

Statistical evaluation and parameters of phlogosis in patients sensitized to cypress

M.T. VENTURA¹, M. GELARDI², R. DI GIOIA¹, R. BUQUICCHIO¹, G. ACCETTURA¹, R.A. TUMMOLO¹, A. ARSIENI¹

¹Department of Internal Medicine, Immunology and Infectious Disease (MIDIM). ²University of Bari; ¹Department of Otolaryngology, University of Bari, Bari, Italy

Received: October 10, 2006

Accepted January 17, 2007

ABSTRACT: To assess the increasing sensitivity to cypress, we evaluated a group of allergic subjects living in Apulia who had been referred for respiratory symptoms to the Section for Allergic and Immunological Diseases (SAID) of Bari University Hospital in the period between January and March 2006, and compared them with a group evaluated during the same months of 2003.

We submitted all patients to Skin Prick Tests (SPT) and Radio-Allergo-Sorbent Tests (RAST). Our data show that the percentage of people sensitised to cypress has increased from 28.6% (2003) to 65.5% (2006). By contrast, we observed no variations in sensitisation to other substances. Since according to data furnished by the Italian Association of Aerobiology (AIA), Monitoring Center of Brindisi 1, there was a variation in the quantity of pollen grains in 2006 as compared to 2003, it seems conceivable that an increase in the amount of pollen grains, together with other factors such as the volume and the brittleness of the grain may influence the increasing sensitization.

In addition, other allergo-immunologic parameters were evaluated for a better evaluation of this allergic phlogosis.

KEY WORDS: Pollinosis, Cypress, Aerobiology, Eosinophil, ECP

INTRODUCTION

"Pollinosis" is a seasonal allergic pathology, also defined as "hay fever", characterized by symptoms like nasal itch, rinorrhea, nasal obstruction, conjunctivitis and sometimes asthma after exposure to pollens in predisposed subjects (1). The pollens form a biological aerosol and, after spontaneous inhalation, penetrate the upper respiratory mucosa and release their allergenic content. Clinical symptoms appear in subjects specifically sensitized to allergens, who synthesize IgE after pollen stimulation (2). The pollens of cypress appear punctually after blooming: they are present in the air from the beginning of January, and with the subsequent flowering of different species belonging to this family, remaining in high concentrations (some hundred granules per cubic meter of air) up to the end of April. In Southern Italy, according to some studies, the minimum has been reported to be equal to 2 % (4-5). Other Authors found that the prevalence

of allergy to cypress in the general population in Italy varies from 9.2% in the North, to 28.2% in Central Italy and 20.1% to the South (3). In Europe the prevalence ranges from 20% in South Italy to 35% in Cordoba, Spain (3-4). The present study was conducted retrospectively with the purpose of evaluating increased sensitization to *Cypress sempervirens* and *Cypress arizonica* in a large group of allergic subjects living in Puglia, Italy, by comparing evaluations made during the winter periods of 2003 and 2006. In addition, some parameters of inflammation were evaluated in order to determine variations of allergic stimulation.

MATERIALS AND METHODS

For this study we evaluated allergic patients with respiratory symptoms referred to the SAID of the University of Bari in the period between January and March 2006 and compared them with patients examined at the same University hospital in the pe-

0393-974X

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Table I - General Characteristics Of The Two Patient Populations

	2003	2006
Patients	230	420
Sex		
Males	103 (44.8)	188 (44.8%)
females	127 (55.2%)	232 (55.2%)
Mean age	33.28	35.24
Family history		
Negative	94 (40.9%)	213 (50.7%)
Positive	136 (59.1%)	207 (49.3%)
Symptoms		
Rhinitis	192 (83.5%)	380 (90.5%)
Conjunctivitis	134 (58.3%)	250 (59.5%)
Asthma	55 (23.9%)	82 (19.5%)
Period		
April-June	50 (21.7%)	65 (15.5%)
January-June	19 (8.3%)	57 (13.6%)
January- March	15 (6.5%)	114 (27.1%)
Perennial	146 (63.5%)	184 (43.8%)
Pollinosis	160	350
Allergic to cypress	66 (41.3%)	275 (78.6%)
Allergic to parietaria	46 (28.8%)	85 (24.3%)
Allergic to graminaceae	64 (40%)	120 (34.3%)
Allergic to olive tree	56(35%)	116 (33.1%)

riod between January and March 2003. The clinical history was evaluated by means of a questionnaire on personal and family data. In addition, information was obtained about any symptoms of pollinosis. All patients were submitted to diagnostic evaluations by means of Skin Prick Tests (SPT) and RAST for inhalant allergens. A group of 20 patients sensitised only to cypress was submitted to evaluation of the ECP in nasal lavage and in serum, to blood eosinophil (EOS) count, and to otorhinolaryngoiatric evaluation by the Otorhinolaryngoiatric Clinic and the study of nasal endoscopy and nasal cytology in our centre, at the same University Hospital.

Skin Prick Test

All patients underwent SPT with a standard panel of inhalant allergens (pollens: graminaceae, composite, parietaria, olive, cypress arizonica, cypress sempervirens; inhalants: D. pteronyssinus, D. farinae, aspergillus and epithelia of dog and cat) (Bayer, Milan-Italy), a positive control (histamine, 10mg/ml), a negative control (glycerinated solution). The results have been interpreted as coded by the European Academy of Allergology and Clinical Immunology (EAACI), subcommission "Standardization of the allergens and Skin Test". The test was considered positive if the mean wheal diameter was

Table II - Clinical And Personal Data Of Patients Sensitized To Cypress

	2003	2006
Patients	66	275
Mean age	32.02	34.77
Sex		
Males	35 (53.0%)	130 (47.3%)
Females	31 (47.0%)	145 (52.7%)
Family history		
Negative	31 (47.0%)	135 (49.1%)
Positive	35 (53.0%)	140 (50.9%)
Symptoms		
Rhinitis	63 (95.5%)	260 (94.5%)
Conjunctivitis	55 (83.3%)	221 (80.4%)
Asthma	12 (18.2%)	46 (16.7%)
Period		
April-June	11 (16.7%)	29 (10.5%)
January-June	19 (28.8%)	52 (18.9%)
January-March	14 (21.2%)	102 (37.1%)
Perennial	22 (33.3%)	92 (33.5%)

3 mm or larger after subtraction of the mean wheal diameter of the negative control (6).

Radioallergosorbent Test (RAST)

To determine specific serum IgE titres for latex, RAST was evaluated according to the manufacturer's instructions as well as the CAP system (Pharmacia, Uppsala, Sweden) (7).

Level of the IgEs

Peripheral blood was obtained by venipuncture and the sera were frozen at 70°C until tested. Specific IgE (CAP-RAST IgE FEIA; Pharmacia) against graminaceae, composite, parietaria, olive, cypress arizonica, cypress sempervirens; inhalants: D. pteronyssinus, D. farinae, aspergillus and epithelia of dog and cat were tested according to standard procedures (7).

Nasal lavage

A relatively non-invasive and well tolerated method was used, able to measure inflammation of the upper respiratory tract (8). Nasal Lavage fluid was taken before treatment with FPANS and at the end of treatment. The subjects were asked to retain

3 ml of 0.9% physiological solution at 36°C for about 10 s, and the operation was then repeated in the other nasal cavity. The lavage fluid was sealed in test tubes and centrifuged at 300 g for 8 min. To inactivate proteolytic enzymes in test tubes were added 5 mmol/l ethylenediaminetetraacetic acid and 0.5 mmol/l phenylmethyl-sulfonyl fluoride. The liquid and cellular phases were separated and the supernatant was stored for successive analyses.

ECP

The ECP levels in the serum and nasal fluid were assessed by the fluoroenzymatic immunoassay (FEIA) with the Pharmacia CAP System (Uppsala, Sweden). In serum, the standard curve ranges between 2 and 200 µg/l; in accordance with the parameters established by the manufacturer, normal levels are thus in the range 2–16 µg/l (9). By contrast, in NL we obtained values ranging between 25.1 and 71.9 µg/l before treatment.

Blood EOS count

Blood EOS were counted by the UNOPETTE Test (Becton Dickinson, NJ, USA) on peripheral blood anti-coagulated with EDTA. The result is ex-

Table III - Eosinophil Count And Ecp In Nasal Lavage (NI) And In Serum

	2003	2006	Normal Subjects
EOS/mm ₃ in peripheral blood	175.1±44 ^a a vs c: <i>P</i> =NS	206±36 ^b b vs c: <i>P</i> =NS	159±52 ^c
EOS/ in nasal citology (Cells on 10 microscopic field)	74 ^a (range 52-87) a vs c: <i>P</i> <0.0001	84 ^b (range 62-91) b vs c: <i>P</i> <0.0001	9 ^c (range 7-12)
ECP in serum (g/l) (mean ± SD)	25.7±7.5 ^a a vs b: <i>P</i> =NS a vs c: <i>P</i> <0.0001	26.8±6.4 ^b b vs c: <i>P</i> <0.0001	8.7±2.3 ^c
ECP in NL (g/l) (mean ± SD) before treatment	41.3±12.7 ^a (range 26.7-65.6) a vs b: <i>P</i> =NS a vs c: <i>P</i> <0.0001	43.4±13.4 ^b (range 44.5-63.8) b vs c: <i>P</i> <0.0001	8.5±1.7 ^c range(7.3-10.5)

pressed as EOS/mm³.

Nasal endoscopy and nasal cytology

Nasal endoscopy was performed under local anaesthesia using a flexible endoscope (Vision Science ENT-2000, Massachusetts, USA).

Particular importance was attributed to the nasal cytology study, performed by scraping with a Rhino-probe® (Apotex Scientific, Inc., Arlington, TX) in the middle part of the lower turbinate, an area characterized by a significant number of cells, in particular ciliated cells. After fixation with methyl alcohol for 4 minutes, the collected material was processed and stained according to the May Grunwald–Giemsa method. This method is suitable both for epithelial mucosa cells (eosinophils, neutrophils, mast cells, lymphocytes and macrophages) and for bacteria and mycotic spores. The cytological material was observed under the Nikon Eclipse E 600 Microscope, at 400 and 1000x magnification. The cell count was performed with the semi-quantitative method (cell count in 10 microscopic

fields, in immersion at 1000X magnification).

Besides the cytological evaluation, in the subjects with immunophlogosis the level of degranulation was considered, both of eosinophils and mast cells. These data were then correlated with the severity of the subjective symptoms (10).

Statistical analysis

On a default model from the National Institute of Statistics a Database of the two samples was, respectively prepared on 420 patients in 2006 and 230 patients in 2003. For each clinical history the following variables were analyzed: age, sex, family history, period of appearance of the symptoms, clinical data, result of the SPTs and the RAST (Pharmacia). The two samples were compared with the Chi-square test; *p* values <0.05 were considered statistically significant.

RESULTS

Among the 420 patients considered in 2006, 350

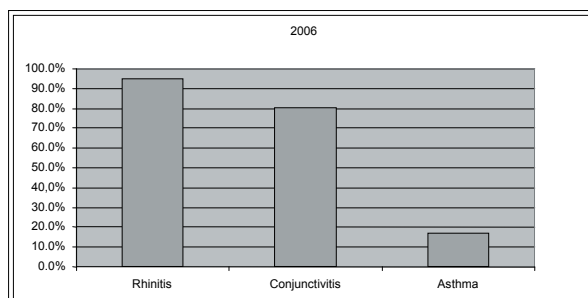


Fig. 1- Clinical symptoms observed in 2006

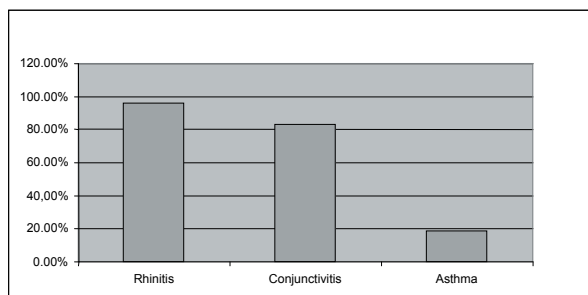


Fig. 2 - Clinical symptoms observed in 2003

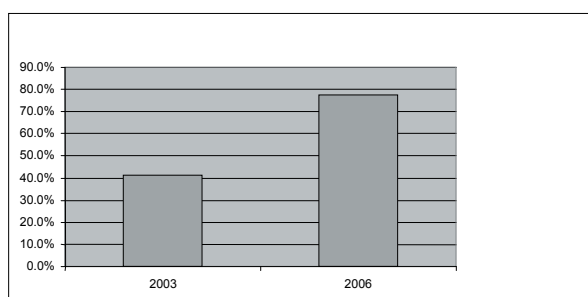


Fig. 3 - Incidence of cypress sensitization

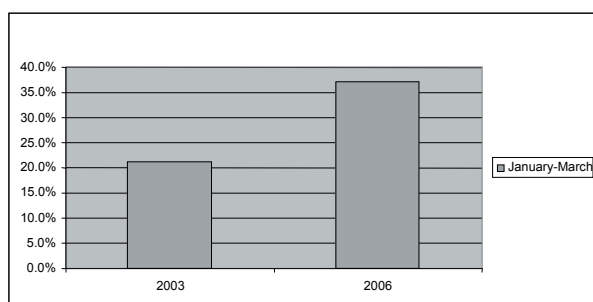


Fig. 4 - Incidence of cypress sensitization between January and March ($p=0.04$)

complained of respiratory problems and had SPT positivity to pollens; in the period between January and March 2003, 230 patients were examined and 160 of them reported symptoms related to a possible pollinosis.

The characteristics of the two populations of

patients are shown in Table I. They are similar in terms of general characteristics: mean age, sex distribution, family history of allergy. Table I shows that the clinical symptoms have similar percentages of incidence in the years 2003 and 2006; as to symptoms, analysis of the data showed that in 2006, 114 subjects (27.1%) had problems in January-March; 184 (43.8%), patients complained of perennial symptoms, while 57 patients (13.6%) had problems from January to June. Finally, 65 (15.5%) patients had symptoms between April and June. In 2003, 146 (63.5%) patients had perennial problems, 15 (6.5%) patients manifested symptoms in the months between January and March, in 19 cases (8.3%) the symptomatic period was between January and June, while 50 subjects (21.7%) manifested symptoms between April and June. Skin Prick Tests (SPT) for inhalants showed that of the whole group of patients affected by allergy to inhalants during 2006, 275 (78.6%) were allergic to cypress, whereas in 2003, 66 (41.3%) patients had skin positivity to cypress. Table II shows the epidemiological data of the patients sensitized to cypress examined in 2003 and 2006: mean age, sex, family history of allergies and symptoms. Oculorhinitis is seen to be the most frequent clinical manifestation, and asthma the least. Among the patients allergic to cypress observed in 2006, 94.5% complained of rhinitis, 80.4% suffered from conjunctivitis and 16.7% from asthma; similar data concerning the incidence of the clinical manifestations was found among the cases of cypress pollinosis observed in 2003, in that 95.5% had rhinitis, 83.3% had conjunctivitis and 18.2% complained of asthmatic symptoms. In 2006 the symptoms were present between January and March in 102 (37.1%) patients, between January and June in 52 cases (18.9%), between April and June in 29 subjects (10.5%) while the other 92 patient (33.5%) had perennial symptoms. In 2003 the period with clinical manifestations was between January and March for 14 patients (21.2%), between January and June for 19 (28.8%), between April and June for 11 (16.7%); the symptoms were perennial in 22 cases (33.3%).

The two samples were compared with the Chi-square test for significance. It emerged that the two samples are similar as regards mean age, family history and symptoms. (Fig. 1, Fig. 2). By contrast, the increased prevalence of sensitization to cypress resulted statistically significant, $p=0.001$ (Fig. 3). The greater incidence of symptoms in the period from January to March was also statistically significant, $p=0.04$ (Fig. 4).

As to the evaluation of the mediator levels in NL, in patients with problems like cypress pollinosis, who are sensitive only to cypress, and are

not in treatment with specific immunotherapy, our data show higher values of ECP than the norm ($P < 0.0001$), both in serum and NL. This was true in 2003 as well as in 2006. Evaluation of the nasal cytology revealed increased eosinophils in the nasal secretion of patients sensitized to cypress ($P < 0.0001$). The values in 2003 overlap those in 2006. By contrast, there were no statistical differences in the values of EOS assessed in peripheral blood in patients evaluated in 2003 and 2006 when compared with normal subjects (Table III).

DISCUSSION

Pollinosis toward *Cupressaceae* in the last few years has acquired the importance of an "emergent" pollinosis (11). In countries with a Mediterranean climate the pollen of Cypress is the most common aeroallergen in the winter and at the beginning of spring. The *Cupressus* (C.) species is native to the oriental regions of the Mediterranean, but has now spread to Italy, where the most common species are *C. sempervirens* (common Cypress) and *C. arizonica*, used for reforestation, as a windbreak and often as ornamental plants (12). In the same family other species are also employed as urban green plants, such as *C. Lawsonii*, that is one of the most efficient for acoustic absorption and is therefore used to pose barriers against noise. The species *Taxus* and *Juniperus* are also the more allergenic of the *Cupressaceae*; this should be kept in mind when exotic species are imported for ornamental purposes such as *Cryptomeria japonica* and some junipers from the American continent (*Juniperus pinchottii* and *Juniperus ashei*) with autumn and winter flowering (13). The greater spread of *Cupressaceae* explains the reason for the increase of this allergy, unknown in Italy until about twenty years ago. Subsequently, it was known as "smaller pollinosis" and today as "emergent pollinosis". The season of cypress pollinosis is centred in winter but varies from species to species (14). In Italy it starts in January and ends in February for *C. arizonica* and in March or April for *C. sempervirens* (15). The flowering of *C. arizonica*, which goes from January to February, overlaps that of *C. sempervirens*, that goes from February to March, and represents the earlier marker of the beginning of the pollen season. In general the maximum pollination has shifted from mid-March to mid-February, and this seems to be correlated to progressive global warming. In the family of *Cupressaceae* the genus *Cupressus* is of greater allergological interest, with 12 different species (16); the *C. sempervirens* and the *C. arizonica* are the most common species in Italy. In the sample of 2006, 275 patients of 420 patients with pollinosis

(65.5% of cases) had positive SPT to cypress; this prevalence is interesting because, according to the literature, the pollen of the *Parietaria* and the Olive Tree, in southern Italy, can be responsible for the high incidence of allergy in the population (17). Moreover, the increase of sensitization for *Parietaria*, *Graminaceae* and olive tree, as evaluated during these two years show a not significant variation. Comparing patients in 2003 and 2006, our study has underlined a statistically significant increase of sensitization to cypress ($p = 0.001$). According to some Authors the increased prevalence of sensitization to cypress could be attributed to two factors: 1) the increased use of *Cupressaceae* as ornamental plants for reforestation, where the rapid growth of pre-existing trees would determine an increased release of pollen particles every year (18) 2) the interaction of cypress pollen with pollution particles (13, 17). However, an increase in the prevalence of SPT positivity to the pollens of *Graminaceae*, *Parietaria* and Olive Tree was not found, even though they are the most important pollens in our geographical area (19). In our opinion, it is possible that these pollens, endowed with a greater allergenic power in comparison to those of cypress, have "exhausted" their abilities to increase the rate of sensitization on the basis of the degree of exposure, in the sense that when a plateau is reached, the limiting factor for the development of the allergic phenotype might not be linked to exposure but exclusively to individual genetic factors or to other, different environmental factors (20). The interactions between airborne particles and the different pollens or allergens with possibly greater local concentrations and presence could be important to justify the increase of sensitization to the pollen of cypress. Airborne particles, pointed out as the most important pollutant for most cities in a 1996 United Nations document, consist of solid and liquid particles of different size and origin, including soot, acids, sulfates and nitrates (21). More than 90% of the solid airborne particles in the big cities derive from the combustion of gas-oil in diesel motors, now termed diesel exhaust particles or DEPs (22). The reported morphological alterations of the pollen granules and the ability to bond with and transport allergens developed by DEPs, could contribute to sensitisation to some allergens (23). Besides, being carried by liposoluble substances, allergens with an easier transepithelial penetration could also achieve a sort of accumulation at local level (24). The greater rate of sensitization to the pollen of cypress finds an echo in the historical study by Ishizaki of *Critomeria japonica* or Japanese cedar, shown to be more harmful in urban than rural areas, regardless of the degree of exposure (13). The pollens of cypress

and cedar could undergo analogous morphological alterations on interaction with airborne particles from car exhausts and their allergens. Also, the cross-reactivity among the greater allergens of the two conifers is well known due to their structural analogy and the presence of a glycosylated residue in Cry j1 (25). Another study demonstrated glycosylation of the allergenic elements of the extract of *C. arizonica*, thanks to their ability to bind different lectins (26). This further supports the hypothesis that Lol p1, a glycosylated protein, binds DEPs and not Lol p5 (not glycosylated), and that the allergens of cypress and Japanese cedar can have analogous relationships with the organic components of DEPs. In addition, respiratory disease, the winter peak of which coincides with that of cypress pollination in particular, could contribute to bring on sensitization to cypress (27). The trend of the cypress pollen count for the period studied, kindly supplied by the Italian Association of Aerobiology (AIA), Monitoring Center of Brindisi 1, reveals increased amounts in the atmosphere of daily counts during the main pollen season in the first quarter of 2006 (about 264/m³ of air) in comparison with the first quarter of 2003 (about 201/m³ of air), that may explain our observations. Another important factor is the volume and brittleness of the grain. In fact, the pollen of cypress is small and weak, and consequently could be subject to rupture and become the source of a particulate aerosol containing pollen allergens (28). This phenomenon was hypothesized by D'Amato et al to explain the increased asthma epidemics observed during thunderstorms (29). The Authors believe that osmotic shock and the consequent release of allergen-rich cytoplasmic fragments could explain this increase. In our case the small-sized, weak cypress grains could better penetrate the upper respiratory tract, causing more symptoms and hence increasing the number of allergic tests and diagnoses (30). No variation in the presentation of symptoms was observed in our study between 2003 and 2006. In fact, in both years rhinitis was the most frequent clinical manifestation, followed by conjunctivitis and finally, asthma, all showing comparable percentages for the two years under study. An interesting point emerged from the comparison of the period of onset of the symptoms observed in the two samples. In fact, in 2006 37.1% of the subjects with sensitization to cypress had symptoms from January to March, whereas in 2003 only 21.2% of the sensitized patients suffered symptoms in this same period. This significant difference points out an increased correlation between sensitization to cypress and the referred clinical history. According to the pollen calendar, the cypress pollination season

starts in January and finishes in March. Nevertheless, it should be borne in mind that each year the dates of maximum pollination can vary by one month, depending on climatic conditions. Different studies have underlined that the peak of maximum pollination has moved from mid-March to mid-February and this data can be correlated with progressive global warming. The pollen season of the *Cupressaceae* is inaugurated by *C. arizonica*, that is pollinated from January to February.

As to the evaluation of the ECP, in the group of 10 patients sensitized only to cypress this parameter is a good basis on which to control allergic inflammation, being more reliable than other parameters such as the number of eosinophils in peripheral blood (31). All 10 patients observed had higher levels of ECP, both in serum and NL. In addition, in all cases there was an increased presence of eosinophils in the nasal secretion, but not in serum. The explanation for this phenomenon is that the level of the ECP indicates that of the allergic phlogosis, because it reflects a state of activation of the eosinophils that release ECP only when they are "activated" (32). In conclusion, in agreement with the data in literature, our study supports an increased sensitization to the pollen of cypress. Our data show that cypress pollen is responsible for respiratory allergy in an increasing number of subjects and that periodic controls of the ECP can enable screening of the clinical conditions of the patient, as well as evaluation of the benefits of the planned therapies.

ACKNOWLEDGEMENTS

We thank Dott. Augusto Arsieni of the Italian Association of Aerobiology (AIA) for the pollen count data furnished.

Reprint requests to:
Mariateresa Ventura, M.D.,
Department of Internal Medicine,
Immunology and Infectious Diseases (MIDIM),
University of Bari, Policlinico,
Piazza G. Cesare,
70124 Bari (Italy).
Tel. ++390805478793 Fax: ++390805593007
e-mail: mt.ventura@allergy.uniba.i

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