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Allergen immunotherapy in polysensitized patient

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Key words
polysensitization; polyallergy; cross-reactivity; specific allergen immunotherapy (AIT)

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Summary
Specific allergen immunotherapy (AIT) is the only therapeutic method with positive impact on natural course of allergic disease - affecting clinical development (including the progression of rhinitis to asthma) and new sensitisations. The actual problem is the increasing number of patients manifesting poly-sensitivity in allergy skin tests and / or in specific IgE tests. Usually, AIT is not recommended in such individuals. The objective we are facing is that in many patients tested as poly-reactive, we have to distinguish in which cases it is a true polysensitization, and when it is due to cross-reactivity of specific IgE antibodies induced by panallergens. This may really determine when AIT may be an appropriate course of action. The article focuses on this problem in more detail, applying the long time Czech and Slovak experience with allergy testing and allergen immunotherapy.

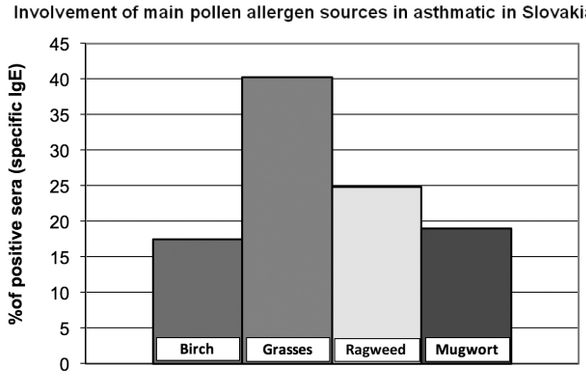
Introduction

Specific allergen immunotherapy (AIT) is the only therapeutic method that may have a positive impact on the natural course of an allergic disease - affecting both the development of new sensitisations, as well as clinical development, including the worsening of symptom severity and the progression of rhinitis to asthma. A serious problem is the increasing number of polysensitized patients and the number of allergy sufferers in whom several clinical manifestations of allergy combine (allergic rhinitis accompanied with bronchial, skin and / or eye symptoms). According to the review of Calderon et al, 50-80% of patients with allergies are polysensitized (1). In patients manifesting poly-sensitivity in allergy skin tests and / or in specific IgE tests, AIT is usually not recommended. However, in many patients tested as poly-reactive in allergy tests, allergen immunotherapy may be an appropriate course of action. Our aim is to focus on this problem in more detail, applying the long time Czech and Slovak clinical experience.

In both our countries, allergy and immunology has been a postgraduate specialization for more than 40 years. Initially, it was a second degree specialty after Intern medicine or Pediatrics as first degree, actually it is a basic specialty. As a result of 40 years expert training (it lasts 5 years and is finished by exam), we have a dense network of specialised outpatient allergy departments (about 6 per 100,000 inhabitants). This makes an essential difference in allergy practice in Slovakia and Czech republic - most of patients with allergic rhinitis and asthma are managed in specialised care, not in general practitioner practice as it is usual in most of European countries. As a result of the broad network of specialised outpatient departments, and also because of local allergy diagnostics and vaccine producer, AIT was broadly used as a first line treatment from the early Sixties of 20th century.

According to the *National centre of health informations* (to which all allergy sufferers are mandatorily reported on yearly basis) the estimated prevalence of patients with allergic diseases is 20%, most of them with respiratory allergy. The spectrum of sensitising allergens is the same as in whole central European region. Most prevalent is grass pollen allergy, with house dust mite allergy covering about 60%; other important allergens are tree pollen (*Betulaceae* family), weed pollen (ragweed, mugwort), epithelia (cat, dog) and moulds (*Alternaria*, *Cladosporium*). The situation in pollen allergy is depicted in **table 1**.

Table 1 *- Main pollen allergens sources in Slovakian asthmatics (according to reference 37).*



Specific allergen immunotherapy – definition

Specific allergen immunotherapy (AIT), also referred to as allergen vaccination, or desensitisation, was defined as a treatment method based on the administration of the causal allergen to sensitised individual (subcutaneously or sublingually) in gradually increasing doses in order to achieve *tolerance*, or at least reduced reactivity to an exposure of given allergen (2,3). However, that definition needs to be reformulated, because in new forms of sublingual immunotherapy, the patient applies a full (final) dose of the allergen from the beginning (or from the 2nd - 3rd day). Specific long-term tolerance is achieved through complex mechanisms involving both humoral and cell-mediated immunity (4,5,6).

Allergen immunotherapy – mode of action

The principal mode of action in sublingual (SLIT) and subcutaneous (SCIT) immunotherapy is equal. The basic mechanism of action is the induction of clinical tolerance of a specific allergen. In successful AIT cellular allergen-specific immunoregulatory mechanisms play a key role, whereas other mechanisms, such as the generation of specific IgG4 blocking antibodies, the decline in specific IgE antibodies, or decreased activity of eosinophils and basophils, are regarded as secondary (4,5,6).
Atopic responses are characterized by the proliferation of antigen-specific lymphocyte subpopulations with a predominantly Th2, Th9 and Th17 cytokine profile types. The impact of AIT leads to the induction of allergen-specific populations of T and B regulatory cells suppressing other subpopulations (4,5). Dendritic cells also play a significant role, especially in the case of the SLIT (7).

Specific allergen immunotherapy – preventive and long-term effect

Allergy is a systemic disease with local symptoms, mostly manifesting in respiratory tract; however, they can also be cutaneous, gastrointestinal or systemic. By contrast to symptomatic pharmacotherapy (H1-antihistamines, nasal steroids), AIT cures allergy as a systemic immune disorder. It is a medical procedure with a direct impact on the pathogenesis of an allergy, and has shown in particular a prophylactic potential, so it may be regarded as a causal treatment (5). Successful AIT not only induces tolerance to a causal allergen, but it also may prevent the formation of new sensitizations and the development of asthma. After 3 years of administration, both SCIT and SLIT may generate a long-term effect, which in most cases lasts for years after its cessation (5,8,9,10,11).

Allergen immunotherapy – indications in allergy to inhaled allergens

AIT may affect the natural history of allergy, particularly among young people, thereby, an *early indication* is advisable. It is therefore worthy to consider questioning the views promoted by the guidelines for the treatment of allergic rhinitis, as well as the guidelines for AIT, which state that immunotherapy shall only be indicated in cases of unsuccessful pharmacotherapy. Early indication of AIT appears to produce the best results (8,12,13).
A clear indication for AIT is the presence of respiratory allergy symptoms, i.e. *allergic rhinitis* and / or *allergic asthma* depending on allergen exposure (2,3,13,14). Sufficient evidence of efficacy (as well as safety) has accumulated, particularly in the cases of pollen and dust-mite allergens (mainly birch, grass, mugwort, ragweed and wall pellitory pollen, the Dermatophagoides pteronyssinus and D. farinae). AIT has also been successfully used in the case of hypersensitivity to allergens of some moulds (Alternaria, Cladosporium) and animal epithelia (cat, dog, and horse epithelia), however, for these allergens, there is little evidence based support (2,3,14).
Indications of AIT in allergic asthma (prevention of onset, treatment of an already existing condition) is currently undisputed from the perspective of clinical immunology (5,6,9,15), howev-

er, the current guidelines for the treatment of asthma are very scarce in terms of AIT. In such important documents as the Global Initiative for Asthma (16), the American Thoracic Society Guidelines for the treatment of asthma (17), or the British Thoracic Society Guidelines (18), AIT is mentioned only marginally, and its possible benefits are not clearly evidenced. The Czech and Slovak allergy-treatment strategy for asthma-management (Czech "Recommendations for the use of AIT" and Slovak "National guidelines for asthma") based on expert panel consensus, attributed AIT more important position than most international counterparts (19,20). Our opinion is supported not only with our long-lasting good clinical experience, but also by many literature data fulfilling the criteria of evidence-based medicine (21,22).

Sensitisation, polysensitization, cross-reactivity, causal allergen, polyallergy

In the case of the patient who generates IgE response to multiple allergens, it is not easy to identify the decisive trigger. Diversity of responses in skin tests, as well as in different specific IgE laboratory tests, is often an obstacle for AIT indication. Our purpose is to examine different clinical situations, and attempt to find a way to rationalise the use of AIT and to clarify a few terms that are frequently used incorrectly (see also **table 2**).

Table 2 *- Crucial terms, patterns of sensitization and indications of specific immunotherapy.*

Sensitization: production of sIgE antibodies to concrete allergen molecule(s)
Allergy: clinical expression of sensitisation to particular allergen (i.e. sensitization leads to symptoms)
Polysensitisation: multiple sensitisation with different allergens:
a) Clinically important only one relevant causal allergen
   - AIT is suitable
b) Two or more causal allergens = polyallergy
   - AIT may be considered after appropriate diagnostic process (consider MBAD)
c) Sensitization to panallergens = allergens present in different (also unrelated) sources of plant and/or animal origin (profilins, polcalcins, tropomyosins, lipid transfer proteins...)
   - Consider MBAD, AIT is questionable (panallergens for AIT not proven)
d) Polyreactivity in allergy tests as a result of sIgE cross-reactivity due to homology of allergen structures
   - Consider AIT (try to choose one allergen)

sIgE: specific IgE antibodies
AIT: specific allergen immunotherapy
MBAD: Molecule Based Allergy Diagnostics

The term *sensitization* means that the patient is producing specific IgE antibodies to concrete allergen molecule(s). Sensitization per se does not automatically mean allergic disease, but it is a risk factor to develop clinical manifestation of *allergy*. Antigen primarily causing sensitization and symptoms we define as a *causal allergen*. Although multiple causal allergens may be present in each patient, not every sensitizing allergen must represent an equivalent trigger of clinical symptoms. In the case of *mono-sensitization*, the patient is sensitized to only one allergen (allergen source), for example to timothy grass or birch pollen or to house dust mite, or to closely related taxonomical family or group of allergen sources, i.e. grasses from *Poaceae* family, trees from *Betulaceae* family, house dust mites, etc. *Poly-sensitization* is a sensitization to three or more allergen sources (e.g., mite, birch, and grass pollen). In patient sensitised to many allergens, also the term multiple-sensitization may be used. In the case of sensitisation to allergens from two unrelated sources (i.e. grass pollen and mites), we use the term *co-sensitization* (23,24). In the case of multiple-positive allergy tests we have to distinguish between real polysensitisation, polyallergy and cross-reactivity. *Polysensitisation* involves sensitivity to multiple different allergen molecules leading to production of specific IgE antibodies of various specificities regardless of symptoms. *Polyallergy* is the term describing the situation, when 2 or more sensitizing allergens are triggers of symptoms.

Cross-reactivity describes the situation when multiple sensitivities are consequence of reactivity of the same specific IgE antibodies with antigenic structures of homologous allergen molecules from different allergen sources. As an example, we can mention three possible patient types with the same positive tests to grass pollen, house dust mite and mould Alternaria (**table 3**). In the case of symptoms only during grass pollen season, it is polysensitisation, but only a grass pollen allergy. If we have the patient with the same test results, but with a clinical picture of perennial rhinitis without seasonal flare up of symptoms, it is a polysensitised patient with mite allergy. In both cases, AIT may be considered. Another patient with the same sensitisation pattern (grasses, house dust mites, Alternaria), with intermittent or persistent symptoms triggered when in contact with each of these allergen sources, is polyallergic and AIT is not suitable.

Another case may be reactivity to *pan-allergens*, i.e. a cross-reactive allergen belonging to a protein family well preserved throughout many widely different species, able to trigger IgE antibody binding (23,24). Typical example of pan-allergen is tropomyosin, causing reactivity to house dust mite extracts and also to different extracts from invertebrate allergen sources such as crustaceans (shrimp, lobster, crab, crawfish), arachnids (house dust mites), insects (e.g. cockroaches), and molluscs (e.g. squid). Of course, not all positively tested allergen extracts denote primary sensitization, but clinical reactivity to all sources

Table 3 *- Different standpoint for immunotherapy in the same pattern of polysensitization.*

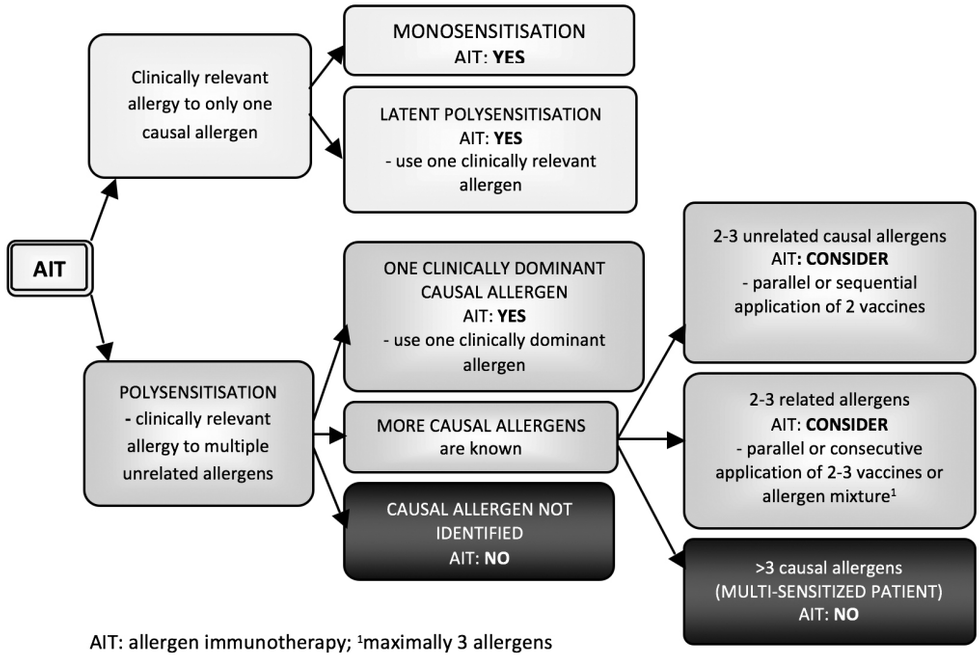
Sensitisation pattern (example)	the same level of positivity to grass pollen, house dust mite, Alternaria in skin and / or laboratory tests		
Symptoms	only during grass pollen season	perennial without seasonal flare up	in contact with each allergen
Indication of AIT	YES (grasses)	YES (mites)	NO (polyallergy)

of tropomyosin cannot be excluded. Panallergens of plant origin comprise profilins, polcalcins, and lipid transfer proteins. For example, profilins were identified in pollen of trees, grasses, and weeds, in plant-derived foods, as well as in latex. Due to its conserved structure, profilin-specific IgE may cross-react with homologues from virtually every plant source (24).

When using diagnostic *allergen extracts*, in patients with multiple positive routine tests we do not know exactly if we see true polysensitisation, or if it is only expression of cross-reactivity of specific IgE antibodies to homologous allergen molecules, or a picture of sensitivity to pan-allergens. However, the assessment of *causality* is crucial. To distinguish this, in addition to a thorough history, we should use *molecule-based allergy diagnostics* (MBAD), to map in detail allergen sensitisation pattern at molecular level (23,24). In MBAD, purified natural or recombinant allergenic molecules (allergen components, hence also term Component Resolved Diagnosis, CRD) instead of allergen extracts are used (25). This may reveal the presence of specific IgE to individual molecules (epitopes) of the allergen and help us to distinguish between allergy to species-specific allergens and cross-reactive allergens, and thus identify the relevant allergen for AIT in polysensitised patients.

Inappropriately indicated AIT in polysensitised individual will be ineffective, and in some cases may lead to deterioration of the condition. Thus, it became common practice to refuse AIT in polysensitised patients. However, even among polysensitised

Table 4 *- Indications for allergen immunotherapy (consensus of expert board from Slovak and Czech Allergy and Clinical Immunology Societies).*



AIT: allergen immunotherapy; [1]maximally 3 allergens

individuals we can find suitable candidates for an effective AIT (**table 2**, **3**, **4** and **5**). Test-detected polysensitisation must be divided into several clinical phenotypes. The *clinical relevance* of test-positive allergens is absolutely fundamental for the indication of AIT.

In case of cross-reactivity, specific IgE antibodies developed in response to the primary sensitizing allergen react with the same or similar molecular structure(s) from other allergen sources. In this context, the term *homologous* allergen is used. Homology expresses what percentage of the amino-acid sequences from which the allergen molecule is constituted is the same among individual proteins. The higher the agreement in the amino-acid sequences, the greater is the probability of specific IgE cross-reactivity.

Clinical relevance and mixing of allergens

Older guidelines do not recommend AIT for polysensitised patients (2). Nevertheless, according to mentioned new diagnostic tools, polysensitisation is only a relative contraindication; however, we need to consider whether multiple specific IgE positivity is the result of real polysensitisation, or the result of a simple cross-reactivity. In the case of sensitivity to different species-specific allergens, we then consider which of the positively tested allergens are clinically relevant, i.e. to what extent they affect patient's clinical symptoms. Even in polysensitized patients, it is possible to determine one relevant allergen or only a couple of them (**table 3**, **4** and **5**).

British authors in large-scale randomized placebo controlled trial demonstrated that polysensitized patients (77% of enrolled) responded to subcutaneous AIT as good as monosensitized ones (26). There were no significant differences between the polysensitized and monosensitized subgroups in terms of efficacy and safety. In another study using 5-grass pollen tablets, a subgroup analysis of 628 adults with different clinical profiles of allergic rhinoconjunctivitis demonstrated that the average rhinoconjunctivitis total symptom score and safety for mono- and poly-

Table 5 - *Examples for allergen immunotherapy decision making (consensus of expert board from Slovak and Czech Allergy and Clinical Immunology Societies).*

Type	Description	Examples of allergens suitable for AIT
1. Monosensitisation	Clinically relevant allergy to one allergen (only one positively tested allergen)	Birch or mixture of birch family pollen Grass pollen (one or mixture) Weeds (with exception of Aster family only single allergens) House dust mites (single or mixture) Moulds (only single)[1] Animals (only single)[1]
2. Polysensitisation with clinically relevant allergy to only one allergen	Other positively tested allergens clinically not relevant, i.e. sensitisation without important symptoms	The same as above
3. Polysensitisation with clinically relevant allergy to more unrelated allergens	AIT may be considered with one dominant causal allergen, or with limited number of allergens (up to 3)	Grasses + Trees + Weeds[2] Mites + other allergens[3] Moulds[4] Animals[4] Moulds and or Animals + Other[5]
4. Polyreactivity in specific IgE tests with allergen extracts expressing cross reactivity with homologous allergen structures	Clinically relevant allergy to only one allergen • In molecule diagnostic only one allergen molecule (component) is dominant • AIT may be considered	Bet v1[6] Phl p1, Phl p5[6] Amb a1, Art v1[6] Der p1, Der p2[6]

[1]Not verified in studies.
[2]Pollen allergens may be mixed together, but not with other allergens.
[3]Mite allergens may be mixed together, but not with other allergens.
[4]It is better not to do mixtures with mould and / or animal allergens, as most of them are enzymes.
[5]Unrelated allergens should be utilised only in separate vaccines (parallel or consecutively).
[6]Most of all profit individuals allergic to main allergen.
AIT: allergen immunotherapy

sensitized patients was identical (27). Also other studies brought similar results. Emminger et al showed in double-blind trial that single-allergen sublingual immunotherapy was found to be clinically effective in both polysensitized and monosensitized patients (28). Authors showed that SLIT with grass tablet containing only Phleum pratense allergen was significantly effective in all three monitored groups: monosensitization to grass pollen, polysensitization to grass and tree pollen and grass pollen and other allergen (but not tree pollen). However, in these studies, the clinical relevance of sensitization to allergens other than that used for AIT was not established.

The other possibility involves administration of multicomponent AIT to polysensitized patients. However, with increasing number of causal allergens, AIT becomes more complicated and its efficacy can decrease. There are several points to think about:

1. Gradual or parallel administration of allergens in multiple vaccines is more time-consuming, financially demanding, demanding on patients' cooperation.
2. In patient manifesting multiple causal allergens, AIT with only one allergen may cover only part of triggers.
3. Possibilities of allergen mixture utilisation are limited:
   - because of protease activity of most of allergen molecules (degradation of the antigenic structures may occur);
   - mixing multiple antigens leads to the occurrence of dilution phenomenon which leads to ineffectiveness due to the insufficient dose of antigen;
   - we should combine only few related allergens, to mix together pollen with dust mite and / or fungal allergens is not advisable.

Not every sensitizing allergen represents an equivalent trigger of clinical symptoms. When selecting allergen(s) for the AIT vaccine, we should take into account how important and how long-lasting are the symptoms caused by each allergen (i.e. assessment of *dominant allergen*). When a positively tested allergen does not cause symptoms, AIT with such allergen is not indicated. In contrary, if exposure to the sensitizing allergen causes symptoms and the period of exposure during the year is long (more then 2-3 weeks), AIT should be considered.

The *correlation* of disease history (daily log of health concerns is desirable), skin prick tests, specific IgE antibodies test results (both allergen extracts and molecule-based diagnostics), and pollen information services helps us to distinguish primary sensitising causal allergen(s), cross-reactivity, polysensitisation and polyallergy. For the indication of AIT we need to consider carefully the nature of symptoms (when, how long and in what environment) and whether and which ones in particular correspond to positively tested allergens. Consequently, we only choose the causal allergens for the formula. Correct evaluation of causality is difficult indeed; yet it is the key to a successful AIT.

Due to fact, that polysensitization is much more prevalent than monosensitization, allergen mixtures (e.g. 2-3 pollen kinds, house dust mite mixture) or parallel application of 2 vaccines are commonly used, but there are no conclusive data on the option of using multi-allergen IT as a treatment choice (29). In the United States, often allergen mixtures of all sensitizing allergens are used, but this is not internationally approved. In 2000 we published the results of an open label study in polysensitized patients (30). Two years of SLIT with allergen mixtures in terms of safety and effectiveness were evaluated. About 10,000 doses were given and no general reactions occurred. Some effect was achieved, but the open label design without comparing arm limits interpretation of results. Only few randomized, double blind and placebo controlled studies with aim to evaluate allergen mixture efficacy, or parallel or consecutive allergen vaccine administration in polysensitised patients were designed and realised.

In 2009, Nelson published a review of studies simultaneously using two or more distinct allergen extracts in either SCIT or SLIT (31). Thirteen studies were identified, 11 using subcutaneous injections, 1 using sublingual administration, and 1 using both. In studies with adequate information, administration of 2 extracts by means of either SCIT or SLIT was effective. In studies using multiple allergens, 3 studies showed clear efficacy, whereas in the other 2 studies lack of efficacy was shown. It was concluded that simultaneous administration of more than 1 allergen extract may be clinically effective; however, more studies are needed, particularly with more than 2 allergen extracts.

One small study in patients allergic to birch and grass pollens, showed that therapy with two extracts is more efficacious than application of only one allergen (32). In Italian open study SLIT, provided evidence that polysensitization is not an obstacle for prescribing AIT and some efficacy in clinical parameters after one year of SLIT in 57 polysensitized children of mean age 11.8 years was demonstrated (33). The aim of a Korean study was to compare the efficacy of SLIT with standardized house dust mite extract in monosensitized and polysensitized patients with allergic rhinitis (34). In the first group, 70 patients were sensitized to house dust mites (HDM) only, in the second group there were 64 patients with HDM allergy simultaneously sensitized to other aero-allergens. In polysensitized allergic rhinitis patients, SLIT with standardized HDM extract produced significant improvements in both nasal symptoms and rescue medication scores comparable to those in monosensitized patients, regardless of other positive allergens.

POLISMAIL was a real-life based multi-centre study designed with aim to evaluate the behaviour of Italian allergists managing polysensitised patients (35). The effect of two-year SLIT was assessed; single allergen extract was used in two-thirds of patients, mix of two allergens was chosen for the rest. Both SLIT with one or two allergen extracts induced significant improve-

ment of the severity grade of allergic rhinitis and asthma and improved quality of life (QoL) in polysensitised patients. This study demonstrates that polysensitization should not represent a counter-indication for prescribing immunotherapy and that the choice to limit SLIT to 1-2 allergen extracts was sufficient and effective in improving symptoms and QoL in polysensitised patients. An important issue is that polysensitization may be associated with a worse clinical picture than that seen in monosensitized patients, particularly in terms of poorer quality of life. However, Ciprandi et al demonstrated, that QoL can be improved in polysensitized patients too, and that the use of just 1 or 2 allergen extracts seems to be sufficient (36).

Few studies concentrate on the length of AIT - for example we have no direct comparison of three and five year duration of AIT. We need to answer the question, whether longer administration of an allergen vaccine leads to a better effectiveness both on symptoms and from a prophylactic point of view. A Czech and Slovak statement is that longer is better, but it is necessary to confirm it.

Conclusion

Immunotherapy distinguishes from pharmacotherapy in specificity and with potential to influence natural course of allergic disease. It has a dual effect: 1) together with anti-allergic drugs it helps to decrease symptoms of allergy, 2) unlike drugs, it may have a prophylactic effect on the disease development and worsening. AIT may reduce appearance of new sensitisation and stabilises clinical course - it limits development from rhinitis to asthma. Both therapeutic and prophylactic effect may last for many years after cessation.

Due to prophylactic potential, AIT should be considered in every patient with respiratory allergy. However, a substantial part of patients which could gain benefit from AIT, do not achieve it because of polysensitisation. Anyway, AIT is not suitable only in patients with multi-sensitization to various unrelated allergens, or in those with undetectable causal allergen. If indicated, we prefer to choose only one allergen for AIT. The available data both on efficacy and induction of immunological tolerance for either mixture or simultaneous application of various allergens is scarce. To validate it, large clinical trials are needed both for SCIT and SLIT multiallergen immunotherapy in polysensitized patients.

With increasing knowledge about allergen molecules and their epitopes, and with growing number of allergen molecules suitable for allergy diagnosis (and for therapy in future?) we can await better and more appropriate indication of AIT. With more precise diagnostics, we can expect a rejection of AIT in some patient indicated to it nowadays; on the contrary, we will use it in many actually not indicated patients. In other words, better efficacy and reduction of unsuccessfully treated patients can be expected. So, in the future, AIT can improve the perspective of many polysensitised patients. However, more studies are needed to confirm AIT efficacy in polysensitised patients - with one causal allergen selected, with multi-component vaccines or simultaneous application of various allergens.

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