We know several AA allergens (Alt a 1-4, 6, 7, 10-12)^{4,5}: the main allergen is Alt a 1; whose high levels in the air result from the counts of the fungal spores transported by the wind¹⁻³. Alt a 1 is a dimer of disulphide-linked subunits that migrate in SDS-PAGE under reducing conditions at apparent $M/r/s$ of 14,500 and 16,000. IgE antibodies to this protein are present in the sera of $>90\%$ of AA-sensitive patients³.

AA can elicit in school-age children immediate or delayed asthma and bronchial hyper-reactivity (BHR)^{6,7}. Exposure to high numbers of AA spores can trigger severe asthma exacerbations and is a risk factor for respiratory arrest in AA-sensitized asthmatic children and young adults⁷. Such exposure to the spores can be aggravated because AA can release more rapidly than other molds the mycelia, potent allergens which in allergic patients have demonstrated significant allergenicity compared to the spores⁸.

It is therefore crucial to early identify the at risk children with SPTs and/or RAST^{8,9} and to submit these patients to SIT¹⁰. It has been shown that SIT is very effective to reduce attacks of asthma and BHR in children with

AA sensitization^{1,10}. Although at 4 years of age AA is the third most common cause of sensitization after Der p and grass pollens¹¹, the prevalence of AA allergy in children is scarcely known owing to the lack of widespread epidemiological studies¹¹⁻¹³, often resulting from multiple sensitizations¹⁴.

Patients and methods

To analyze the above issue we have prospectively studied all children with asthma or allergic rhinitis attending our Allergy and Clinical Immunology Division between 4 January 1990 and 31 December 1997, all with the following prerequisites:

1. To suffer from asthma and/or allergic rhinitis since at least 3 years;
2. Having not received an appreciable benefit, in the previous years, from preventative treatments using DSCG or Nedocromil sodium, or ketotifen, with the consequent necessity of using a symptomatic treatment with antihistamines, bronchodilators and/or corticosteroids;
3. Residence in Rome county;
4. SPTs and RAST positive for molds;
5. Absence of any other important disease.

As usually, the diagnosis of atopic diseases in the children was done as follows: Family and personal history, physical examination, SPTs and/or RAST to the most common inhalant allergens.

We assessed whether the children were "at risk" of atopic disease because of a positive family history of atopy since one or both parents and/or other siblings suffered from asthma, or allergic rhinitis.

Appropriate emergency equipment and medications were available on site. Antihistamine drugs and oral/topical steroids were stopped at least two weeks before SPT application. Skin testing was done at baseline by the prick method by a doctor trained in allergy with the co-operation of a qualified nurse. The skin was marked with a ballpoint pen for the allergens to be tested. The babies were then tested with: histamine hydrochloride (1 mg/ml) as a positive control and isotonic saline as a negative control. We continued with a battery of inhalant allergens, including Dermatophagoides pteronyssinus, Alternaria alternata, Lolium perenne, Olea europea and Parietaria offic-

nalis (SARM, Roma, Italy). The diagnostic extract of each individual allergen was placed on the volar surface of the forearm as drops through which the skin was superficially pricked with a straight pin for one second. A new pin was used for each prick test and then discarded, and the drop of the extract was then wiped off about one minute after the prick¹⁵.

SPTs were read at 20 minutes and considered positive as follows:

- + when the wheal was the half of the histamine wheal;
- ++ when the wheal was equal to the histamine wheal;
- +++ when the wheal was two-fold the histamine wheal;
- ++++ when the wheal was more than two-fold the histamine wheal¹⁶.

We took for positive only children with a +++ or ++++ reaction, that is a wheal \geq 3 mm with an area = 7 mm² (cut-off). So we considered as positive only the children with a mean wheal diameter of 3 mm or larger than the negative (saline) control¹⁷. A positive (histamine, 1:1000) control was performed to ensure the absence of any antihistamine drug interference¹⁶. Concerning Alternaria extracts, we strictly correlated SPT results with the clinical conditions of the individual child, each followed-up at least for three years.

Specific IgE antibodies and determination of specific IgE levels were done by radioallergosorbent test (Phadezym RAST, Pharmacia Diagnostics). RAST results are expressed in "RAST Units" (PRU = Phadebas RAST Unit) as follows:

- 1st class = IgE levels < 0.35 IU/ml,
- 2nd class = IgE levels between 0.35 IU/ml and 0.7 IU/ml,
- 3rd class = IgE levels between 0.7 IU/ml and 17 IU/ml,
- 4th class = IgE levels higher than 17 IU/ml.

We considered as positive only children with 3rd or 4th class results. The children were excluded from the study when the correlation between clinical history, in vivo and in vitro results, and diagnosis was doubtful.

The record-charts, as it is usual in our Division, were filled for each child by an allergist according to the data referred to from the accompanying parents. In particular we asked about the type, onset, duration of disease, drug treatment and possible previous SIT. For the diagnosis of asthma, 3 episodes of wheezing

without fever were required. For the diagnosis of allergic rhinitis, nasal discharge and/or blockage occurring continuously for at least 4 weeks plus the typical pale aspect of allergic mucosa on rhinoscopy, without any sign of infectious rhinitis in other relatives was required.

We further evaluated: (1) prevalence of AA positivity among children positive to inhalants; (2) sensitisation only to AA without positivity for other inhalants; (3) concordance between SPTs and RAST for allergy to AA; (4) incidence of SIT prescription among children sensitized only to AA.

Statistical analysis:

The statistical calculations were performed using the X² test.

Informed consent:

Informed consent was obtained from parents of each child.

Results

We have evaluated in total 6840 children aged 1-9 years, all with asthma or allergic rhinitis. All required preventative treatments using DSCG or Nedocromil sodium, or ketotifen, or severe exacerbations demanded a symptomatic treatment with antihistamines, bronchodilators and/or corticosteroids

Almost all children were resident in Rome or in Rome County, and 96.9% of them tested positive for inhalant allergens, except AA:

1. Sensitization to AA without positivity for other inhalants:
among the 6840 children, 213 were positive to AA (3.1%) as part of a multiple sensitization;
2. Prevalence of AA positivity among children with respiratory allergy:
only 89/6840 children (1.3%) had a mono-sensitization to AA;
3. Concordance between SPTs and RAST for allergy to molds:
a concordance between SPTs and RAST was present in 21/89 (23.6%) children, since only 21 had both SPTs and RAST positive to AA ($p = 0.0009$);
4. Number of children treated with SIT:
we also learned that 9/89 children (10.1%) were currently subjected to SIT;
5. Family history:
family history was positive in 82.9% of parents of the 89 children;

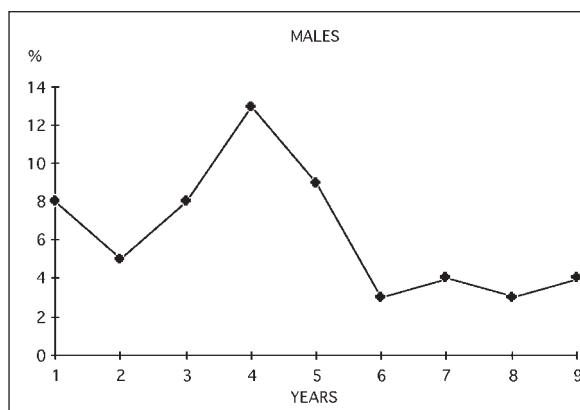


Figure 1. Percentage of males reacting positively to Alternaria SPTs by age groups.

6. Sex:

among the 85 children, 57 were males = 64.04%) and 32 females (35.96%) ($p = 0.0002$), aged 1-9 years;

7. Age of onset of sensitization to AA:

as it is shown in Figures 1 and 2, the peak of AA sensitivity was at age 4 for males, and at age 5 for females. Subdividing the children into age groups, more males were 1-4-year-old, compared to females ($p = 0.0001$);

8. Clinical manifestations:

the clinical manifestations are summarized in Table I, and Table II shows the seasonal prevalence. Asthma alone or associated with other atopic diseases was significantly present in the children ($p = 0.0001$). AA sensitization was prevalent in autumn-winter compared to spring-summer presentation: $p = 0.0023$.

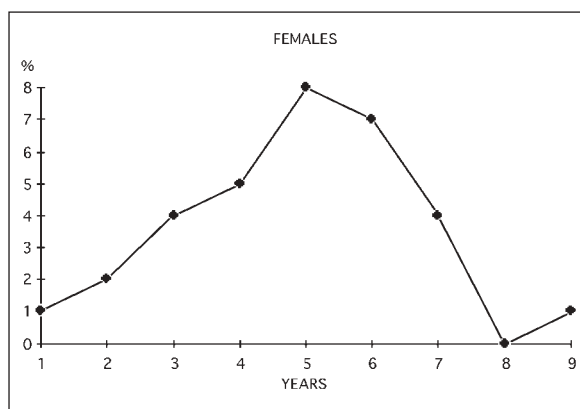


Figure 2. Percentage of females reacting positively to Alternaria SPTs by age groups

Table I. Clinical manifestations in children with mono-sensitization to AA.

Symptoms	No. of children	%
Asthma	49	55.1
Asthma and allergic rhinitis	19	21.3
Allergic rhinitis	15	16.8
Asthma and atopic dermatitis	6	6.7

$p = 0.0001$ (Asthma alone or associated versus allergic rhinitis alone)

Discussion

This is the first pediatric epidemiological study evaluating the presence of AA mono-sensitization in a very large range of allergic children (6840).

Moreover, this study shows that the sensitization to AA appears to be a significant independent risk factor for pediatric asthma: 74/89 children (83.1%) had asthma alone or associated with allergic rhinitis alone ($p = 0.0001$) (Table I). Sensitization to AA is thus one of the most significant factors in respiratory allergy and the growing interests in it are its involvement in severe asthmatic reactions. While the mean year incidence comprises 855 children, this is fluctuating: e.g. in 1991 we have found in 977 children a 3.7% incidence of sensitization. Probably due to the more frequent multiple sensitizations, AA allergy appears to be underdiagnosed and few studies consider only children who have monosensitization to AA¹¹.

Five important results of the present study must be stressed: (1) the very high positivity of family history, making this affection a genetic one in children, (2) the very high proportion of boys compared to girls (57/89 =

64%), (3) the earlier onset in males also compared to females, a more frequent perennial incidence, (4) the higher incidence of asthmatic manifestations and (5) the prevalent autumn-winter compared to spring-summer presentation. These results, all marked by significant statistical differences, again stress the severity of respiratory allergy due to AA sensitization. Formerly El-Sharif et al¹⁸ demonstrated that paternal asthma and maternal hayfever significantly tripled the risk for their children to have wheezing.

SPTs should be considered the most reliable diagnostic parameter in children (gold standard), as demonstrated by their concordance with the severe clinical manifestations shown by all children with positive SPTs to AA (prevalence of asthma and of perennial symptoms) (Tables I and II). Kelso et al have confirmed that the concordance between SPTs and RAST results is present only in a part of cases¹⁹. Evaluating the RAST standard, the mRAST (modified RAST) and the CAP (chemiluminescent assay) in comparison with SPTs, mRAST is the most sensible, the RAST standard is the most specific, and mRAST and CAP are the most effective²⁰. In asthmatic children a concordance was found in 54.5-93% with SPTs and RAST for Der p, pollens, Fel d, Can d, and molds including AA²¹. A multicenter study reported that CAP sensitivity is equal to 95.5%, and the specificity is equal to 98.1%, with a range between 93.4% for Der p and 99.5% for AA²². Further, CAP appears to be a useful tool for measuring RAST to AA allergens⁶.

Moreover, we have demonstrated that sensitization to AA is higher in other Italian studies. Partly the discordance among these studies are correlated to the difficulty of taking into account only monosensitized patients. In a recent study, a multiple sensitization to inhalant allergens, including AA, was found in 187 children, 18 of whom were monosensitized (9.6%) to AA¹³. In another study among 391 children, 28 were sensitized to molds (7.1%) but it was not specified how many children were sensitized to AA¹². In Southern Australia, in two pediatric studies, a percentage of sensitization to AA between 4.4% and 6.2% was found in one study²³, and between 4% and 15.2% in another³: the figures were highest in children living in dry, hot inland towns, while the lowest figures were

Table II. Seasonal manifestations in children with monosensitization to AA.

Symptoms	No. of children	%
Perennial	55	61.8
Seasonal	34	38.2
autumn-winter	25	28.1
spring-summer	9	10.1

Perennial vs seasonal: $p = 0.0016$, autumn-winter vs spring-summer: $p = 0.0023$

reported in children living in hot, humid towns on the coast. Consequently, such reports appear to show that a damp climate may be critical to develop mold colonies, but a dry climate favors spore detachment and the consequent potential to trigger sensitization. We stress that indoor, humid places, can be frequent, from 33 to 86% of cases from unpublished data of our Department.

In a widespread epidemiological study including more than 4,000 people encompassing the whole USA population the percentage of sensitization to AA varied from 3% in the younger age group and 7.5% in older children and young adults aged 12 to 17 and 18 to 24 years. However, the authors found allergic rhinitis only in association with asthma²⁴. We deem therefore that large numbers of children are necessary to determine a reliable percentage of incidence.

Studies have stressed the increase in asthma prevalence^{14,23-28}, AA allergy may turn out to be very dangerous in children, being associated with acute asthma that requires emergency treatment²⁹. Its significant prevalence can derive from an increased allergenicity depending from the quicker release than in other molds of potent allergens, such as AA spores and mycelia^{8,10}. It has been stressed that AA is able to trigger asthma attacks, and even anaphylactic shock: the exposure to AA is a risk factor of respiratory arrest in asthmatic children and young adults⁷. Further, exposure to fungal spores can adversely affect the daily respiratory status of some asthmatics³⁰ with fatal cases significantly correlated with spore counts³¹ >1.000/m³. Moreover, the correlation among family and personal history, physical examination, SPTs, and clinical manifestations appears to be operative also in a recent study¹⁴, as well as between allergy and the presence of asthma²⁴ (55.1% in the current study). Several studies have confirmed that AA is one of the most important risk factor for the development and the persistence of asthma³²⁻³⁵. A 6-year-old child with IgE-mediated sensitivity to *Alternaria* developed an acute, life-threatening asthma attack during the peak *Alternaria* season³².

Although we have found only 1.3% of 6840 children with monosensitization to AA, the severity of this condition suggests that all children with suspected inhalant allergy should be tested with standardized AA aller-

gen extracts, and this mold should be included in the standard panel for the diagnosis of respiratory allergy³⁶. We deem that following-up children with asthma related to AA sensitization, and evaluating the course of their illness, helps to eliminate false-positive SPTs to AA. Such data also show that exposure to environmental molds can play a role in AA sensitization, and should be considered in prevention strategies. Children with severe clinical manifestations should be treated with SIT, which in AA allergic children is very effective¹⁰. Oral immunotherapy can also be effective: a recent clinical trial³⁷ stressed that, in children treated with immunotherapy, cutaneous and bronchial reactivity were significantly reduced, while there was an increment of IgG4 levels. We suggest to children with AA allergy to wear a medical bracelet alert, and that parents of the youngest children are provided with a cellular phone.

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