



Multiallergen-specific immunotherapy in polysensitized patients: where are we?

Allergen-specific immunotherapy administered by the subcutaneous route was introduced a century ago and has been shown to be effective in the management of allergic rhinitis and asthma. More recently, the sublingual administration of allergen extracts has become popular, especially in European countries, and has also demonstrated efficacy in respiratory allergic diseases. Both modes of allergen administration during immunotherapy have been shown not only to reduce symptoms and the need for medication, but also to prevent the development of additional sensitivities in monosensitized patients, as well as asthma development in patients with allergic rhinitis, with a long-lasting effect after the completion of several years of treatment. Almost all of the well-designed and double-blinded, placebo-controlled studies evaluated treatment with single-allergen extracts. Therefore, most meta-analyses published to date evaluated immunotherapy with single allergen or extracts containing several cross-reactive allergens. As a result, in general, multiallergen immunotherapy in polysensitized patients (mixture of noncross-reactive allergens) is not recommended owing to lack of evidence. Although some guidelines have recommended against the use of multiallergen mixtures, allergists commonly use mixtures to which the patient is sensitive with the rationale that effective immunotherapy should include all major sensitivities. Literature on this subject is scarce in spite of the widespread use worldwide. Here, this issue will be extensively discussed based on currently available literature and future perspectives will also be explored.

KEYWORDS: allergen-specific immunotherapy ■ allergic rhinitis ■ asthma ■ polysensitization ■ subcutaneous immunotherapy ■ sublingual immunotherapy

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Allergen-specific immunotherapy (SIT) administered by the subcutaneous route was introduced a century ago [1,2] and has been shown to be effective in the treatment of patients with allergic rhinitis (AR) and asthma [3–5]. More recently, administration of allergen extracts (drops or tablets) by the sublingual route in the management of AR and asthma has become popular with proven efficacy in both AR [6] and asthma [7].

Both sublingual and subcutaneous routes of allergen SIT have been shown not only to reduce symptoms and the need for medication, but also to prevent the development of new sensitizations in monosensitized patients [8–10], and asthma development in patients with AR [11,12].

Moreover, both routes have been shown to exert long-term efficacy following completion of several years of treatment [8,13–16].

On the other hand, most of the randomized controlled subcutaneous (SCIT) and sublingual immunotherapy (SLIT) studies have been performed with single allergens or cross-reactive allergen extracts in monosensitized patients, whereas epidemiological and clinical trials show that polysensitization is more prevalent in the general population [17–19], and in patients

consulting allergists [20,21]. This high prevalence of polysensitization raised the questions as to whether:

- Monoallergen immunotherapy (IT) is effective in polysensitized patients;
- Multiallergen SCIT and SLIT are effective [22–24].

A number of SLIT and SCIT studies published to date have been performed in polysensitized patients treated with monoallergen extracts that have demonstrated clinical efficacy [25].

On the other hand, the efficacy of multiallergen IT in polysensitized patients has been debated. There have been few studies that have specifically evaluated the efficacy of multiallergen SCIT or SLIT. The available studies on SLIT in polysensitized patients are few with respect to SCIT and are different with respect to the use of allergen mixes and administered doses [22–24].

Here, we discuss the evidence for multiallergen IT by both routes in polysensitized patients. Studies using extracts containing several closely related allergens (grass pollen mixture extracts) were considered as single-allergen IT, whereas mixtures or simultaneous treatments with little

or no cross-reactivity as multiallergen IT (mites, tree pollens, grass pollens, animal dander and weed pollens).

Multiallergen SIT in polysensitized patients

Despite the widespread use of multiallergen IT, a few studies can be found in the literature. A recent review by Nelson identified only 13 studies in which two or more unrelated allergens were simultaneously administered [26]. Results of three out of 13 trials using both single- and two-allergen extracts were not reported separately for one- and two-allergen treatment. In the remaining ten studies in which results were reported separately, SCIT was used in 11, SLIT in one and both modes in another. Nelson reported his results by stratifying studies as those using:

- Single- and two-allergen extracts;
- Single- and four-allergen extracts;
- Multiallergen extracts.

Based on data from studies with adequate information, Nelson reported that sublingual or subcutaneous administration of two simultaneous extracts was effective. In studies using multiple allergens, three showed clear efficacy, unlike the other two, possibly owing to inadequate doses or omission of clinically relevant allergen extracts [26]. This work highlighted the need for well-designed and -powered, controlled studies on multiallergen IT in polysensitized patients.

More recently, Calderon *et al.* evaluated multiallergen SIT in polysensitized patients [27]. The group also stressed the need for further investigations both on SLIT and SCIT before validating its efficacy in practice.

Here, studies using multiallergen IT to treat patients who were sensitized to more than one cross-reactive allergen will be briefly reviewed. Studies evaluating only single-allergen SLIT [23,28–31] or SCIT [32] in polysensitized patients will not be discussed. In this article, use of either mixture or simultaneous separate allergen extracts will be discussed based on efficacy, safety and immunological responses.

SCIT studies comparing multiallergen IT with monoallergen IT or placebo

The first study evaluating multiallergen SCIT in 173 polysensitized children with asthma randomized them to receive either placebo (saline) or low, medium, or highest tolerated dose of allergen mixture extracts, including

all inhaled allergens to which the children were sensitized on skin testing [33]. Results were reported after 4 years of treatment and after all children had reached 16 years of age (14-year study). Evaluation in the 4th year demonstrated that 18% of children in placebo and low-dose IT groups were free of asthma symptoms, whereas 66 and 78% were free of symptoms in the medium- and highest-dose IT groups, respectively. Details on sensitization and the amount of allergens given in each treatment group were not reported in this study (Table 1) [33].

In a double-blind, placebo-controlled (DBPC) trial, Lowell and Franklin included 24 adults who previously received ragweed IT with ongoing ragweed-induced allergic rhinoconjunctivitis symptoms [34]. Patients were randomized to receive either a mixture of all relevant allergens including ragweed or a mixture of all relevant allergens minus ragweed. Tree, grass and plantain pollens were included in the allergen mixture extracts. During pollen season, medication and symptom scores were significantly lower in the group receiving all relevant allergens including ragweed when compared with the minus ragweed group. The same investigators compared high- and low-dose ragweed extracts included in multiallergen extract in 24 adults who were symptomatic in spite of previous ragweed IT [35]. The group receiving a high dose of ragweed (maximum tolerated dose) demonstrated significantly lower symptom scores. Those two studies suggest the importance of including the highest dose of allergen extract when using mixtures of allergen extracts.

Bousquet *et al.* conducted a DBPC SCIT study on 70 adults with AR with and without asthma sensitized to orchard grass alone or orchard grass plus other allergens [36]. Patients sensitized to orchard grass alone were randomized to receive either orchard grass SCIT or placebo, while polysensitized patients were randomized to receive SCIT with orchard grass and up to three other extracts they were sensitized to, or placebo. SCIT was administered in a rush protocol. Clinical improvement was only detected in those who were monosensitized to orchard grass and were receiving single-allergen SCIT. No benefit over placebo was detected in those receiving multiallergen SCIT. In another pediatric DBPC SCIT study, Adkinson *et al.* included 121 children with moderate-to-severe perennial asthma [37]. Children were randomized to receive either

Table 1. List of studies using multiallergen immunotherapy to treat patients who were sensitized to more than one cross-reactive allergen.

Study (year)	Design	Subjects; condition	Allergens	Mixture/ simultaneous	Findings	Ref.
Johnstone and Dutton (1968)	RCT	173 children; asthma	All allergens (+) on SPT	Mixture	Remission of 78% in the highest allergen concentration group	[33]
Lowell and Franklin (1965)	DBPC	24 adults; ARC	Pollen mixture (+) ragweed Pollen mixture (-) placebo	Mixture	SS↓ in pollen mixture + ragweed group	[34]
Franklin and Lowell (1967)	DBPC	24 adults; ARC	High ragweed + multiple pollens vs low ragweed + multiple pollens	Mixture	SS↓ in high ragweed + pollen group	[35]
Bousquet <i>et al.</i> (1991)	DBPC	70 adults; AR ± asthma	Orchard grass vs placebo Orchard grass + parietaria, plane tree and olive tree	Mixture	Clinical improvement over placebo only in orchard grass group	[36]
Adkinson <i>et al.</i> (1997)	DBPC	121 children; asthma	HDM, grass, alternaria, aspergillus, cladosporidium and cat/dog vs placebo	Mixture	SS, MS, BHR and PEF no difference	[37]
Hedlin <i>et al.</i> (1999)	DBPC	29 children; asthma	Birch/Timothy + cat/HDM vs birch/Timothy + placebo	Mixture	BHR improved in multiallergen group	[38]
Alvarez-Cuesta <i>et al.</i> (2005)	DBPC	53 adults; AR and/or asthma	Orchard grass + olive tree vs placebo	Mixture	SS↓, MS↓ and QOL↓ in active group	[39]
Moncayo Coello <i>et al.</i> (2003)	RCT	54 children; ARC	Cat, HDM, German + American cockroach vs pharmacotherapy	Mixture	Results not reported separately	[40]
SCIT + SLIT studies						
Cirla <i>et al.</i> (2003)	RCT	36 adults; ARC ± asthma	SCIT grass mix + SLIT placebo vs SCIT grass mix + SLIT birch/hazel	Simultaneous	Cough↓ Conjunctival symptoms↓ Asthma medication↓ BHR↓ Nasal reactivity↓ in combined SCIT + SLIT group	[41]
SLIT studies						
Marogna <i>et al.</i> (2007)	Open-label RCT	48 adults; ARC + asthma	Birch pollen Grass pollen Birch + grass pollen Pharmacotherapy	Simultaneous	SS↓ MS↓ BHR↓	[42]
Amar <i>et al.</i> (2009)	DBPC	54 adults; AR	Timothy vs Timothy + 9 pollens vs placebo	Mixture	SS, MS, nasal reactivity, sp IgG4 and sp IgE did not change in multiallergen group	[43]
Swamy <i>et al.</i> (2012)	DBPC	30 adults and children; AR + asthma	Timothy + HDM vs placebo	Simultaneous	SS↓ MS↓ SPT reactivity↓ Nasal reactivity↓ sp IgG4↑ sp IgE↓ Basophil activation↓ Allergen sp Foxp3 memory cells	[45]

AR: Allergic rhinitis; ARC: Allergic rhinoconjunctivitis; BHR: Bronchial hyper-reactivity; DBPC: Double-blind placebo-controlled; HDM: House dust mite; MS: Medication score; PEF: Peak expiratory flow; QOL: Quality of life; RCT: Randomized controlled trial; SCIT: Subcutaneous immunotherapy; SLIT: Sublingual immunotherapy; sp: Specific; SPT: Skin prick test; SS: Symptom score.

placebo or up to seven extracts (including house dust mite (HDM), grass, alternaria, aspergillus and cladosporium, but not cockroach, cat or dog, even sensitized). There was no significant difference between SIT and placebo groups

based on symptom medication scores, peak expiratory flow values and bronchial hyper-reactivity to methacoline.

Hedlin *et al.* compared birch or Timothy grass IT plus either cat or HDM, or placebo in

29 asthmatic children in a DBPC manner [38]. After 3 years, the group receiving multiallergen IT (pollen and HDM or cat) demonstrated significant improvement in allergen-induced bronchial hyper-responsiveness.

Another DBPC SCIT study included 53 adults with seasonal AR and/or asthma sensitized to orchard grass and olive tree pollens [39]. Patients received either a mixture of orchard grass and olive tree pollens or placebo. At the end of 1 year, symptom, medication and quality of life assessment scores were significantly lower in the active group. In a randomized controlled trial, 54 children with allergic rhinoconjunctivitis were treated with a mixture of up to four allergens including cat and HDM, and German or American cockroach [40]. After 6 months, significant improvement in allergic symptoms and medication use was detected in the active group compared with the pharmacotherapy group. Conversely, results with ≥ 1 allergen were not separately reported.

SCIT/SLIT studies comparing multiallergen IT with single-allergen IT or placebo

Thirty-six patients with rhinoconjunctivitis with or without mild asthma were recruited in a randomized controlled study [41]. Patients received either preseasonal grass SCIT alone or preceded by birch/hazel SLIT. After 2 years, both groups demonstrated a decrease in symptom scores and a need for antihistamines during the grass pollen season. Conversely, a reduction in cough and conjunctival symptoms, as well as use of asthma medications, were significantly increased in the combined IT group compared with preseasonal SCIT alone. Moreover, the threshold dose of grass pollen during nasal provocation was significantly higher in the combined IT group and bronchial hyper-reactivity to methacholine only improved in this group. The authors concluded that birch/hazel SLIT improved the outcome of grass pollen SLIT subsequently administered (TABLE 1).

SLIT studies comparing multiallergen IT with single-allergen IT or placebo

The first SLIT study on multiallergen SLIT was conducted on 48 adults with rhinoconjunctivitis and mild asthma in an open-label randomized controlled manner [42]. Patients were sensitized to birch and grass, and randomized to receive SLIT for birch, grass or both birch and grass, as well as pharmacotherapy only (controls). In

the birch and grass group, SLIT was given in separate vials taken sublingually at the same time. Following a 4 year period, all three IT groups revealed significant improvement in symptom and medication scores compared with baseline and controls. Symptom and medication scores significantly improved with grass plus birch SLIT or with single-allergen SLIT during both the grass and birch seasons [42]. Another SLIT study conducted by Amar *et al.* compared single- (Timothy) with multi-allergen SLIT including the same dose of Timothy [43]. Fifty-four patients were randomized to receive either Timothy extract, multiallergen SLIT or placebo for 10 months. None of the treatment groups demonstrated any efficacy based on symptom scores when compared with placebo. The authors suggested that the treatment year was close to the driest year ever recorded in Denver, which significantly decreased grass pollen counts. It is likely that low grass pollen counts in this treatment year may have contributed to the lack of significant differences in the symptom and medication scores. In addition, the 10-month duration of the treatment may not have been long enough to detect any significant differences in clinical parameters. It has been previously reported that the onset of SLIT is usually detected between 4 and 12 months of treatment (TABLE 1) [44].

A more recent study evaluated the efficacy of dual SLIT in children and adults sensitized to both Timothy grass and HDM [45]. This was a randomized, double-blind, controlled Phase I study. The subjects received either Timothy grass and HDM dual SLIT (i.e., by using separate vials), or placebo. At the end of the 12 months, subjects treated with dual SLIT (Timothy grass and HDM) demonstrated significantly decreased symptom/medication scores, allergen-specific nasal skin reactivity, basophil activation and specific IgE levels, and increased allergen-specific IgG4 levels, in addition to the induction of allergen-specific tolerance, when compared with placebo. The active group demonstrated significantly increased Timothy grass and HDM-specific IgG4 levels, reduced specific IgE levels and basophil activation. In addition, dual SLIT promoted epigenetic modifications of allergen-specific suppressive CD4⁺ CD25 high CD127 low CD45 RO⁺ fox P3⁺ memory Tregs. This study was the first to demonstrate that long-term tolerance might be induced by multiallergen SLIT [45].

Taken together, multiallergen SCIT studies published to date used mixtures of allergens

in one vial, whereas there are no SCIT studies using simultaneous separate vials [33–40]. There is only one study using SLIT and SCIT of different allergens simultaneously with positive results [41]. Meanwhile, review of the multiallergen SLIT studies demonstrated that two studies used simultaneous treatment with separate vials of different allergens [46] whereas in the study in which a mixture of noncross-reactive allergens were used, multiallergen SLIT given for 10 months could not demonstrate efficacy [43].

Multiallergen immunotherapy: mixture or simultaneous allergen extracts

It is important to determine whether use of a mixture of noncross-reactive allergens or simultaneous allergen extracts from separate vials is more effective. Although low in number, SCIT studies published to date have demonstrated efficacy with mixtures in a vial [33–40]. However, the available multiallergen SLIT studies are not comparable because two used separate simultaneous vials for two allergens with positive results [40,42], whereas negative results were reported in a study in which a mixture of ten allergens in the same vial was used. [43].

It has been suggested that multiallergen immunotherapy may have the disadvantage of dilution of extracts in the vial, which may result in the reduction of an effective dose of individual allergens [47]. In addition, for sublingual administration, it is suggested that the possibility of a limited absorption capacity of the sublingual area may result in competition between allergens for absorption [48]. Therefore, these aspects should carefully considered before deciding to initiate multiallergen IT for the treatment of respiratory allergic diseases. There are important issues regarding allergen mixes that should be addressed; these are dilution, degradation and absorption of allergens in mixtures [26]. The potency of allergen extracts are generally expressed as the content of major allergen per unit volume or per administered dose. For many of the standardized extracts effective allergen doses have been established and as long as this amount is placed in the final mixture, dilution of any allergen will not occur. However, this may not be valid for weaker extracts such as molds, cockroach, dog, cat and HDM in which larger amounts of each must be added to provide the adequate effective dose [26]. The second problem with multiallergen mixtures is the protease activity of some allergens, which may degrade the other allergens in the vial.

This activity is usually found in fungal and cockroach extracts. Therefore, inclusion of these allergens in mixture proportions is generally not recommended [49–51].

Limitation of the absorption of allergens in multiallergen extracts applies only to sublingual administration of allergens as the sublingual mucosa is not normally an absorptive surface. Therefore, during application of multiple allergens, the amount of the protein that can be absorbed over a given period of time may be limited. Another suggested problem associated with the sublingual region is the possibly limited number of APCs in the region. In a previous study, 64 patients with allergic rhinoconjunctivitis sensitized to grass or birch pollens were assigned to one drop three-times daily for 2 years, or one drop twice or once daily for 1 year, followed by one drop three-times daily for the 2nd year, or no treatment (control group) [52]. Medication use and skin reactivity to allergens were determined at the end of the 1st and 2nd years. Only the 3 × 1 group demonstrated a decrease in medication use and skin reactivity at the end of 1 year. The 2 × 1 and 1 × 1 groups demonstrated similar changes in year 2. Although the investigators concluded that the number of sublingual allergen applications is correlated with efficacy, the cumulative dose of the administered allergen was different between the groups in the 1st year. Therefore, it is not possible to draw this conclusion without comparing results with a group receiving the same dose of allergen administered once daily.

Immunologic responses induced by multiallergen IT

The allergen-specific immunologic response to multiallergen IT administered either subcutaneously or -lingually is not well defined owing to the low number of studies. In particular, studies comparing monotherapy with multiallergen IT in patients receiving IT are warranted. In a SLIT study, Amar *et al.* compared placebo, monotherapy with Timothy extract and multiallergen mixture, including the same dose of Timothy extract, based on titrated nasal challenges and skin prick tests (SPTs), as well as Timothy-specific IgG4 and IgE, along with cytokines released by the Timothy-stimulated lymphocyte proliferation, in addition to clinical parameters [43]. Although there were no significant differences in the clinical parameters following 10 months of treatment, subjects treated with Timothy

monotherapy, but not with Timothy-containing multiallergen IT, demonstrated significantly improved nasal challenge results when compared with placebo. In addition, Timothy-specific IgE levels were significantly higher in both mono- and multi-allergen treatment groups than placebo, while specific IgG4 and a decrease in Timothy-induced IFN- γ were only detected in the monotherapy group. Timothy-induced IL-4, -5 and -10 levels did not show any difference. The increased specific IgG4 levels only in the monotherapy group suggests that Timothy monotherapy is more efficient than multiallergen SLIT. These results suggest the interference of allergens when multiple allergens are coadministered as a mixture.

A very recent DBPC study conducted by Swamy *et al.* compared symptom medication scores, SPT and nasal challenge test results, allergen specific IgE and IgG4 levels, and immunologic parameters such as basophil activation and methylation of CpG within FoxP3 memory Tregs in 30 patients sensitized to both HDM and Timothy grass [45]. SLIT was administered dually but not as a mixture, that is, by using separate bottles of each allergen for 12 months. The group receiving dual Timothy grass plus HDM SLIT was compared with

the placebo group; however, there was no monotherapy group in this study. The active group demonstrated reduced responses to both Timothy grass and HDM allergens based on results of SPT and nasal challenges. An increase in Timothy grass- and HDM-specific IgG4 levels, and decreased allergen-specific IgE levels were detected in the active group. In addition, the active group demonstrated decreased basophil activation and the allergen-specific CD4⁺ CD25^{high} CD127^{low} Cd45RO⁺ foxP3⁺ memory cells (allergen-specific suppressive memory Tregs) demonstrated reduced DNA methylation of CpG sites within the Foxp3 locus. In a previous study, the decrease in methylation of CpG sites of the FoxP3 locus was associated with increased stability of FoxP3 expression [53]. Therefore, this study demonstrated dual SLIT-induced tolerance by epigenetic modifications of FoxP3 in memory Tregs [45]. This Phase I study suggests that SLIT performed with multiple allergens simultaneously administered, rather than mixed, could be an effective treatment for polysensitized patients, which deserves larger long-term studies.

Taken together, it is hard to draw a conclusion from the three available SLIT studies (one with mixtures and two with simultaneous

Executive summary

Allergen-specific immunotherapy in polysensitized patients

- Polysensitization is more prevalent than monosensitization.
- Current data are scarce on polysensitized patients treated with subcutaneous (SCIT) and sublingual (SLIT) immunotherapy.
- Studies using multiallergen immunotherapy in polysensitized patients are overviewed briefly.

SCIT studies in polysensitized patients

- Allergen mixes have been used in studies to date.
- Most studies demonstrated decreased symptoms or medication use.

SLIT studies in polysensitized patients

- Both allergen mixes and simultaneous allergen administration have been used.
- Simultaneous administration of house dust mite and pollen seems to be clinically efficient with induction of allergen-specific tolerance.

SCIT + SLIT in polysensitized patients

- There is only one study that demonstrates improvement in clinical symptoms as well as skin, nasal and bronchial reactivity.

Mixture or simultaneous allergen extracts

- All SCIT studies used a mixture of allergen extracts in a vial.
- SLIT studies used both mixtures and simultaneous allergen administration.
- Studies are needed to prove efficacy of both mixtures and simultaneous administration.

Immunological responses

- Simultaneous SLIT administration resulted in induction of allergen-specific suppressive memory Tregs for the two administered allergens.

Future perspective

- Further studies are needed to validate the use of multiallergen immunotherapy.
- Clarification of using either mixture or simultaneous allergens is essential.
- Multiallergen immunotherapy will target the majority of patients.

allergens) on how to use allergens in polysensitized patients [42–45]. Studies on SCIT in polysensitized patients to date used mixtures of allergens and reported clinical efficacy, whereas immunological responses have not been evaluated. Limb *et al.* evaluated specific IgE levels of 82 children who received either mixture SCIT or placebo 8–16 years following treatment discontinuation [54]. There were no significant differences between groups in terms of specific IgE levels, but a significant reduction in skin test sensitivity.

Conclusion & future perspective

Although polysensitization is much more prevalent than monosensitization in patients with allergic diseases, there are no conclusive data on the option of using multiallergen IT as a treatment choice. Studies published to date report some positive results but are few in number [33–40,42–45]. In addition, allergen mixes are mostly used in the SCIT studies, whereas

in SLIT studies, both mixes and simultaneous administration of allergens have been evaluated.

Therefore, the available data both on efficacy and induction of immunological tolerance for either mixture or simultaneous application of various allergens is scarce. Large clinical trials are needed for both sublingual and SC multiallergen IT in polysensitized patients to validate it as a treatment option, as the majority of the patients attending allergy clinics are polysensitized.

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