

EFFICACY OF SUBLINGUAL COSEASONAL IMMUNOTHERAPY WITH A MONOMERIC ALLERGOID IN CUPRESSACEAE POLLEN ALLERGY - PRELIMINARY DATA

R. Ariano, MD (1), R.-C. Panzani, MD (2), G. Mistrello, MD (3)

SUMMARY:

Background: Respiratory allergy to conifers pollens is a growing phenomenon all over the world (Mediterranean area, USA, Japan). In France and Italy *Cupressus sempervirens* is the commonest offender.

Objective: To check the efficacy of specific immunotherapy by the sublingual route using a monomeric allergoid of *Cupressus arizonica* pollen.

Material and Methods: 20 patients and 10 controls were randomly selected. The diagnosis was assessed on a clinical hivernal symptomatology of allergic rhinitis, and/or asthma, positive skin test and RAST, and nasal provocation test to *Cupressus arizonica*. The extract was a monomeric allergoid titrated in allergenic units (AU). The treatment was coseasonal from the beginning of december to the end of march. It was not conducted double blind.

Results: The evaluation of the allergenic potency of native and modified *Cupressus arizonica* extracts have shown that the modified extract has a decreased skin test IgE binding capacity.

According to symptom, drug consumption scores and nasal provocation test, there was a statistical improvement in the active group (compared to the control group) without any side effect.

Discussion: The use of a modified *Cupressus arizonica* extract, which is easier to prepare than *Cupressus sempervirens* and which is a monomeric allergoid, has shown that by the sublingual route, this therapeutic approach is safe and that the first results were satisfactory without any side effect.

This good tolerance was anticipated by the fact that, checked on 16 volunteers by skin test, the allergoid gave a less important local reaction on skin testing than the native extract.

Conclusion - This study has shown that:

- The KCNO modified *Cupressus arizonica* extract has a reduced IgE binding capacity ;
- This monomeric allergoid is safe and effective administrated by the oromucosal route in clinical respiratory allergy to *Cupressaceae* and devoid of any side effect.

Key-words: Cupressaceae pollen - Sublingual immunotherapy - Monomeric allergoid.

INTRODUCTION

The inhalation of *Cupressus sempervirens* and other cupressaceae pollens is becoming an increasing cause of pollinosis, particularly in the Mediterranean area (1-7). Reasons for such an increase, which are largely unknown could be explained, partly as consequence of a major exposure to Cypress pollens due to an extensive plantation of these trees in the recent past, and partly by a modification of pollens under the influence of pollution as demonstrated recently by Midoro-Horiuti and one of us (8), and by Lahoz et al. (9). In contrast with this

increase, the difficulties encountered in preparing reliable extracts because of the low proteinic and high carbohydrates contents of the pollen of *Cupressus sempervirens*, made the diagnosis and specific immunotherapy a tricky task.

However, since we knew from the beginning of our study that there was an extensive cross reactivity between the different species of cupressaceae which extends itself to Taxodiaceae (11-13) and since the extract of *Cupressus arizonica* is easier to prepare, we used this extract for specific immunotherapy. However a major allergen of *Cupressus sempervirens*, Cup s 1, has been purified (14).

Besides, new therapeutic approaches have helped to carry out an improved specific immunotherapy. Firstly, the sublingual route has alleviated the burden of treatment. Secondly, the use of monomeric aller-

(1) U.O. Medicina Generale. Ospedale di Bordighera. A.S.L n°1 Imperiese.

(2) Centre de recherches en Allergologie. 7 bis, chemin de la batterie du Roucas Blanc, 13007 Marseille. Fax: 33(491)379708; email: Raphael.panzani@tiscali.fr

(3) Lofarma S.p.A Milano

goid extracts has decreased the risks of systemic effects and has shown their therapeutic potential in animals (15).

The aim of our study was:

- to compare the *in vivo* allergenic activity of *Cupressus arizonica* pollen extracts in its native and modified forms;
- to evaluate the safety and clinical efficacy of *Cupressus arizonica* monomeric allergoid extract in Cupressaceae allergy.

MATERIALS AND METHODS

In spite of the predominance of *Cupressus sempervirens* in Italy compared to *Cupressus arizonica*, the use of the second one is justified on the ground that there is an extensive cross reactivity between these two species.

Preparation of native and chemically-modified Cupressus arizonica extracts.

Cupressus arizonica pollen extract was prepared as previously described. Briefly *Cupressus arizonica* pollen was defatted before being extracted with 0.125 M NH_4CO_3 (5% p/v) for 4hrs at 4°C under stirring. The suspension was centrifuged at 20,000 rpm for 1 hr at 20°C. The supernatant was dialysed against distilled water and ammonium sulphate was added to obtain an 80% saturated solution. After 4hrs of stirring, precipitated proteins were recovered and redissolved in water at 1/10 of the initial volume. Finally protein content of precipitated *Cupressus arizonica* extract was determined according to Bradford (16) using the commercial BioRad Protein Assay Dye Reagent (BioRad, Milan, Italy) and BSA as reference standard. Subsequently 5ml of such extract, conditioned with 20mM phosphate buffer, pH 7.2, was passed through a Sephadex G25 column (D10, Amersham, Pharmacia, Biotech, Milan, Italy). Excluded peak was recovered and submitted to chemical modification. Briefly solid sodium tetraborate and potassium cyanate were added to 2.5ml of gel-filtered extract in order to obtain final concentrations of 0.1M and 1M, respectively. After 20 hrs at 40° C, the salt excess was eliminated by gel-filtration on G25 column and excluded peak (allergoid extract) was recovered and dialysed against NaCl, 0.9% and 0.275% NaHCO_3 (prick solution). Native extract was treated the same way, except the addition of potassium cyanate, substituted by KCl to maintain the same ionic force. Both native and allergoid extracts were then serially diluted in prick solution contain-

ing glycerol (50%) and phenol (4% as final concentration) and used for *in vivo* evaluation of allergenic potency by skin prick test (SPT).

Allergenic extract

We used a monomeric allergoid of *Cupressus arizonica* (LAIS, Laboratorio Farmaceutico Lofarma, Milan). The allergen was modified by reaction with potassium cyanate at neutral pH to substantially decrease, by substitution of epsilon-amino group of lysine residues, its capacity to react with IgE, as measured by radioallergosorbent test inhibition. The preparation for treatment was titrated in allergenic units (AU) and standardized by radioallergosorbent-test inhibition compared with an in house reference titration.

Patients

30 patients were selected from an outpatients population (20 active group, 10 control group). The main inclusion criteria were: rhinoconjunctivitis and/or asthma during the Cupressaceae pollen season, positive skin prick tests and RAST (Cap System Pharmacia & Upjohn) to *Cupressus sempervirens* and *Juniperus ashei*, specific nasal provocation tests to *Cupressus arizonica*. The patients were randomly selected into two groups: group A (treated with immunotherapy), group B (placebo group). The two groups were homogeneous as far as clinical data, gender, mean age are concerned (table 1). All patients enrolled in the study wrote their informed consent.

Clinical characteristics	Active group	Placebo group	p
Number	20	10	
Gender			
Male	14	5	
Female	6	5	
Mean age	32.70 (sd 11,85)	36.20 (sd 12,73)	p < 0,46 ns
Onset pathology in years	4,2 (sd 2,3)	4,8 (sd 2,9)	p < 0.68 ns
Conjunctivitis	3	2	p < 0.21 ns
Rhinitis	13	6	p < 0,90 ns
Asthma	4	2	p < 0.45 ns
Polysensitized	11	6	p < 0.32 ns
RAST	3,7 (sd 0,47)	3,6 (sd 0,52)	p < 0.61 ns

Table 1: Clinical data.

Symptom and drug scores

Patients were asked to record on a diary card both clinical symptoms and drug intake during the pollen season. Symptoms such as, itching and watering of the eyes; sneezing, itching nasal obstruction and secretion; cough, dyspnea, wheezing and sputum were graded as follows: 0 = absent; 1 = mild, 2 = moderate, 3 = severe. Drug consumption was also scored: the score was 1 for each administration of local drug and 2 for each administration of systemic drugs.

Skin testing, nasal provocation test and serum specific IgE to *Cupressus arizonica* pollen

■ **Skin prick tests (SPT)** were performed with a commercial extract (Lofarma, Milan, Italy). The diluent of prick solution was used as a negative control and histamine (10 mg/ml) as a positive control. The reaction was considered positive if the weal diameter was at least 3mm once subtracted the value of the control solution. SPT tests for the *in vivo* evaluation of allergenic potency have been performed using native and modified *Cupressus arizonica* extracts at different concentrations (from 1:2 to 1:16). In this case the average diameter of the weal induced by each dilution was calculated according to the formula $(D+d)/2$, where D is the maximum diameter and d the perpendicular diameter at the midpoint.

■ **Specific nasal provocation test (NPT)** was carried out with *Cupressus arizonica* extracts (Lofarma, Milan, Italy) standardized, with the RAST inhibition method, in Allergenic Unit (AU) at 50, 100 and 200AU/ mL. The patients had to be symptoms-free. Test performed with the instillation by pressurized at into one nostril of increasing concentration at interval of 15 minutes. A control test with the dilution buffer was performed the opposite site. Threshold dose of allergen determined from to peak inspiratory utilized the Youlten peak inspiratory flow meter (PIFRn meter). It offers some advantage over the expiratory flow rate meter especially when used in provocation tests and it is easier to handle compared with standard rhinomanometry. The results are expressed as the mean values of three consecutive registrations. Tests was considered positive when inspiratory flow drop of 20% or an onset of at least two rhinitic symptoms among sneezing, itching, obstruction and rhinorrhea, were observed after administration into a nostril of a puff (130 ul) of extract, starting from that one at lower concentration (17).

Serum *Cupressus arizonica*-specific IgE determination was evaluated by RAST (Sferikit, Lofarma, Milan,

Italy) while serum *Cupressus sempervirens* and *Juniperus ashei*-specific were determined IgE by CAP System (Pharmacia, Uppsala, Sweden).

Pollen counts

■ **Pollen counts** were performed daily during the period of study with a 7-day recording volumetric spore trap (Hirst trap - VPPS 2000- Lanzoni, Bologna) placed on the top of our hospital about 20 m above the ground. The results were expressed as weekly mean values of grains/m³ of air (g/m³). They show that in 2003 there was a stronger peak than in 2002 during march (figure 1).

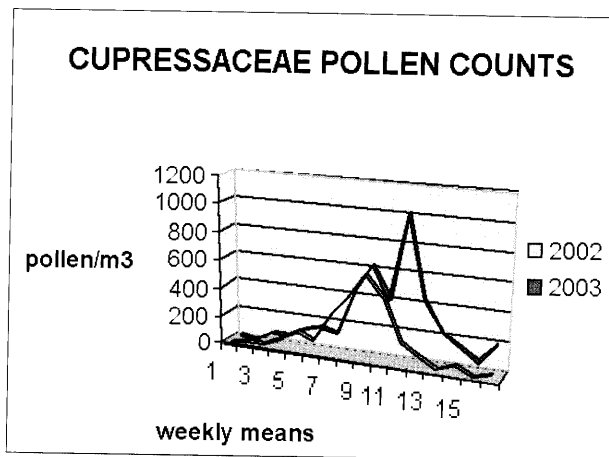


Figure 1.

Treatment

Immunotherapy consisted of aqueous solutions for oromucosal administration in drops: Specific sublingual immunotherapy (SLIT). The vaccine was titrated in Allergenic Units (AU), an arbitrary unit originally established for products to be administered by the nasal route and defined as 1/40 of the mean provocative dose by specific nasal challenge. The treatment was administered as a preseasonal and seasonal course according to a fast-induction schedule from the beginning of december to the end of march using two doses, 500 and 3000 AU.

The schedule used for the treatment of patients belonging to group A was the following:

- **Initial therapy for fast induction:** 500 AU/ml = 25 AU/drop: 1°, 2°, 3°, 4° day: 1 drop twice a day; 5°, 6°, 7°, 8° day: 4 drops twice a day then 3000 AU/ml = 150 AU/drop 9°, 10°, 11°, 12°, 13° day: 2 drops twice a day; 14°, 15°, 16° day: 6 drops twice a day;
- **Maintenance therapy** 3000 AU/ml = 150 AU/drop: 6 drops once a week until the end of march.

Patients from group B received a standard drug therapy. The prescribed drugs were: Oral or local H1 antihistamines, local or oral corticosteroids, local chromones.

Patients began the treatment all at the same time in the first days of december 2002 and continued uninterruptedly until the end of march.

Patients from group A received SLIT, while those of the group B received placebo and pharmacologic therapy. The study was designed in a double-blind, placebo-controlled. The symptoms scores was collected before the start of immunotherapy (march 2002) and after (march 2003). Specific nasal provocation tests was carried out, after pollen season, in april 2002 and april 2003.

Statistical analysis

Statistical evaluation was done by the Mann-Whitney U-test (two-tailed probabilities) for the intergroup comparison and the Wilcoxon signed rank test for intragroup comparisons at the different times of observation. During the month with the most peak of pollen count (February in both years) the comparison between the two years evaluating, with Wilcoxon test, the mean monthly symptom and drugs consumption, showed significant difference for both parameters for the Group A: Symptoms scores: 9,30 (ds 3,03) versus 4,95 (ds 2,16) $p < 0.001$; Drugs consumption: 5,95 (ds 1,54) versus 3,00 (ds 1,03) $p < 0.001$.

There was no significant difference in group B.

Results

In vivo evaluation of allergenic potency of native and modified Cupressus arizonica extracts.

A comparison between native and modified extracts, at different dilutions, has been performed by skin prick tests on 16 volunteers. The weal size was marked directly on the skin and then transferred through transparent acetate sheet onto paper to measure the respective diameters. The results are showed in the table 2. As we can see, modified Cupressus arizonica extract shows a significant reduction of the skin response in respect to the native one, for all the considered dilutions, suggesting that the chemical modification is able to decrease the IgE-binding capacity of extract.

Immunotherapy

In table 3 and 4 are shown the comparisons between symptom and drug scores in active and placebo

group before and after ITS. At the beginning, the active and placebo groups were comparable for all the considered parameters. During the subsequent Cupressaceae pollen season (year 2003) a statistical evaluation based on Wilcoxon test indicated that the symptom scores as well as drug consumption were significantly lower in the active group; on the contrary no difference was observed in the placebo group. The same results apply to nasal provocation test with an obvious increase in the nasal tolerance of doses of allergens in the active group compared to the placebo group (table 5). A further statistical comparison between active and placebo groups using Mann-Whitney U-test confirmed that, during the previous pollen season (year 2002), no differences in terms of symptom and drugs consumption scores were observed between them; on the contrary significant difference in both parameters were observed between

DILUTIONS	NATIVE	S.D.	MODIFIED	S.D.	P *
1: 2	9,38**	2,06	4,88	1,41	< 0.01
1: 4	8,25	2,57	4,25	1,69	< 0.01
1: 8	6,88	2,22	4,13	1,41	< 0.01
1: 16	6,00	1,86	3,63	0,89	< 0.01

Table 2: Comparison between native and modified cupressus arizonica extracts. Weal diameters average.

*Mann-Whitney U-test

** Mean weal diameter in mm

SD: Standard deviation

	Before SIT	After SIT	Statistical analysis
Active group (n. 20)	9,30 (sd 3.03)	9,30 (sd 3.03)	$p < 0.01$
Placebo group (n. 10)	11.00 (sd 2.11)	10.40 (sd 1.96)	$p = 0.052$
	$p = 0.060$	$p < 0.01$	

Table 3: Symptom scores between active and placebo group before and after SIT.

	Before SIT	After SIT	Statistical analysis (Mann-Whitney U test)
Active group (n. 20)	5.95 (sd 1.54)	3.00 (sd 1.03)	$p < 0.01$
Placebo group (n. 10)	6.00 (sd 1.33)	7.20 (sd 1.06)	$p = 0.051$
	$p = 0.055$	$p < 0.01$	

Table 4: Drug consumption scores in active and placebo group before and after SIT.

	ACTIVE GROUP(N. 20)		PLACEBO GROUP(N. 10)		STATISTICAL ANALYSIS
	BEFORE	AFTER	BEFORE	AFTER	
MEAN (10 mcg/ml)	10.12 (sd 2.13)		11.50 (sd 2.45)		P = 0.87 ns
		25.78 (sd 5.62)		9.36 (sd 8.76)	P < 0.01 *
Difference	+ 15.66		- 2.14		P < 0.01 **

Table 5: Changes in nasal sensitivity to *Cupressus arizonica* pollen by provocation tests.

* Wilcoxon test

** Mann-Whitney U-test

active and placebo groups in the 2003 pollen season, suggesting SIT with *Cupressus arizonica* allergoid could be effective in the treatment of patients affected by *Cupressaceae* pollen allergy. All patients completed the study and no local or systemic side effects has been reported, confirming that this therapy, even if administered according a fast schedule, was well tolerated. We have used the test With Mann-Witney U-test for the comparison between group A and group B and, while we have not found differences in the scores of the first year, we have found, instead, significant differences in the following year is for the symptoms that for drugs ($p < 0.001$).

DISCUSSION

Specific Immunotherapy to conifers based on subcutaneous injection of allergenic extracts has been used for decades to treat allergic diseases (18-22). The possible risk of inducing severe side effect reactions combined with the discomfort for patients who underwent this treatment, has led to explore safer and possibly self-administrable therapeutic modalities, intranasal or in particular the swallow oral technique (SLIT) (23). Chemical modification of extracts with KCNO, under experimental conditions promoting the selective substitution of the E-aminogroups of the lysine residues of the proteinic molecules, appears to be an ideal way to develop products meeting these requirements. Such products, known as monomeric allergoids, are characterised by a reduction of their allergenic potential and a preservation of their immunogenic capacities as well as molecular dimension. Previous experimental studies in animals have demonstrated that KCNO modified *C. arizonica* extract showed biological, immunological and chemical-physical characteristics analogous to that ones of other KCNO modified allergenic extracts. Also, in mice, this extract elicited specific IgG antibodies able to recognize the native allergen.

The first aim of our study was to demonstrate by skin testing that *Cupressus arizonica* allergoid extract showed lower in vivo IgE-reactivity than its native, non modified, counterpart. The results have shown that the differences between the two groups were statistically significant for all the considered dilutions. Taken together these observations indicate that the chemical molecular changes involve much probably IgE-B cell epitopes of the allergens, leaving nearly unaltered their T-cell epitopes and therefore their immunogenic properties. This particular profile of modified extract, combined with its in vivo lower IgE reactivity as demonstrated by SPT tests, made it particularly suitable to specific immunotherapy. In the recent last years local SIT, particularly the one based on the sublingual/oromucosal administration of monomeric allergoid, has been well accepted as an alternative to injectable SIT (24). A relevant contribute in the therapeutic effect of local SIT can derive to the fact that in KCNO allergoid extract monomeric molecular dimension of the allergenic proteins is preserved, facilitating their absorption through the mucosal membrane. Previous pharmacokinetics studies in humans suggest that the administration of modified extract as orosoluble tablets may involve both local and systemic immunity (25-26).

The second important aim of our study was to evaluate the clinical effectiveness of *Cupressus arizonica* allergoid when administered by sublingual route in an aqueous extract: nasal and orosublingual immunotherapy have been accepted by the WHO. Not many controlled clinical studies exist in literature on the assessment of the efficacy of specific immunotherapy to cure patients with a *Cupressaceae* pollen allergy. Encouraging results have been obtained in trials conducted in USA against Mountain Cedar (*Juniperus ashei*) pollinosis and in Japan against *Cryptomeria japonica* and *Chamaecyparous obtusa*. The originality of the present work consists in the use of KCNO modified *C. arizonica* extract, formulated as droplets and administered by the oromucosal route. In addition to the use of this monomeric allergoid, the self-administration of the vaccine is an obvious advantage for the patients. The choice of *Cupressus arizonica* extract derives from previous study demonstrating that it can be considered as the most representative of the *Cupressaceae* species in terms of allergenic relevance and cross reactivity (27), we have already published preliminary data on the efficacy of *Cupressus sempervirens* pollen by subcutaneous immunotherapy (28), and a double-blind study, during three years, with mixed extract of *Cupressaceae* and *Taxodiaceae* (29). In both cases the results were good.

In the present study, according to the following criteria, symptom scores, drug scores, threshold of nasal reactivity by nasal provocation test, there is a statisti-

cally important difference in favour of the active compared to the control group. Although it is a non double blind preliminary study, these results placebo controlled are in favor of this type of SIT in Cupressaceae respiratory allergy. The treatment, although carried out in a seasonal fast schedule, was well tolerated without any side effect. It is moreover interesting to observe that the good results were obtained during a pollination season characterised by an high *Cupressus spp* pollen grain counts as determined by our local Aeroallergen Monitoring Centre (fig. 1).

It is interesting to observe that the administration of the extract can be made during the blooming season (february and march).

CONCLUSION

In conclusion, our study demonstrated that 1) KCNO-modified *Cupressus arizonica* extract has a reduced IgE-binding capacity compared to the native one; 2) This extract in droplets and administered by the oromucosal route, is safe and effective in reducing clinical symptoms of patients affected by *Cupressus sempervirens* pollen allergy. Using a coseasonal schedule we demonstrated that comparing the symptom and drug scores and the results of the nasal provocation tests between the active and control groups we obtained a statistically important difference in favour of the active group. It is of course a preliminary study before studying a larger number of patients.

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References

- Panzani R.-C. - L'allergie respiratoire aux pollens de coniferes. *Rev Fr Aller* 1962;3:164-8.
- Panzani R., Centanni G., Brunel M. - Increase of respiratory allergy to the pollens of cypresses in the South of France. *Ann. Allergy* 1986; 56: 460-63.
- Panzani R., Zerboni R., Ariano R. - Allergenic significance of Cupressaceae pollen in some parts of the Mediterranean area. In Allergenic Pollen and Pollinosis. In: Europe -Edited by G. D'Amato, F. M. Spiekma, Bonini S. Blackwell Sc. Pub.1991; p.81-84.
- Ariano R. - Allergia respiratoria al polline di cupressacee. *Folia Allergol. Immunol. Clin.* 1988; 35: 275-284.
- Panzani R.-C., Ariano R. - Allergenic significance of cupressaceae and taxodiaceae pollen in some parts of the mediterranean and all over the world. *Indian J Aerobiol.* 1999;12:1-10.
- Caiaffa M.-F., Macchia L., Strada S., Bariletto G., Scarpelli F., Tursi A. - Airborne Cupressaceae pollen in Southern Italy. *Ann Allergy*, 1993;71:45-50.
- Bousquet J., Cour P., Guerin B., Michel F.-B. - Allergy in the Mediterranean area. I: Pollen counts and pollinosis in Montpellier. *Clin Allergy* 1984;4:249-258.
- Togawa A. Panzani R.-C., Garza M., Goldblum R.-M., Midoro-Horiuti T. - Isolation and characterization of the major allergen from italian cypress (*Cupressus sempervirens*) pollen. *J Allergy Clin Immunol* 2004, abst. 1099 S300.
- Cortesano I., Civantos E., Aceituno E., del Moral A., Lopez E., Lombardero M., del Pozo V., Lahoz C. - Cloning and expression of a major allergen from *Cupressus arizonica* pollen, Cup a 3, a PR-5 protein expressed under polluted environment. *Allergy*. 2004, 59(5) 485-90
- Afferri C., Butcheroni C., Pironi L., Puggioni E.-M., Orlandi A., Barletta B., Tinghino R., Ariano R., Panzani R.-C., Di Felice G., Pini C. - Comparison between the native glycosylated and the recombinant Cup a1 allergen: role of carbohydrates in the histamine release from basophils 2002;32(11):1620-7
- Panzani R.-C., Yasueda H., Shimizu T., Shida T. - Cross reactivity between the pollen of *Cupressus sempervirens* (common cypress) and of *Cryptomeria japonica* (japanese cedar). *Ann Allergy* 1986; 57:26-30.
- Barletta B., Afferri C., Tinghino R., Mari A., Di Felice G., Pini C. - Cross-reactivity between *Cupressus arizonica* and *Cupressus sempervirens* pollen extracts. *J. Allergy Clin Immunol*, 1996;98:4,797-804.
- Schwietz L.-A., Goetz D.-W., Whisman B.-A., Reid M.-J. - Cross-reactivity among conifer pollens. *Ann. Allergy Asthma Immunol.* 2000;84:87-93.
- Monsalve R.-I., Barber D., Panzani R.-C., Villalba M., Rodriguez R. - Purification and characterization of Cup a 1, major allergen of *Cupressus sempervirens* pollen. Abst.290, *European Congress Allergol Paris* 2003:91-92.
- Mistrello G., Roncarolo D., Zanoni D., Zanotta S., Amato S., Falagiani P., Ariano R. - Allergenic relevance of *Cupressus arizonica* pollen extract and biological characterization of the allergoid. 2002;129(4):296-304.
- Bradford M.-M. - A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-254.
- Wihl J., Malm L. - Rhinomanometry and nasal peak expiratory and inspiratory flow rate. *Ann. Allergy* 1988;61:50-5.
- Pence H.-L., Mitchell D.-Q., Greeley R.-L., Updegraff B.-R., Selfridge H.-A. - Immunotherapy for mountain cedar pollinosis. *J Allergy Clin Immunol* 1976;58:39.
- Miller F.-F., Smith R.-E., Lawson W.-J. - Repository emulsion therapy for mountain cedar pollinosis : a double blind study. *J. Allergy* 1964;37:7.
- Reid M.-J., Schwietz L.-A., Wishman B.-A., Moss R.-B. - Mountain cedar pollinosis: can it occur in nonatopics ? *N. Engl. Reg. Allerg. Proc.* 1988;9:225.
- Ito Y., Takahashi Y., Fujita T., Fusuyama S. - Clinical effects of immunotherapy on Japanese cedar pollinosis in the season of cedar and cypress pollination. *Auris Nasus Larynx*, 1997;24(2):163-70.
- Ramirez D.-A. - The natural history of mountain cedar pollinosis. *J. Allergy Clin. Immunol.* 1984;73:88-93.
- Charpin D., Dron M., Fardeau M.-F., Massabie-Bouchat Y.-P., Hugues B., Fabre C., Vivinus S., Pegliasco H., Vatrinet C., André C. - Clinical efficacy of *Juniperus ashei* specific immunotherapy in European Cypress rhinoconjunctivitis: a double-blind placebo-controlled trial. EAACI Congress 2003, abst 11.
- W.H.O. Position Paper. "Allergen immunotherapy: therapeutic vaccines for allergic diseases", 1998.
- Mistrello G., Rapisarda G., Falagiani P. - Detection of IgE-binding activity in serum after intranasal treatment of normal rabbits with *P. judaica* extract. *Allergy* 1991;46:52-8.
- Mistrello G., Roncarolo D., Gentili M., Zanoni D., Falagiani P. - Modified Par j 1 allergen from *P. judaica* pollen and its rate of absorption in rats. *Immunol Lett* 1994;40:31-36.
- Di Felice G., Caiaffa M.-F., Bariletto G., Afferri C., Di Paola R., Mari A., Palumbo S., Tinghino R., Salliestro F., Tursi A., Macchia L., Pini C.: Allergens of Arizona cypress (*Cupressus arizonica*) pollen: Characterization of the pollen extract and identification of the allergenic components. *J Allergy Clin Immunol* 1994;94:547-555.
- Ariano R., Spadolini I., Panzani R.-C. - Efficacy of sublingual specific immunotherapy in Cupressaceae allergy using an extract of *Cupressus arizonica*. A double blind study. *Allergol. et Immunopathol.* 2001;29(6):238-244.
- Ariano R., Panzani R.-C., Augeri G. - Double-blind placebo controlled specific immunotherapy with mixed cupressaceae taxodiaceae pollens in respiratory allergy to *cupressus sempervirens*. *Allergol et Immunopathol* 1997;25,1:23-29.