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# Component-resolved diagnosis-assisted prescription of allergen-specific immunotherapy: a practical guide

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## SUMMARY

*Allergen specific immunotherapy remains the only means to change the natural history of allergic disease. Thanks to the recent advances in molecular biology a large spectrum of purified allergen molecules are presently routinely available for diagnostic purposes. This review represents a practical guide on how to use these new diagnostic tools in order to detect precisely the primary sensitizing allergen sources in subjects showing a multiple sensitization to seasonal and/or perennial airborne allergens, thus avoiding the diagnostic mistakes that have been probably associated with the prescription and administration of several ineffective immunotherapies up to a recent past.*

## Introduction

Allergen specific immunotherapy (SIT) is the only therapeutic approach able to change the natural history of airborne allergic diseases. Its efficacy has been demonstrated by hundreds of properly performed studies worldwide and is presently unquestioned. The WHO position paper, as well as the guidelines by the European Academy of Allergy and Clinical Immunology, state that SIT is indicated in patients sensitized to allergens that cannot be avoided and who suffer from long-lasting and sufficiently severe symptoms (1,2). Of course, the efficacy of immunotherapy relies on the fact that the right allergen(s) is/are administered to the allergic patient.

## Extracts-based diagnosis

Natural allergenic extracts have been the milestone of allergy diagnosis of respiratory allergy for more than one century. They have been progressively improved in terms of purity, sensitivity, and standardization up to the point that current extracts can be confidently considered to contain most, if not all, allergen proteins present in the various respiratory allergen sources. Currently, a negative or positive skin test or in-vitro test with one extract of an airborne allergen source shows a sensitivity and predictive value that is frequently close to 100%. However, despite their undeniable merits, natural extracts carry one intrinsic defect that cannot be eliminated: they are mixtures of

both allergenic and non-allergenic proteins and, more importantly, in most cases every single extract contains several allergens. As a consequence, extracts may show some variability in the relative concentration of the single allergens from one batch to another and, more importantly, positive extract-based tests don't tell us which allergen proteins in the single allergen sources are the sensitizing ones. This is a major problem if one considers that some allergenic proteins are present in an homologous form in most, if not all, allergen sources of a certain sort (whether pollens, mites, moulds, or animal dander), and that such proteins are largely cross-reacting. Immunologic cross-reactivity is the result of the presence of widespread phylogenetically conserved proteins showing homologous epitopes. Thus, if the patient is sensitized to one single allergen source or to a limited number of allergen sources such defect of extract-based diagnosis has little or no influence on clinical decisions in terms of diagnostic precision and subsequent allergen specific immunotherapy prescription. In contrast, if the patient is sensitized to many allergen sources, for instance > 4 distinct pollens (3), the story is different, particularly in view of the fact that pollen seasons of different sources are frequently overlapping. It is possible to state that the main problem for the practical allergologist facing multi-sensitized patients is represented by the possible presence of a co-recognition of cross-reacting allergen proteins in distinct allergen sources and that his/her objective must be to identify the primary sensitizing allergens.

### Cross-reactivity: seasonal allergens

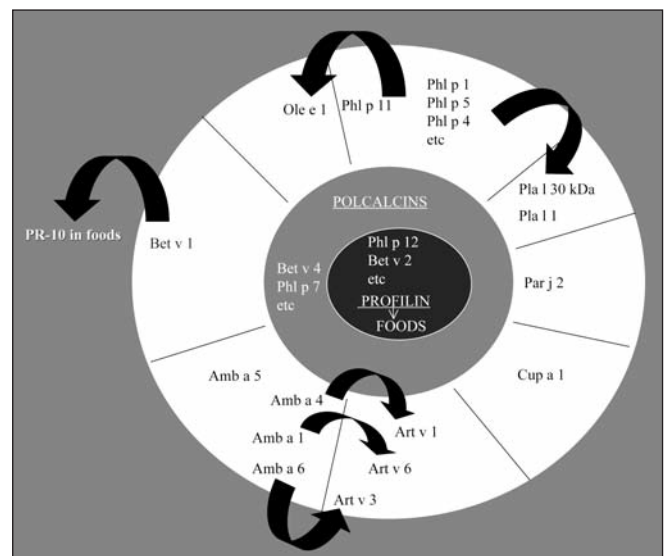
Among seasonal airborne allergens, the situation of possible cross-reactivity is more complex than one could figure out, as it involves not only the so-called pan-allergens (see beyond), but also allergen proteins present in single pollen families. Our current knowledge can be depicted as in figure 1.

#### *Cross-reactivity within pollen families and between different pollen families*

The external ring of the "target" is divided into sections each representing one of the main allergenic pollen families (*Graminaceae*, *Compositae*, *Ambrosia*, *Urticaceae*, *Plantaginaceae*, *Fagales*, *Oleaceae*, *Cupressaceae*). Within each family allergens are largely cross-reacting; thus, one single pollen species can be used as a representative of the whole

family. So, in our figure *Phleum* represents all grasses, birch represents all *Fagales* [hazel, oak, hornbeam, alder, beech], mugwort represents about 13.000 other members of the *Compositae* family [4]), cypress (*Cupressus arizonica*) represents other Cupressaceae (*Cryptomeria japonica*, *Thuja* spp, and *Juniperus* spp), olive represents also ash (*Fraxinus* spp) and privet (*Ligustrum vulgare*), and so on. Some exceptions exist to this rule: for instance, both mugwort and ragweed belong to the *Compositae* family but show distinct major allergens; as a consequence, they are separated in our figure. Similarly, the ragweed group includes both short and giant ragweed (*Ambrosia artemisiifolia* and *Ambrosia trifida*, respectively), although it has been shown that the two pollens are not completely overlapping in terms of allergenicity (5); thus, *Ambrosia artemisiifolia* has been chosen as the representative of the family as this is the main allergen present in northern Italy, but this might not hold true for all geographic areas. Most of the "representatives" contain several allergen proteins but in the external ring only those that can be considered as markers of genuine sensitization to a certain pollen species are shown. Within the external ring cross-reactivity may also occur

**Figure 1** - Image of a target summarizing the current knowledge about cross-reactivity within pollen allergens. The external ring is divided by pollen species; the main markers of genuine sensitization are shown. The middle ring includes polcalcins, Phl p 7 and Bet v 4 being the representative of this largely cross-reacting group of pan-allergens. The center of the target includes the profilin, Bet v 2 and Phl p 12 being the representatives of these plant pan-allergens. The arrows indicate possible cross-reactivities between pollen allergens other than pan-allergens.



between allergens present in different plant families. The best known example in this sense is the cross-reactivity between the major allergens of Fagales pollen, and homologous proteins (the so-called PR-10, pathogenesis-related proteins group 10) present in a number of plant-derived foods (see ref. 6 for a revision). But cross-reactivity has been described in other cases as well. For instance, it is well known from clinical practice that about 30% of grass pollen-allergic patients score positive on skin and in-vitro testing with olive pollen in areas where olive trees are virtually absent. Van Ree et al. found a cross-reactivity between a minor grass pollen allergen (the group 11 allergen), and the major olive pollen allergen, Ole e 1 that might explain this finding, particularly in areas where olive pollen is scarce or absent (7). Similarly, grass pollen-allergic patients are often reactive against plantain (*Plantago lanceolata*) whereas Plantain sensitization in the absence of grass pollen sensitization is extremely rare, and this has been ascribed to cross-reactivity phenomena (8). The cross-reactivity between ragweed and mugwort is a more complex story. Both plants belong to the same botanic family but their major allergens are distinct. However, the presence of common allergen structures other than profilin in mugwort and ragweed pollen had been observed as early as 14 years ago (9), and recently some degree of cross-reactivity between both major and minor allergens of ragweed and mugwort has been shown. Infact, homology between Amb a 6 and Art v 3 (10), Amb a 4 and Art v1 (11), and Amb a 1 and Art v 6 (12) has been observed. Notably, the IgE cross-recognition between Amb a 6 and Art v 3 appears to be unidirectional, as it occurs only in patients primarily sensitized to ragweed but not in those primarily sensitized to mugwort (10).

#### *Pollen pan-allergens: Polcalcins*

The middle ring of the target in our figure includes polcalcins (pollen calcium-binding proteins), a family of highly cross-reacting pollen pan-allergens. Calcium-binding proteins containing 2 EF-hands, 3 EF-hands, and 4 EF-hands are virtually present in pollen from all plant species. Patients sensitized to polcalcins invariably score positive on both SPT and in-vitro tests with virtually all pollen extracts. Phl p 7, the ryegrass polcalcin seems the most cross-reactive of the group (13). The 2 polcalcins presently available for diagnostic purposes (Phl p 7 and Bet v 4, the birch polcalcin) are excellent marker of sensitization to this group of proteins in subjects showing multiple pollen sensitization (14).

#### *Pollen pan-allergens: Profilin*

The central part of the target in figure 1 is occupied by another plant pan-allergen: profilin, a structural protein present in the cytoskeleton of all vegetable species, including pollens and plant-derived foods. Due to the high homology between profilins from different allergen sources, sensitized patients will score positive on both in-vitro and in-vivo tests with most pollen extracts and with a number of plant food extracts as well (15, 16). Possible exceptions are represented by *Parietaria* and cypress profilins, that seem to show a lower degree of homology with the other members of this protein family (17,18). Two profilins are presently available for routine component-resolved diagnosis: Phl p 12, a grass profilin, and Bet v 2, the birch profilin. They both are excellent markers of IgE hypersensitivity to the whole group of homologous proteins. Further, profilin hypersensitivity has been recently detected in-vivo by skin tests with a purified extract of date palm pollen profilin with excellent results (18).

#### *Detecting the primary sensitizing pollen in practice*

The external ring of the target tells us whether the patient is really polysensitized to different pollen sources. A growing number of recombinant or natural allergen molecules are presently available for in-vitro testing, and this has led to a revolution in the diagnosis of allergic diseases. However, it is possible to take advantage from the fact that most allergen sources contain one major allergen that scores invariably positive in primarily sensitized patients; this will allow to keep the number of detections to a minimum. Thus, Art v 1, Amb a 1, Par j 2, Bet v 1, Ole e 1, and Cup a 1 are markers of primary sensitization to mugwort, ragweed, pellitory, birch, olive, and cypress pollen, respectively, whereas the major plantain pollen allergen, Pla l 1, is still missing in available routine assays. Although grass pollen contains many genuine allergens, allergy in the absence of sensitization to the group 1 and/or group 5 allergens is an exceptional event (19, 20); thus, Phl p 1 and Phl p 5 used in parallel represent an excellent means to demonstrate primary grass pollen hypersensitivity.

In conclusion, it is possible to detect genuine sensitization to all the main pollen species by only 8 assays; such diagnostic workup along with clinical history, which remains the milestone of allergological evaluation, will lead to prescribe the correct immunotherapy even in the most complex cases of multi-sensitization.

## Cross-reactivity: mites, moulds & animal dander

### Moulds

At least two cross-reacting allergens, enolase and manganese superoxide dismutase, have been detected in moulds. The glycolytic enzyme enolase present in many moulds shows extensive cross-reactivity between *Cladosporium herbarum*, *Alternaria spp*, *Candida albicans*, *Aspergillus fumigatus*, *Penicillium citrinum*, *Fusarium solani*, and *Rhodotorula mucilaginosa* (6, 21). Manganese superoxide dismutase has been detected as a major allergen in *Aspergillus fumigatus*, and seems to cross-react to homologous enzymes in different prokaryotes and eukaryotes, including *Saccharomyces cerevisiae*, natural rubber latex, and even man (6).

Needless to say, mould-allergic patients sensitized to enolase will score positive on in-vivo and in-vitro test with extracts of virtually all moulds that are routinely assayed. However, as in the case of pollens, the detection of genuine markers of sensitization (Alt a 1 for *Alternaria spp*, Asp f 1 for *Aspergillus spp*, Cla h 8 for *Cladosporium herbarum*) will lead to a correct diagnosis, and to a correct immune treatment. On the other hand, *Alt a 6* from *Alternaria spp* will diagnose enolase sensitization (although this allergen is presently available only on the ISAC microarray platform and not yet on the ImmunoCAP).

### Mites

Mites contain several cross-reacting allergens. Group 1 mite allergens, the cysteine proteases Der p 1 and Der f 1, induce both species-specific and cross-reactive IgE antibodies (except with Blo t 1, the major allergen of *Blomia tropicalis*), whereas group 2 allergens, Der p 2 and Der f 2, cross-react with Eur m 2 from *Euroglyphus maynei*. Mite tropomyosin (Der p 10 and Der f 10), a highly conserved pan-allergen in invertebrates, cross-reacts with the homologous allergen in crustaceans, mollusks, worms (e.g. *Anisakis*), insects (e.g., cockroach), cephalopods and arthropods. There is a general consensus that the detection of IgE to group 1 and/or group 2 mite allergens is a marker of genuine mite sensitization. This data along with a clear clinical history will easily lead to the prescription of a proper allergen specific immunotherapy

### Animal dander

Serum albumins are minor allergens in mammals, including cat (Fel d 2), dog (Can f 3), cow, horse (Equ c 3), pig, and

rodents, but are largely cross-reacting (22-24). Patients sensitized to serum albumin may score positive for a number of different mammals on both SPT and in-vitro test (25). Also in this case, however, clinical history along with the detection of IgE to the major species-specific allergens (Fel d 1, Can f 1, etc) will easily lead to a correct diagnosis and to the choice of the right specific immunotherapy.

### Immunotherapy in multiple hypersensitivity and conclusion

Recent studies showed that most patients sensitized to pollen the pan-allergens profilin and/or polcalcin are also truly multi-sensitized (i.e., these subjects show both co-sensitization and co-recognition of seasonal respiratory allergens) (26). The effectiveness of allergen specific immunotherapy in multi-sensitized subjects has been questioned in the past. However, a randomized controlled trial on mountain cedar allergy performed > 20 years ago unequivocally showed that allergen SIT is equally effective in patients with single or multiple sensitization, provided that the allergen administered is the right one. We have now the right means to avoid diagnostic mistakes.

### Declaration of conflict of Interest

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