

Prevention of new sensitizations in monosensitized subjects submitted to specific immunotherapy or not. A retrospective study

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Summary

Background Specific immunotherapy is the only currently available allergen-orientated treatment able to modify the natural history of respiratory allergic diseases. Safety and clinical efficacy of this treatment are well documented, but evidence about the ability to reduce new sensitizations is still poor.

Objective We report a retrospective study conducted in order to assess the prevention of new sensitizations in monosensitized subjects treated with specific immunotherapy vs. monosensitized patients treated with anti-allergic drugs.

Methods 8396 monosensitized patients with respiratory symptoms were selected according to an open, retrospective design. Group A included 7182 patients submitted to specific immunotherapy (and anti-allergic drugs when needed) for 4 years and then treated with drugs for at least 3 years. Group B included 1214 patients treated only with drugs for at least 7 years. All patients underwent prick test with a standard panel of allergens and total and specific IgE determination before and after 4 years of treatment and again 3 years later.

Results Groups were well balanced. Polysensitized subjects were 23.75% in Group A and 68.03% in Group B after 4 years ($P < 0.0001$) and 26.95% and 76.77%, respectively, after 7 years ($P < 0.0001$). Asthmatic subjects were more prone to develop polysensitization in comparison to subjects suffering only from rhinitis (32.14% instead of 27.29% after 4 years, 36.5% instead of 31.33% after 7 years; $P < 0.0001$). Specific IgE decreased by 24.11% in Group A and increased by 23.87% in Group B ($P < 0.0001$). Total IgE decreased by 17.53% in Group A and increased by 13.71% in Group B ($P < 0.0001$).

Conclusions Specific immunotherapy was observed retrospectively to reduce new sensitizations in monosensitized subjects suffering from respiratory allergic diseases.

Keywords: allergy, monosensitization, polysensitization, prevention, respiratory symptoms, specific immunotherapy

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Introduction

Allergen-specific immunotherapy (SIT) was first used in medical practice at the beginning of the last century [1] for the treatment of patients suffering from respiratory allergic diseases. The efficacy and safety of SIT has been

questioned until recently. In 1998 the WHO Position Paper [2] and the EAACI Position Paper [3], on the basis of a careful revision of the available literature, stated the safety and efficacy of SIT performed by injections or by local (nasal, sublingual) administrations under adequate conditions. Further studies supporting efficacy and safety of both injective and local SIT have been published afterwards [4–11].

A long-term effect of SIT after a 3-year treatment has also been reported [12–15].

Several studies run in the last decades have been dedicated to the natural history of the respiratory allergic diseases, showing its evolution towards asthma and polysensitization.

Because SIT is able to modify the immune response [2,3], a modification of the natural history after such treatment in patients suffering from allergic rhinitis can be expected but the assessment requires a large number of carefully selected patients and a very long follow-up. Only a few papers have documented the ability of SIT to prevent or at least delay the development of asthma in patients suffering from allergic rhinitis [16–18].

The ability of SIT to prevent the development of new sensitizations in monosensitized patients has been so far documented in only one paper involving 44 children [19].

We therefore designed a protocol to study retrospectively the development of polysensitization in a large population of monosensitized patients submitted to SIT for 4 years in comparison to a parallel group of monosensitized patients treated with anti-allergic drugs only.

Materials and methods

Study design

The study was planned as a retrospective parallel group open study including patients suffering from allergic rhinitis and/or asthma followed-up as outpatients in the period 1980–99.

Inclusion criteria

Patients were considered eligible for the study under the following conditions:

- age at least 14 years
- clinical history (at least one year) of allergic rhinitis and/or mild to moderate asthma (symptoms from at least twice a week to daily; FEV₁ from not below 80% to at least 70% predicted)
- monosensitization to one of the following respiratory allergens: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Compositae* mix (Artemisia, Ambrosia),

Corylaceae-Betulaceae mix (Alder, Birch, Hazel), grass mix, *Olea europaea*, *Parietaria judaica*

- positive skin prick test weal to the relevant allergen ($\geq$ histamine 10 mg/mL)
- *in vitro* specific IgE to the relevant allergen $\geq$ class 4

Study groups

Patients were subdivided into Group A; including patients submitted to SIT, and Group B; including patients not submitted to SIT.

Each Group was further subdivided into two subgroups, including patients suffering only from rhinitis without any asthmatic symptom at enrolment (A1 and B1), and patients who had clear asthmatic symptoms with or without rhinitis at enrolment (A2 and B2).

SIT was proposed after diagnosis to all patients, but was accepted only by patients subsequently included in Group A. Patients unavailable to accept SIT (mainly for economical or compliance reasons) were included in Group B.

Sit

Patients belonging to Group A have been submitted to SIT with the relevant allergen for 4 years, with native allergens pharmaceutically prepared as injective depot treatment and commercially available from different producers. The induction phase was performed according to the producer's recommendations and was followed by a perennial schedule with maintenance injections at 4-week intervals according to a standard procedure followed in our clinic which consists of the administration of a dose of 0.7 mL all year long for perennial allergenic extracts whereas for seasonal allergenic extracts, the dose was reduced to 0.3 mL during the pollen season.

All patients were asked to sign an informed consent form before being submitted to SIT.

Skin prick tests

All patients attending our clinic, suffering from respiratory symptoms and sensitized to at least one inhalant allergen, have been routinely submitted to skin prick tests 4 years (T1; end of SIT for Group A) and 7 years (T2) after the first diagnosis (T0). The skin prick tests were performed by trained allergologists in our centre according to a routine procedure and using a standard panel of respiratory allergens including mites (*D. farinae* and *D. pteronyssinus*), *Parietaria judaica*, grass mix, *Compositae* mix (Artemisia and Ambrosia), *Corylaceae-Betulaceae* mix (Alder, Birch, Hazel), *Olea europaea*, *Alternaria tenuis*, positive and negative control. Tests were done out of the season in

Table 1. Demographic data of patients (T0)

	Number of patients	Mean age (years)	Sex	
			Female (%)	Male (%)
<i>SIT</i>				
Subgroup A1 (rhinitis)	2938	22.96	1657 (56.4)	1281 (43.6)
Subgroup A2 (asthma and rhinitis)	4244	23.45	2351 (55.4)	1893 (44.6)
Group A (total)	7182	23.25	4008 (55.8)	3174 (44.2)
<i>DRUGS</i>				
Subgroup B1 (rhinitis)	499	21.56	285 (57.1)	214 (42.9)
Subgroup B2 (asthma and rhinitis)	715	23.03	407 (56.9)	308 (43.1)
Group B (total)	1214	22.42	692 (57.0)	522 (43.0)

pollen-sensitized patients and in symptom-free periods in patients sensitized to mites. All patients were instructed not to take drugs during the 3 weeks before the test.

Commercial allergenic extracts from different suppliers have been used during the investigation here described.

Total and specific IgE

A sample of blood was taken from each patient at T0 and T2 for the *in vitro* determination of total IgE and allergen-specific IgE according to a routine procedure followed in our centre.

Different analytical methods, according to what was state-of-the-art at each period, have been used during the investigation (RAST, ELISA).

Drugs

All patients, whether treated with SIT or not, were prescribed and instructed to use the same anti-allergic drugs for the control of the respiratory symptoms.

Different drugs have been used during the study period, according to their commercial availability. Patients were administered antihistamine tablets, inhaled β -2 adrenergic agonists, and topical and systemic steroids.

Environmental avoidance measures

All patients positive to mites, whether submitted to SIT or not, have been instructed to take standard environmental measures to decrease the allergenic load (i.e. *no use of humidifiers, frequent vacuum cleaning, washing sheets with*

water at $\geq 55^\circ\text{C}$ at least once a week and removal of carpets, soft toys and plants from the patient's bedroom).

Statistics

A parametric test (*t*-test) was used for total and specific IgE statistical analysis. Chi-square test for independent data was used for analysis of rates between groups.

P-values < 0.05 and < 0.01 were considered, respectively, as statistically significant and highly statistically significant.

Statistical analysis was done with a standard statistical package (BMDP, Los Angeles, CA, USA)

Results

Demographic data

A total of 8396 patients monosensitized to mites, grass, olive, *Compositae*, *Corylaceae-Betulaceae* or *Parietaria* have been selected for the study.

Groups and subgroups were well balanced for sex and pathology (Table 1).

Sit

Allergens used for SIT are summarized in Table 2.

Most patients (4018/7182, 55.95%) were treated with pollen extracts, mainly represented by *Parietaria judaica* (3092/4018, 76.95% of patients submitted to SIT with pollen extracts).

Mite extracts were administered for SIT to 3164/7182

Table 2. Allergens used for SIT

	Genus or Group	Number of patients (%)
<i>Seasonal allergens</i>	<i>Parietaria</i>	3092 (76.95)
	Grass	817 (20.33)
	<i>Olea</i>	57 (1.42)
	<i>Compositae</i> (mix)	28 (0.70)
	<i>Corylaceae-Betulaceae</i> (mix)	24 (0.60)
	Total pollens	4018 (55.95)
<i>Perennial allergens</i>	Mites	3164 (44.05)

patients (44.05%), whereas grass extracts were administered to 817/7182 patients (20.33%).

Parietaria, mites and grass extracts together accounted for more than 98% of SIT.

Development of new sensitizations

Only 1706 out of 7182 (23.75%) monosensitized patients treated for four years with SIT (Group A) showed at least one new sensitization as compared to 826 out of 1214 (68.03%) patients of the parallel group (Group B) who developed new sensitizations whereas, 3 years later, 1936/7182 (26.95%) patients in Group A and 932/1214 (76.77%) patients in Group B developed new sensitizations (Table 3). Both comparisons were highly significant ($P \leq 0.0001$, Table 4, upper part).

Patients suffering from only rhinitis, treated with SIT or not, appeared significantly less prone to develop new sensitizations 4 or 7 years after the first observation ($P \leq 0.0001$ to $P = 0.0086$ for all comparisons; Table 4, upper part).

Patients submitted to SIT during the previous four years

Table 3. Polysensitized patients after 4 (T1) and 7 (T2) years

	Number of patients	Polysensitized T1 (%)	Polysensitized T2 (%)
<i>SIT</i>			
Subgroup A1 (rhinitis)	2938	620 (21.10)	702 (23.89)
Subgroup A2 (asthma and rhinitis)	4244	1086 (25.59)	1234 (29.07)
Group A (total)	7182	1706 (23.75)	1936 (26.96)
<i>DRUGS</i>			
Subgroup B1 (rhinitis)	499	318 (63.72)	356 (71.34)
Subgroup B2 (asthma and rhinitis)	715	508 (71.05)	576 (80.56)
Group B (total)	1214	826 (68.04)	932 (76.77)

Table 4. Statistics on the evolution of polysensitization

Comparison between groups or subgroups (absolute values at T1 and T2)	P-value	
	T1	T2
A vs. B	< 0.0001	< 0.0001
A1 vs. A2	< 0.0001	< 0.0001
B1 vs. B2	0.0086	0.0002
A1 vs. B1	< 0.0001	< 0.0001
A2 vs. B2	< 0.0001	< 0.0001
(A1 + B1) vs. (A2 + B2)	< 0.0001	< 0.0001
Comparison between groups or subgroups (evolution from T1 to T2)	P-value	
	T1	T2
A vs. B	< 0.0001	
A1 vs. A2	0.043	
B1 vs. B2	0.012	
A1 vs. B1	< 0.0001	
A2 vs. B2	< 0.0001	
(A1 + B1) vs. (A2 + B2)	0.01	

Group A: SIT; Group B: drugs; Subgroups A1 and B1: rhinitis only; Subgroups A2 and B2: asthma and rhinitis

were, with a high statistical significance, less prone to develop new sensitizations during the following three years in comparison to patients treated only with drugs (A vs. B, $P \leq 0.0001$; Table 4, lower part); in fact, 230 patients out of 5476 (4%) patients still monosensitized after 4 years of SIT showed at least one new sensitization after 3 years as compared to 106/388 (27.32%) patients of the drug group.

This was also true for patients suffering from only rhinitis submitted to SIT or not (A1 vs. B1) and for patients suffering from asthma and rhinitis submitted or not to SIT (A2 vs. B2) in the same period ($P \leq 0.0001$ for both; Table 4, lower part).

According to the calculated average annual rate of

Table 5. Specific and total IgE (kU/L)

	Specific IgE mean value (SD)		Total IgE mean value (SD)	
	T0	T2	T0	T2
<i>SIT</i>				
Subgroup A1 (rhinitis)	40.501 (15.804)	31.319 (12.788)	197.621 (93.020)	164.654 (74.065)
Subgroup A2 (asthma and rhinitis)	44.009 (16.635)	33.011 (13.016)	208.801 (91.819)	171.026 (66.837)
Group A	42.576 (16.391)	32.319 (12.949)	204.233 (92.468)	168.423 (69.946)
<i>DRUGS</i>				
Subgroup B1 (rhinitis)	38.507 (15.425)	48.902 (16.975)	201.003 (90.404)	224.232 (98.250)
Subgroup B2 (asthma and rhinitis)	41.202 (13.789)	50.201 (13.915)	204.506 (76.860)	235.562 (81.945)
Group B	40.094 (14.538)	49.667 (15.254)	203.066 (82.678)	230.905 (89.145)

Group A: SIT; Group B: drugs; Subgroups A1 and B1: rhinitis only; Subgroups A2 and B2: asthma and rhinitis

development of new sensitizations from 0 to 4 years, 1 out of 4 (24.8%) monosensitized patients treated with drugs as compared to only 1 out of 15 (6.6%) monosensitized patients treated with SIT developed a new sensitization per year in this period.

The average annual rate of new sensitizations from 4 to 7 years was lower in value but still higher in patients treated with drugs as compared to patients treated with SIT (1 out of 10, i.e. 10.1% and 1 out of 72, i.e. 1.4%, respectively) and also higher in asthmatic patients as compared to patients with rhinitis.

Total and allergen-specific IgE

Average values for each Group and subgroup at time T0 and T2 are shown in Table 5.

Allergen-specific IgE were significantly higher in Group A and subgroups as compared to the corresponding Group B and subgroups at T0 ($P = 0.009$ for A1 compared to B1; $P < 0.0001$ for other comparisons).

Total IgE were similar at T0 in Group A and B ($P = 0.682$), and also in subgroups (A1 vs. B1, $P = 0.437$; A2 vs. B2 $P = 0.235$).

Allergen-specific IgE decreased significantly in Group A (-24.11%) and subgroups (-22.63% for A1; -25.00% for A2) and increased significantly in Group B (+23.87%) and subgroups (+27.00% for B1; +21.84% for B2) after 7 years ($P < 0.0001$ in all cases).

Total IgE paralleled the trend already shown for specific IgE in Group A (-17.53%) and Group B (+13.71%) and

subgroups (-16.69% for A1 and -18.09% for A2; +11.56% for B1 and +15.19% for B2) in all cases with a high statistical significance ($P < 0.0001$).

Because of these differential trends, specific and total IgE in group and subgroups A were significantly lower at T2 as compared to the corresponding B Group and subgroups ($P < 0.0001$ in all cases).

Patients suffering from asthma and rhinitis at enrolment had higher specific and total IgE in comparison to patients belonging to the same group and suffering only from rhinitis, before and after the treatment. The difference was highly significant at T0 and T2 for specific and total IgE in patients belonging to Group A ($P < 0.0001$). For patients belonging to Group B the difference was highly significant at T0 for specific IgE ($P = 0.002$) but not for total IgE ($P = 0.475$). In the same Group at T2 the difference was not significant for specific IgE ($P = 0.140$) and significant ($P = 0.028$) for total IgE.

Patients submitted to SIT and polysensitized after 7 years (1936/7182) had significantly higher levels of both specific and total IgE at T0 when compared to patients still monosensitized at T2 (239.52 as compared to 191.18, $P < 0.0001$ for specific IgE; 52.80 as compared to 38.78, $P < 0.0001$ for total IgE).

Discussion

Because cat was not included in our standard prick test panel of allergens at the beginning of the period considered in our study, we excluded cat monosensitized patients from

our analysis. Patients monosensitized to *Alternaria* were also excluded because of the low reliability until recent years of this extract [20–22].

Patients monosensitized to *Parietaria judaica* are largely represented in our survey because this pollen is the main one in our region (Sicily, Mediterranean Coast of Southern Italy).

Allergens and materials for *in vivo* and *in vitro* assessments have undergone substantial improvement during the last two decades but the large number of patients included in both groups of our study over the same period of time is expected to compensate for the possible differences due to such unavoidable variations. In other words, absolute rates could have been overestimated, but comparisons between parallel groups are expected to give reliable results.

Many longitudinal studies have focused on the increase of the sensitization rate from childhood to adulthood [23–26], but only three recent reports analysed the evolution from mono to polysensitization. One paper reached the conclusion that the relative increase in polysensitized patients was age-related [27]. A further paper by the same investigators assessed the development of polysensitization in 72 out of 165 (43.6%) previously monosensitized children after 2–10 years from the first diagnosis [28]. Both studies were run on children treated only with drugs.

Children monosensitized to mites (age below 6 years) were studied for the development of polysensitization in one double-blind placebo-controlled study [9]. All patients belonging to the control group (22 children) developed at least one new sensitization after 3 years as compared to only 10 children belonging to the active group including 22 children. Our results are in good agreement with the three papers mentioned above taking into consideration that the mean age of our subjects was 22.42 years.

The efficacy of SIT is no longer a matter of debate [2,3], but the cost/benefit ratio can be further improved if this therapeutical approach turns out to be able to prevent both the development of asthma and further sensitizations.

We believe that our data, obtained in two very large and well-balanced groups of patients, strongly support this last point. Furthermore, differently from studies run in highly selected groups of patients with treatments prepared *ad hoc* and administered according to special schedules, our results have been obtained with commercially available allergen preparations administered according to standard schedules to patients not selected according to special criteria.

Patients submitted to SIT showed a significantly lower number of new sensitizations not only during the 4-year treatment, but also during the 3-year period after its interruption, as compared to the parallel group of patients treated only with drugs.

These results mean that SIT has a preventive effect towards new sensitizations, not only while it is performed, but for at least three years after its interruption.

New sensitizations were significantly more likely to take place in patients suffering from asthma and rhinitis as compared to patients suffering only from rhinitis.

The average annual rates of new sensitizations in the period 0–4 years were higher than values from 4–7 years (6.6% and 1.4% in Group A and 24.8% and 10.1% in Group B, respectively). In our opinion, this means that there is an early risk to develop a new sensitization after the first one. Because SIT-treated patients develop new sensitizations at a 4-fold lower average annual rate from 0–4 years and at a 7-fold lower average annual rate from 4–7 years than patients treated with drugs (6.6% vs. 24.8% and 1.4% vs. 10.1%, respectively), SIT should be considered very early after the first sensitization has taken place to prevent further sensitization(s).

These results in the SIT group are somewhat surprising because a lower average annual rate should have been expected during instead of after SIT. According to our data, SIT is only able to decrease the absolute rate but not the normal trend towards new sensitizations. In our opinion, this point deserves further investigation.

Data about specific and total IgE are in good agreement with the observed trend of polysensitization.

Patients belonging to Group A and subgroups (SIT) were well balanced with patients of Group B and subgroups for total IgE but not for specific IgE. The absolute difference between groups was small (6.16%) but nonetheless significant because of the high number of samples analysed. This means that Group A started from a worse condition than Group B if we consider specific IgE concentration as a signal of the clinical condition of patients.

Patients suffering only from rhinitis had significantly lower specific and total IgE levels than patients suffering from asthma and rhinitis at T0 in both Groups. This again stresses the link between IgE concentration and the clinical condition of patients.

After 4 years of SIT and 3 years of drugs patients showed a sharp decrease of both specific and total IgE (–24.11% and –17.53%, respectively), as compared to a sharp increase of the same two parameters in patients treated only with drugs for 7 years (+23.87% and +13.71%, respectively). The decrease of specific and total IgE was higher in patients submitted to SIT and suffering from asthma and rhinitis as compared to those suffering only from rhinitis. A decrease in specific and total IgE is a common finding in SIT studies and therefore our data are in good agreement with previous experiences.

Patients belonging to the SIT group who developed new sensitizations at T2 had initially higher levels of specific

and total IgE as compared to patients still monosensitized at T2 (+25.11% and +36.15%, respectively). According to our data, an increase of both specific and total IgE in Group B (only drugs) was paralleled by an higher rate of new sensitizations, while the decrease of both specific and total IgE in Group A (SIT) was accompanied by a lower rate of new sensitizations.

It is known that SIT is able to decrease IL-4 production by peripheral blood mononuclear cells (PBMC), in this way decreasing both Th2 differentiation and IgE production [29]. SIT has also been shown capable of reducing IL-5 and IgE production after stimulation with Der f 1, and this reduction parallels the clinical outcome of the treatment [30]. The induction of tolerance to the allergen [31] and the development of a state of specific anergy in peripheral T cells by IL-10 [32] are other immunological changes associated with SIT. Polysensitized patients have been shown able to produce higher levels of IL-4 after a specific stimulation as compared to monosensitized patients [33] and they also showed higher levels of both specific and total IgE [34]. On the other hand, local production of IgE in the lung lasting 7–14 days after one antigenic challenge has been shown [35] and the same event can be expected during the natural exposure to the relevant allergen(s).

Our results are in agreement with, and give further support to, these findings.

Monosensitized patients suffering from asthma and rhinitis, with high levels of specific and total IgE, and not submitted to SIT were the best candidates for polysensitization as opposed to patients suffering only from rhinitis with low levels of specific and total IgE and submitted to SIT. Moreover, monosensitized patients not treated with SIT were candidates to specific and total IgE levels increase.

As in our clinical experience, SIT seems to be less effective in polysensitized patients and in patients suffering from asthma and rhinitis. In our opinion, SIT should be performed as early as possible in monosensitized patients suffering only from rhinitis to prevent polysensitization and the development of asthma. Similar experiences on the preventive effects of SIT in reducing the onset of further sensitizations have been reported only in children [36,37] as well as the preventive effect on the development of asthma [17,18].

Data about the development of asthma in our personal experience, not considered in this paper, will be dealt with in another report.

We believe that the results here presented, obtained in a very large sample of patients followed up for at least seven years, are an important contribution to the current knowledge about the outcomes of specific immunotherapy in reducing the onset of further sensitizations in monosensitized patients who undergo this kind of treatment.

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