

Prevalence of sensitization to *Alternaria* in allergic patients in Italy

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Background: The actual prevalence of sensitization to *Alternaria* is not known, partly due to the unreliability of diagnostic extracts.

Objective: To assess skin positivity to extracts of *Alternaria* in a wide population of Italian patients suffering from respiratory symptoms using a biologically standardized extract.

Methods: A total of 2942 patients were skin prick tested with *Alternaria*, and a panel of common inhalant allergens. Blood samples for specific IgE quantitation were taken both from patients positive and from patients negative (control group) to *Alternaria* extract.

Results: Three hundred six patients (10.4%, ranging from 1.8% in Turin to 29.3% in Cagliari) were positive to *Alternaria*; 37 were sensitized to only this mold, while the remaining 269 were sensitized to at least one other allergen. Of the *Alternaria*-positive patients, 79.7% suffered from rhinitis and 53.3% from asthma, either alone or associated with other symptoms.

Conclusions: We suggest that, at least in Italy and in countries with similar climatic and environmental situations, standardized *Alternaria* extract should be included in the panel commonly used in investigating the allergen responsible in patients suffering from respiratory allergy.

Ann Allergy Asthma Immunol 1998;80:71–6.

INTRODUCTION

Many genera of Hyphomycetes (*Fungi imperfecti*, with only asexual repro-

duction) are regarded as potentially important allergen sources. *Alternaria*, *Cladosporium*, *Drechslera*, *Epicoccum*, *Fusarium*, and *Aspergillus* are considered to be the most relevant ones.¹ They are potentially present in outdoor as well as in indoor environments, mainly in humid places and dwellings. The Basidiomycetes (mushrooms, puffballs, rusts, smuts, etc.) are also now being studied by allergolo-

gists.² The most thoroughly studied species are *Alternaria alternata* (formerly known as *Alternaria tenuis*) and *Cladosporium (Hormodendrum) herbarum*, probably because of the large and easily identified number of spores collected by aerobiologic sampling.

The air diffusion of fungal spores depends on the time of the day, the season, the atmospheric conditions, the geographic area^{3,4} and the distance from the source of emission, as well as other factors.^{5,6} *Alternaria alternata* is currently considered the mold most widely responsible for reaginic-based allergic syndromes, rhinitis and bronchial asthma, even if there is less prevalence of positive reactions compared with pollens and house dust mites.^{7,8} The less frequent positive skin tests may be due to less allergenicity or inadequate extract purification rather than reduced exposure, for maximum daily *Alternaria* counts can reach 1,000 spores/mm³ in August. Exposure to the airborne spores of this mold can trigger severe exacerbations of asthma and is a risk factor for respiratory arrest in children and young people suffering from bronchial asthma.⁹

The actual prevalence of such sensitization is not fully known due to the lack of widespread epidemiologic studies. From the allergologic point of view little is known about molds compared with other inhalant allergens. We know, however, that a protein concentration at least 30 times higher than that employed for instance, with mite extracts, must be used to obtain comparable allergenic potency at least in the case of *Cladosporium*.¹⁰ Intrader-

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mal tests, where high allergen concentrations are not required to identify sensitivity, have been therefore traditionally employed for mold allergy diagnosis. During recent years improvements in production, purification, and standardization techniques have provided reliable prick test extracts for molds.¹¹⁻¹⁴ We have, therefore, planned our trials according to the first three steps recommended by SIAIC (Italian Society of Allergy and Clinical Immunology) for the diagnosis of allergy.¹⁵ The steps commonly employed by Italian allergologists are (1) clinical history of the patient, (2) prick testing with a biologically standardized extract of *Alternaria alternata*, and (3) in vitro determination of *Alternaria alternata*-specific IgE.

The in vitro quantitation of specific IgE antibodies was planned for all patients positive to this allergen by skin prick testing; a number of negative patients were also included to obtain information about sensitivity of the skin test.

The aim of our study was, therefore, to determine the prevalence of skin positivity to *Alternaria* by means of the skin prick test method in a wide population of patients who underwent an allergologic evaluation performed according to the recommendations of SIAIC.

The results of the skin test and in vitro determination were also compared to determine the correlation between the two tests.

MATERIALS AND METHODS

Population

This study involved 20 Italian Allergologic Centers, selected in order to have results that were significant for the whole of Italy. Each center undertook therefore to perform at least 100 allergologic evaluations according to a common experimental protocol. All patients of both sexes, affected by respiratory pathology likely to be of an allergic nature and arrived at the selected centers in consecutive order during January to May, 1994 to have special allergologic examinations, were

included in this study. Patients undergoing specific immunotherapy for a past or present *Alternaria* allergy with infectious diseases or patients affected by malignant or toxic-dysmetabolic diseases (such as diabetes) as well as pregnant women were excluded. Patients showing the presence of severe cutaneous symptoms or a negative skin reaction to histamine, or patients treated with drugs that might interfere with the cutaneous response and all other cases in which internationally acknowledged diagnostic procedures could not be performed were excluded.^{16,17}

A questionnaire containing personal details and the type and duration of pathology was filled in for every patient included in this study. In all centers the questionnaire compilation was performed by an allergologist who also verified the consistency of anamnestic data and prick test results. Then, the same doctor confirmed the diagnosis of allergic illness. All cases where correlation or diagnosis were uncertain were excluded from the study. All centers followed the same experimental protocol approved by the Italian Health Authorities.

Skin Tests

Alternaria extract (supplied by Alergia e Immunologia Abellò SA, Madrid, Spain) was obtained by extracting spores and mycelium from a pure selected culture of *Alternaria alternata* with 50 millimolar aqueous NH_4HCO_3 . After purification by means of diafiltration, the allergenic composition of the extract was characterized by electrophoresis and immunoblotting, versus an in house reference extract whose biologic potency had been determined in vivo.¹⁸

For this purpose 48 patients with a clinical history suggesting sensitization to *Alternaria alternata*, who had never been subjected to specific immunotherapy before, and with specific serum IgE to *Alternaria alternata*, were prick tested with four different concentrations of the allergen extract (highest concentration 8 mg protein/mL, progressive dilution 1:10). Histamine (10

mg/mL) and physiologic saline were employed as controls, while the test with each allergen concentration was performed in duplicate. The wheal provoked by each test was measured, the geometric mean for each concentration was calculated, and the mean wheal areas were plotted against concentration on double-logarithmic paper. According to the method followed, the nominal potency of 100 BU/mL is expected to give wheals with an area of approximately 75 mm² (9.7 mm of diameter) in the average allergic population.^{19,20} The diagnostic extract also contained 50% vol/vol glycerol, 0.4% wt/vol phenol, and 0.9% wt/vol sodium chloride.

All selected patients were tested with the *Alternaria* standardized extract and with a set of the inhalant allergens most commonly used in Italy: grass mix (including *Avena sativa*, *Dactylis glomerata*, *Festuca pratensis*, *Hordeum vulgare*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Secale cereale*, *Triticum sativum*, *Anthoxanthum odoratum*, *Cynodon dactylon*, *Holcus lanatus*, *Phragmites communis*), wall pellitory (*Parietaria judaica*), mugwort (*Artemisia vulgaris*), dust mites (*Dermatophagoides pteronyssinus* and *farinae*), cat dander, *Aspergillus* species, *Cladosporium*, and olive pollen (*Olea europaea*).

The diagnostic extract of each individual allergen was placed on the volar surface of both forearms so that the space between each test site was of at least 3 cm. This was done to avoid interference between allergens. Prick tests were performed by the same operator in each center, using a sterile plastic disposable lancet with a 1.25 mm tip²¹; the drop of the extract was then wiped off about one minute after the prick. Histamine (10 mg/mL) and glycerol-saline solution were used as positive and negative controls respectively. The assessment was obtained 20 minutes after the prick; a wheal of <7 mm² was considered a negative reaction. In order to have a correct, uniform assessment of the skin positivity, the same allergologist who per-

formed the *in vivo* tests outlined the profile of every wheal using a fine-point marking pen. The mark then was transferred by adhesive tape to the questionnaire of each *Alternaria* skin test positive subject.

In Vitro Tests

Blood samples of about 10 mL for the *in vitro* quantitation of specific IgE antibodies were taken from all patients skin-positive to the *Alternaria* extract. *Alternaria*-specific IgE was detected by RAST using reference standards supplied in commercial kits by Pharmacia (Uppsala, Sweden) and a monoclonal radioactive-labelled IgE-specific antibody (HE2-Hamlet).²² Paper discs were prepared according to the method described by Ceska and co-workers²³ employing an *in house* reference extract of *Alternaria alternata*¹⁸ as stated above. Coupling was performed with a protein concentration of 40 µg/disc. The same *in-house* reference extract had been used to standardize *Alternaria alternata* prick-test diagnostics employed in this study. All *in vitro* IgE quantitations were performed at the end of the trial in the same laboratory with the same reagents on sera samples kept frozen.

Data Analysis

Levels of *Alternaria*-specific IgE and wheal areas provoked by *Alternaria* and the controls (histamine and glycerosaline solution), evaluated by means of planimetry by the same operator for all tests, were statistically analyzed by a single specialized center not participating in this study. A statistical analysis (McNemar test) was carried out on the data concerning skin positivities to prick tests in the patients examined, taking either the absolute value of 7 mm²²⁴ or the area ratio (allergen wheal area/histamine wheal area) higher than 0.25¹⁵ as cut-off limit. The correlation between the wheal area and specific IgE was calculated using the linear correlation test.

RESULTS

This study included 2942 patients, 1443 men and 1488 women. In 11 cases the sex was not stated in the

questionnaire. The ages ranged from less than 1 year to 73 years, with a higher frequency of skin positivities at ages between 21 and 30. Skin positivity (wheal area at least 7 mm²) to *Alternaria* was detected in subjects with ages ranging from 4 to 60 years. Symptomatology was present from a minimum of less than 1 year to a maximum of 37 years. Positivity to *Alternaria* was detected in 306 patients (10.4%), but it varied considerably among the different centers from a minimum of 1.8% (Turin) and 1.9% (Genoa) to a maximum of 24% (Lecce) and 29.3% (Cagliari) (Fig 1). Of subjects skin-positive to *Alternaria*, 55.2% had positive family histories for allergy.

By subdividing the sample population into age groups, the highest percentages of skin positivity to *Alternaria* were found at ages ranging from 0 to 10 and 11 to 20 years, with values of 17% and 18% respectively (Fig 2). In contrast, as an absolute value, the skin positivity to *Alternaria* was found more frequently in ages ranging from 11 to 20 and 21 to 30 years, with a greater prevalence in males (Fig 3).

Among patients positive to the prick test, monosensitization to *Alternaria* was detected in a total of 12.1% of

cases, whereas the remaining patients (87.9%) showed concomitant sensitization to one or more allergens (Fig 4). As far as the clinical picture of skin-positive patients to *Alternaria* is concerned, 53.3% of the patients had bronchial asthma, which was in 13.7% of the patients the only manifestation of allergy. Rhinitis was detected in 79.7% of the patients, but only 17.6% of these suffered from rhinitis alone (Fig 5). Prick tests with the *Alternaria* extract and the level of specific IgE were compared in 284 patients only (233 positive and 51 negative to the skin test) because of refusal of blood tests by many patients. The correlation between *in vivo* and *in vitro* tests was the following: both positive in 133 patients (133/233 = 57.1%) and both negative in 51 cases (51/51 = 100%). In 100 patients (35.2%) the *in vitro* did not confirm the *in vivo* test positivity, whereas in no cases was the RAST positive in subjects negative to the skin test. The test of linear correlation between skin positivity and RAST levels was not significant. Assuming an allergen wheal area/histamine wheal area ratio higher than 0.25 as cut-off, positivity to *Alternaria* extract was detected in 313 patients. This difference was not significant. Only in 15 patients

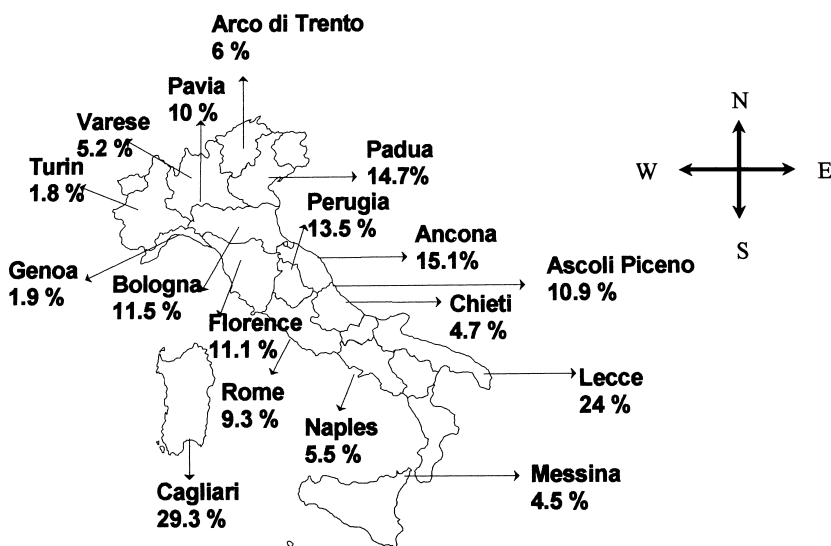


Figure 1. Geographic distribution of centers taking part with percentages of subjects with positive reactions to *Alternaria* skin test.

did the two tests give different results; among these, two prick test-negative patients (negative according to the area ratio criterion but positive according to the absolute wheal dimension criterion) were RAST class 2. The lack of correlation between skin absolute reactivity (wheal area at least 7 mm²) and the RAST results, as already commented, was confirmed also in the case of the area ratio criterion.

DISCUSSION

One of the most important mold-induced pathologies in allergology is sensitization to *Alternaria* and the growing interest in it is due to the probability that it is involved in severe asthmatic reactions.⁹

The percentages of sensitization revealed in the atopic population vary considerably according to different studies.^{3,4,8} These discordancies could be linked partly to the purity of the diagnostic extracts employed.¹²⁻¹⁴ Furthermore, it is often difficult to correlate the detected positivity to *Alternaria* with the seasonal symptomatology because of frequent polysensitization. As a general rule, patients monosensitized to *Alternaria* are a small minority.²⁵

Two wide-ranging case-record pediatric studies by J K Peats et al³ report a prevalence of positivity to *Alternaria* that varied from 4% to 15.2%. The percentage is higher in children living in hot, dry inland towns, compared with those living in hot, humid towns on the coast. In a similar pediatric study carried out in Southern Australia, J K Peats and J Woolcock⁴ reported percentages of sensitization to *Alternaria* that varied between 4.4% and 6.2%, and that were higher in hot, dry, inland towns than in two humid towns, one on the coast and the other inland. The reported data seem to indicate that, even if a damp climate is required to develop mold colonies, spore detachment and the consequent ability to induce sensitization, are favored by a dry climate.

In a wide-ranging epidemiologic study of more than 4,000 people covering the entire USA, P T Gergen and

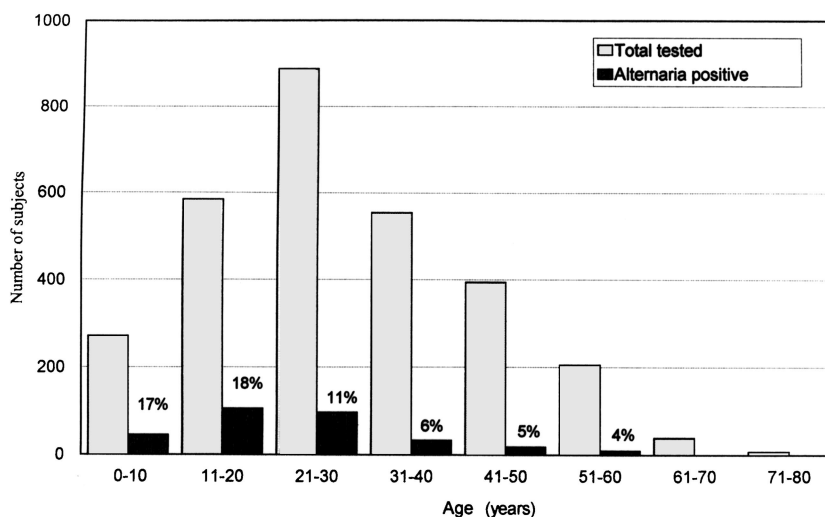


Figure 2. Percentages of subjects reacting positively to *Alternaria* skin test compared with number of subjects tested by age-groups.

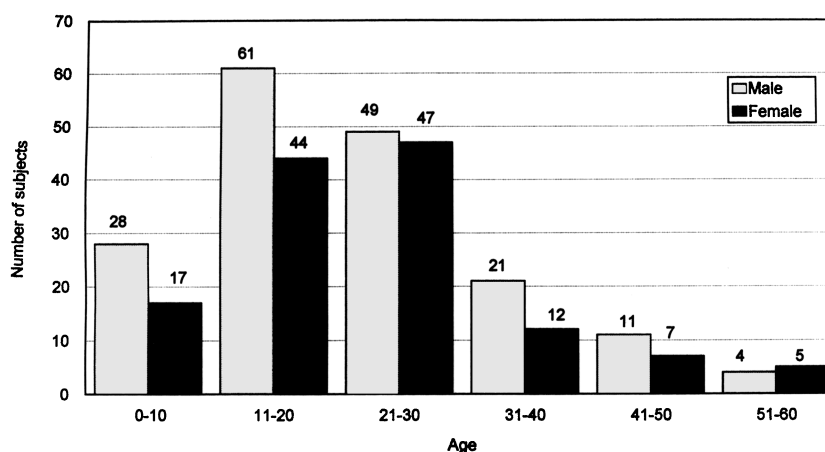


Figure 3. Subjects reacting positively to *Alternaria* by sex and age-group.

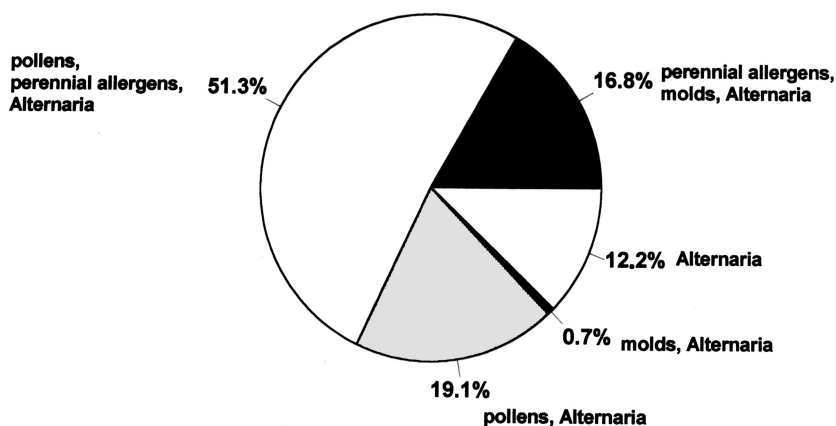


Figure 4. Skin test positivity.

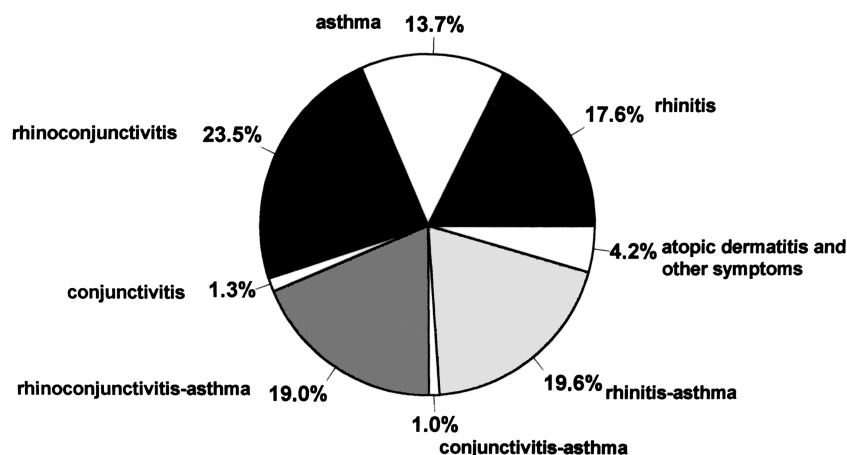


Figure 5. Type of symptoms in subjects with positive reactions to *Alternaria* skin test.

P C Turkeltaub⁸ reported percentages of sensitization to *Alternaria* of approximately 3% in the age group 6 to 11 years and of 7.5% in the two age groups 12 to 17 and 18 to 24 years. Furthermore, this study revealed a statistical correlation between allergy to *Alternaria* and the presence of bronchial asthma. On the contrary, rhinitis was found only in association with asthma.

The importance of the current study derives from the fact that it is the first Italian epidemiologic assessment that evaluates the presence of sensitization to *Alternaria* in a very wide range of allergic patients (approximately 3,000 subjects).

Furthermore, the Allergological Centers involved in the research were selected in order to represent the different climatic conditions of the nation. In fact, Italy is characterized by northern regions, which are generally colder, and by southern regions, which are warmer, while humidity is prevalent in inland areas versus coastal towns. Finally, a higher number of towns than those in previous studies^{3,4} were included in this research. The results allow the following observations. Sensitization to *Alternaria* is more frequent among subjects less than 30 years of age, as generally occurs for other allergic inhalant-induced diseases. Furthermore, the existence of a family predisposition to generic aller-

gens in approximately 50% of positive patients is true also with respect to molds.

As far as the disease pattern correlated with skin-positivity to *Alternaria* is concerned, in our study rhinitis had a uniform prevalence (approximately 80%) but bronchial asthma was also present in more than 50% of cases. The prevalence of allergy to *Alternaria* (approximately 12% on average) observed was higher than that of previous studies. The percentage varied significantly throughout the national territory and ranged from a minimum of less than 2% in Turin and Genoa to a maximum of more than 30% in Cagliari.

The percentage differences detected are difficult to interpret, as they do not always seem correlated with the climatic conditions (temperature and humidity) of the geographic area studied. In fact, in southern coastal towns (Naples, Lecce, Messina, and Cagliari) that have similar climates, mild in winter and hot and windy in summer, clearly different positivities (from 5.5% to 29.3%) were detected. Similar climatic conditions characterize the coastal town of Genoa (Northern Italy), where a very low percentage of positive subjects was detected (1.9%). In Northern and Central Italy inland towns (Perugia, Florence, Bologna, and Pavia), which are cold in winter and hot and humid in summer, there is a more uniform tendency towards skin

positivity with medium-to-high values (from 10% to 13.5%). The foothill towns of Northern Italy (Turin, Varese, and Trento), cold in winter and less hot and humid in summer, had rather low percentages (from 1.8% to 6%).

As we have already seen pointed out in the literature, our study confirmed that monosensitization to *Alternaria* is present in a low percentage of subjects, who are also usually positive to other seasonal or perennial allergens. This is very important, because only in rare monosensitized subjects can the clinical importance of sensitization to *Alternaria* be correctly verified by comparative monitoring of symptoms and exposure to the allergen without influences by other concomitant sensitizations.

The statistical analysis did not show significant differences between the cutaneous tests and the level of specific IgE. At least in the case of *Alternaria*, the positive or negative result of a skin prick test performed with a well characterized and biologically standardized extract does not require any in vitro confirmation. Mold extracts and mold allergen molecules are rich in carbohydrates²⁶; these compounds are known to interfere with the linking of allergen molecules to solid supports employed for IgE in vitro analysis.²⁷ This could, at least partly, account for the differences encountered between results of in vivo and in vitro diagnosis in our research on mold allergy.

These results confirm that skin tests should generally be considered the most reliable evidence (gold standard) among those available at present for the diagnosis of allergy, even though some authors advise the performance of intradermal tests to investigate sensitization to molds.¹⁶

The results of this research should encourage further studies of the clinical significance of sensitization to *Alternaria* in patients monosensitized to this mold by determining correlation between symptoms and exposure.

Finally the prevalence of *Alternaria alternata* sensitization in Italy is high enough to suggest inclusion of this

mold in the standard panel for the diagnosis of respiratory allergies.

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