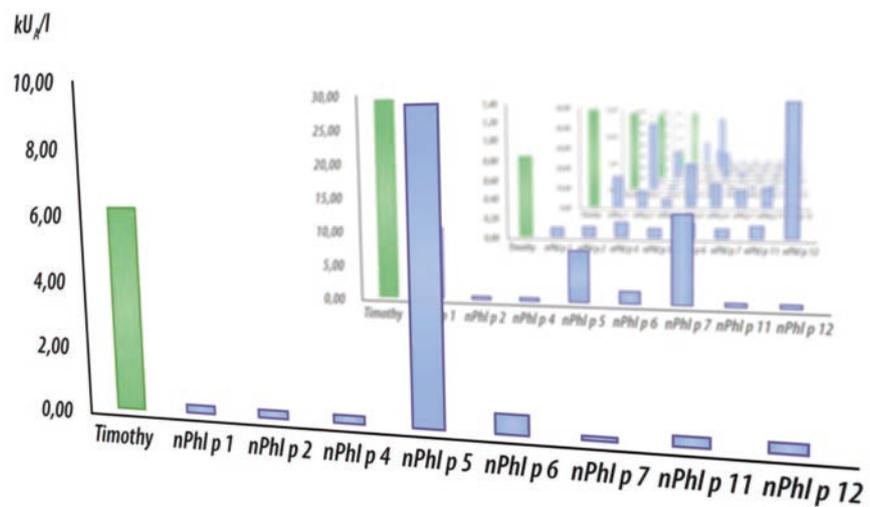# Journal

*Immuno*Diagnostics

Improving the diagnostic work-up of pollen allergic patients

For successful specific immunotherapy (SIT) treatment of pollen allergies the patient should be treated with an appropriate extract containing the actual symptom-triggering proteins in sufficient quantities. Molecular allergology (MA) helps to pin-point the actual allergenic molecule(s) responsible for the symptoms which is crucial for optimal treatment of patients. In this review, MA and its benefits in the work-up of patients indicated for SIT are described and recent publications on the subject are reviewed.

CAPture presents recently published articles on different risk markers in allergic diseases; ω-5-gliadin in wheat allergy, baseline serum tryptase in food allergy-induced anaphylaxis and inhalant allergen-specific IgE in asthma.

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SCIENTIFIC

The benefit of MA in diagnosing pollen allergies

This year's first issue of ImmunoDiagnostics Journal reveiws how molecular allergology can support the indication of specific immunotherapy treatment (SIT) of pollen allergic patients.

Approximately one third of people in the western world suffer from rhinitis which in many patients is triggered by pollen allergies. For these patients SIT represents the only way to modify the course of the disease. Unfortunately SIT is not always effective, sometimes due to inadequate diagnosis and sub-optimal choice of treatment.

For a correct decision on treatment and choice of extract it is vital to identify the actual disease-trigger(s). As trees, grass and weeds have overlapping pollen seasons in many geographical areas, and also since sensitization tests based on allergen extracts can give misleading results, conventional extract based testing is not sufficient. The more in-depth analysis obtained when using a molecular allergology based approach reveals the sensitization profile of the patient on the protein level, which together with the knowledge of the characteristics of these proteins improve the identification of patients likely to be helped by SIT treatment. An even stronger correlation between diagnostic findings and the therapeutic intervention could be achieved in future, if combined with a better description and standardization of the extracts that are used for the treatment.

I hope that the review will give you an overview of the benefits of MA in the work up of pollen allergic patients, some new insights and/or serve as a starting point for further reading.

CONTENTS

CAPture – A selection of recent allergy papers

SYNOPSIS

- Children (n=351) and adults (n=390) with current asthma were recruited.
- Specific IgE to house dusts mites, cockroach, cat, dog, molds (*Alternaria*, *Aspergillus*) and murine urine and total IgE were quantified using (ImmunoCAP®, Phadia Laboratory System, Thermo Fisher Scientific, Uppsala, Sweden) applying a cut off of 0.35 kU$_A$/l.
- Total and allergen specific IgE were log transformed for quantitative analyses.
- Dichotomous analysis: rat and cockroach sensitization in children correlated with an increased risk of ED visits, while in adults the same was shown for cat and mite sensitization.
- Analysis of sensitization as a continuous variable showed that rat, cockroach and *Aspergillus* in children, and mites and the sum of all sIgE measured in adults, was significantly associated with increased risk of asthma-evoked ED visits.

Citation: Arroyave WD et al. The relationship between a specific IgE level and asthma outcomes: Results from the 2005-2006 National Health and Nutrition Examination Survey.
J Allergy Clin Immunol In Practice. 2013; 1:501-8.

IgE levels to indoor allergens are risk markers of asthma emergency department visits and wheeze

This study investigates whether IgE-levels to nine indoor allergens are predictive of current asthma and emergency department (ED) visits in asthmatic subjects. Patients were recruited from the American NHANES study, and their IgE levels to indoor allergens were analyzed as continuous variables and related to the occurrence of wheeze and ED visits due to asthma.

Probability curves demonstrated that asthma ED visits were significantly associated with increasing levels of IgE to rat, cockroach and Aspergillus in children. In adults, house dust mite-specific IgE levels, as well as the sum of individual sIgE levels were strongly associated with increased asthma and ED visits. A significant albeit lower association of total IgE with ED visits was seen in adults.

Based on the predicted probabilities calculated a 10 kU$_A$/l increase in allergen-specific IgE gives odds ratios (OR) of 2.7 and 4.1 for ED visits in cockroach and rat sensitized children respectively. In adults, a similar increase in mite-specific IgE gives an OR of 3.1.

The authors conclude that the asthma outcomes measure correlate with increasing levels of specific IgE to indoor allergens. The rather high confidence intervals shown might be due to differences in allergen exposure since the sensitization was related to current clinical readouts.

SYNOPSIS

- Children with a doctor's diagnosis of wheat allergy and sensitized to wheat extract were recruited for oral wheat challenge. (n=24, median age 5 years).
- Serum IgE to wheat extract, ω-5 gliadin and gluten-derived hydrolyzed wheat proteins (Meripro) were measured by ImmunoCAP® with a detection limit of >0.1 kU$_A$/l.
- Twelve of the 24 patients (50%) passed the wheat challenge test.
- CD sens test with wheat extract was positive in 87.5% (n=21) of the cases.
- Children with positive challenge test had significantly higher sIgE levels to wheat extract (p<0.01), ω-5 gliadin (p<0.005) and HWP (p<0.005) than those negative in challenge.
- No patients with negative challenge test had ω-5 gliadin-sIgE above 0.21 kU$_A$/l.

Citation: Nilsson N et al. Combining analyses of basophil allergen threshold sensitivity, CD-sens, and IgE antibodies to hydrolyzed wheat, ω-5 gliadin and timothy grass enhances the prediction of wheat challenge outcome.
Int Arch Allergy Immunol. 2013;162:50-7.

IgE to ω-5 gliadin and HWP are markers for wheat allergy in children and adolescents as shown by oral wheat challenge test

Allergy to wheat grains is among the most common food allergies in early childhood. Allergic reactions to ingested wheat are caused by wheat-specific proteins; however, co-sensitization to grass pollen gives rise to positive wheat extract tests since wheat is a grass. The aim of the present study was to, in children with suspected wheat allergy, relate the outcome of oral wheat challenges, specific IgE-levels to ω-5 gliadin and hydrolyzed wheat proteins (HWP) and CD-sens results (a basophil activation test) to each other.

Using a decision point at >0.21 kU$_A$/l sIgE to ω-5 gliadin and/or >6 kU$_A$/l sIgE to HWP resulted in 100 % sensitivity and 91.7% specificity when comparing to the outcome of the oral wheat challenge test (calculated from presented data). No patients with negative challenge test had ω-5 gliadin-sIgE levels above 0.21 kU$_A$/l. CD-sens values showed a significant correlation with the level of wheat extract sIgE (r = 0.64) as well as sIgE to HWP (r = 0:59). However, a response in the CD-sens assay did not discriminate between positive and negative wheat challenge test since all assessable tests were positive. In conclusion, this study shows the importance of ω-5 gliadin-sIgE as a marker for wheat allergy in children and adolescents, and that sIgE to HWP also indicates wheat food allergy in this age group.

SYNOPSIS

- Food allergic children with proven sensitization, clear-cut clinical history and positive open challenge test were recruited (n=167, median age 2.5-3 years), as well as 113 age and gender matched healthy controls.
- Serum IgE to food allergens and baseline serum tryptase were measured by ImmunoCAP®.
- BST levels were significantly higher in food allergic children with a clinical history of anaphylaxis compared to healthy children.
- The BST levels were significantly higher in allergic children with moderate/severe reactions compared to children with no/mild reaction.
- Children with tree nut/peanut allergy had significantly higher baseline tryptase than milk/egg allergic children.

Citation: Sahiner UM et al. Serum basal tryptase may be a good marker for predicting the risk of anaphylaxis in children with food allergy.
Allergy 2014;69:265-8.

Elevated baseline serum tryptase levels are associated with higher risk of anaphylaxis in children with food allergy

Elevated levels of baseline serum tryptase (BST) in patients with *Hymenoptera* venom allergy is associated with high risk of anaphylaxis, and a reference level associated with systemic reaction has been suggested (11.4 ng/ml). As for food allergy, the relation between elevated BST levels and increased risk for anaphylaxis has not been studied.

The aim of the present study was to compare the BST levels in food allergic children with and without earlier anaphylactic reactions. BST was significantly (p=0.009) higher in food allergic children with a clinical history of anaphylaxis compared to healthy children. Furthermore, the BST levels were significantly higher in allergic children with moderate/severe reactions compared to children with no/mild reaction (p=0.004).

Cut offs of 5.7 ng/ml and 14.5 ng/ml were associated with 50% and 90% predicted probabilities for a clinical history of moderate to severe anaphylaxis, respectively. The BST level was significantly associated with a risk (OR 1.3) of moderate to severe anaphylaxis in food allergic children.

The authors suggest that an elevated baseline serum tryptase level may predict moderate/severe anaphylaxis in childhood food allergy.

Molecular allergology in diagnosis and management of pollen allergy

The only currently available, curative treatment for allergic rhinitis and asthma is specific immunotherapy (SIT). SIT treatment is, however, not always successful, and a reason for treatment failure is often an inadequate diagnosis of the allergy. To improve the accuracy of diagnosis in pollen allergic individuals, molecular allergology (MA) can be applied in the work-up. This approach provides a more comprehensive sensitization profile of the patient, which helps identify patients who are likely to benefit from SIT and to select the most appropriate treatment for each patient[1-4].

Pollen allergy, or pollinosis, is one of the most common chronic allergic conditions, with an estimated prevalence of up to 20% in European countries[5]. Several studies indicate that the incidence is increasing[6-9]; figures from the United States government for 2011 indicate that 16.9 million adults (7.3% of the population) were diagnosed with pollen allergy in the preceding 12 months[10, 11].

In many pollen allergic individuals with severe symptoms of allergic rhinitis, treatment with antihistamines or corticosteroids is insufficient to prevent a decline in health-related quality-of-life that is associated with poor work and educational performance[12-15]. It has been widely reported that individuals with seasonal allergic rhinitis have an increased probability of developing asthma[16-18]; rhinitis and asthma are now considered manifestations of the same disease according to the 'United Airways' hypothesis[19-21].

Specific immunotherapy (SIT; see box) is effective in treating pollen allergy[22-27] and is indicated when symptoms are severe and poorly controlled by optimal pharmacological treatment, and when accompanied by mild or moderate asthma[28, 29]. SIT has also been shown to prevent the progression of seasonal allergic rhinitis to asthma[30-34].

For SIT to be effective, individuals must be treated with sufficient amounts of the sensitizing allergen[35-37]. However, SIT is not effective in all patients, which often results from allergy misdiagnosis[38]. Traditionally, the sensitizing allergen is identified from the patient's allergenic case history, and skin prick tests (SPT) and *in vitro* specific IgE measurements using crude allergen extracts[1, 39]. However, extract-based tests provide no information on the nature of the molecules involved in the immunological recognition[40], and are insufficient to identify the primary sensitizing molecule(s)[1, 3]. Diagnostic tests with allergen extracts often indicate that a patient is sensitized to multiple allergen sources, which can reflect true co-sensitization to two or more allergen sources, or cross-reactive binding of IgE to very similar allergenic molecules present in the extracts[1, 3].

Specific immunotherapy (SIT)

SIT is a therapy for allergic diseases that consists in administering increasing doses of the sensitizing allergen with the aim of inducing immune tolerance. The most common route of allergen administration is by subcutaneous injection (SCIT); however, tablets containing allergen extracts for sublingual administration (SLIT) are approved in the European Union and North America and some other countries[18, 85]. SIT induces changes in humoral immunity, reducing the production of allergen-specific IgE and increasing production of specific IgG (mainly IgG4) from B cells. IgG4 is believed to act as a blocking antibody inhibiting IgE-facilitated allergen presentation and histamine release[73, 86, 87]. These effects are thought to depend on inhibition of allergen-specific Th2 responses, a switch from Th2- towards Th1-type responses, and induction of regulatory T-lymphocytes that produce IL–10 and TGF–β and down-regulate the pro-inflammatory effects of many immune cells[87]. SIT also reduces the allergen-specific inflammatory response, decreasing the number of infiltrating immune cells and production of inflammatory mediators at the site of allergen exposure[73]. These changes contribute to long-lasting immune tolerance that persists after SIT is discontinued [24, 26, 34, 88].

Allergen components in pollen

An extract of an allergen source, such as birch or timothy grass pollen, typically contains several allergenic molecules that are known as allergen components. These are often referred to as 'major' and 'minor' allergens, based on the proportion of atopic individuals who show sensitization to them (above or below 50% in a defined population)[41]. In most atopic individuals, the major allergen is the original sensitizing molecule and is responsible for producing the clinical symptoms[1, 37]. In pollinosis, the major allergens are 'specific' or marker allergens for the allergen source.

Minor allergens can also be specific markers for their allergen source. However, many belong to protein families that are widely present in the plant kingdom and are highly conserved across plant families. Therefore, they give rise to IgE antibodies that cross-react with similar proteins in related and also unrelated species. These allergens are often referred to as 'panallergens'[42-44] and in pollen the most important groups are profilins, polcalcins and carbohydrate cross-reactive determinants (CCDs) (see box).

Figure 1. *Profilins and polcalcins are panallergens commonly responsible for cross-reactivity between pollen that may confound extract-based test results.*

Highly cross-reactive allergens in pollen

Profilins: a family of small, highly conserved molecules with more than 75% sequence homology, even between members of distantly related organisms[89]. Profilins, such as Bet v 2 and Phl p 12 have a high degree of homology and are present in tree, grass and weed pollen and in plant-derived foods[42, 90]. Primary sensitization to profilin usually results from grass pollen exposure, and IgE cross-reactivity is associated with multiple pollen sensitizations in extract-based diagnostic tests.

Polcalcins: pollen-specific allergens from the calcium-binding protein family. Polcalcins are not believed to be involved in pollinosis-associated food allergies[91]. Timothy grass Phl p 7 is the most cross-reactive of the polcalcins[91].

Carbohydrate cross-reactive determinants (CCDs): carbohydrate moieties of plant and insect glycoproteins that are the most frequent epitope structures for IgE[92]. CCDs are widespread in pollens, foods and insect venoms and have highly conserved structures, which leads to a high degree of IgE cross-reactivity[92]. Cross-reactive IgE to CCDs can cause positive results to plant allergens of no apparent clinical significance in extract-based tests in pollen-sensitized patients[93-95].

Although specific components are marker molecules for a certain pollen species, some are also valid surrogate markers for other closely related pollens of the same plant family. For example, the major birch allergen component Bet v 1 can also be used to diagnose sensitization to alder. Over 90% of patients with sensitization to grass pollen have IgE against the specific components Phl p 1 and/or Phl p 5 from *Phleum pratense*[45, 46], but as these components have a high similarity between different grass species they can be used to diagnose sensitization to any other temperate grasses[46].

Diagnostic considerations

Diagnostic tests with allergen extracts containing both specific and cross-reactive allergens often indicate poly-sensitization to several different pollens[47-50]. Although cross-reactive components are rarely responsible for clinical symptoms, they can cause strong positive reactions in extract-based SPTs and *in vitro* specific IgE measurements[37], making it difficult to identify the allergen responsible for clinical symptoms and to distinguish between patients who are truly poly-sensitized to multiple allergen sources and positive reactions caused by cross-reactions[1, 3]. In some geographical areas, such as southern Europe, where pollen seasons occur in close succession and often overlap, sensitization to cross-reactive components makes identification of the primary sensitizing allergen particularly challenging[38, 51].

A molecular approach to allergy diagnosis

New diagnostic allergy tests that use purified or recombinant allergen components enable a detailed, quantitative evaluation of the patient's IgE antibody profile. This component-resolved diagnostic (CRD), or MA, approach measures the amount of specific IgE binding to purified natural or recombinant specific and cross-reactive allergen components in a solid-phase binding assay and can identify the individual molecules to which a patient is sensitized. Commercial assays can measure specific IgE binding to a single allergen component in one test, or simultaneously to multiple allergen components on a micro-array; results are interpreted using knowledge of allergen components' role as primary sensitizers or cross-reacting components of no, or limited, clinical significance[44, 52]. MA can therefore reveal if positive reactions in extract-based tests are due to true poly-sensitization or to cross-reactive components (Figure 2). Today, a large number of recombinant and purified specific and cross-reactive components of common tree, grass and weed pollens are available and can be used to obtain a detailed sensitization profile of pollen allergic patients[53] (see Table 1).

Table 1. *Characteristics of specific and cross-reactive pollen allergen components available for CRD.*

Characteristics	Tree pollen				Grass pollen		Weed pollen					Markers of cross-reactivity
	Olive (*Olea europaea*)	Birch (*Betula errucosa*)	Cypress (*Cupressus arizonica*)	Plane (*Platanus acerifolia*)	Timothy grass (*Phleum pratens*)	Bermuda (*Cynodon dactylon*)	Mugwort (*Artemisia vulgaris*)	Parietaria (*Parietaria judaica*)	Plantain (*Plantago lanceolata*)	Ragweed (*Ambrosia elation*)	Saltwort (*Salsola kall*)	All pollen species
Major specific components	rOle e 1	rBet v 1	nCup a 1*	rPla a 1	rPhl p 1 rPhl p 5b	nCyn d 1*	nArt v 1 nArt v 3	rPar j 2	rPla l 1	nAmb a 1	nSal k 1*	
Minor specific components	rOle e 7 rOle e 9	rBet v 6			nPhl p 2 nPhl p 4* rPhl p 6 rPhl p 11							
Minor, cross-reactive components		rBet v 2 (profilin) rBet v 4 (polcalcin)			rPhl p 12 (profilin) rPhl p 7 (polcalcin)							profilin polcalcin MUXF3 (CCD)

Purified components carrying CCDs that may bind CCD-reactive IgE antibodies not associated with allergic symptoms.
Bet v 1 is also a marker for sensitization to other trees of the *Fagales* family (hazel, alder, oak, beech and hornbeam).
Ole e 1 is also a marker allergen for the diagnosis of ash (*Fraxinus*) pollen allergy.
Phl p 1/Phl p 5 and Cyn d 1 may be used as a specific allergen component tests also for other temperate and tropical grasses, respectively.
Par j 2 displays only limited cross-reactivity with other pollen and food non-specific lipid transfer proteins.

MA can improve the selection of patients for SIT

SIT is an expensive treatment that typically lasts 3 to 5 years[35]. Correct identification of the primary sensitizing allergen(s) to enable selection of truly eligible patients is important for a successful treatment outcome and for cost-effective patient management. A post hoc simulation study calculated substantial cost-savings from the use of MA in the diagnostic work up of pollen allergic patients indicated for SIT[54]. In addition, patients who underwent SIT following diagnosis with SPT and MA were reported to have a better quality-of-life than those diagnosed using SPT alone, which resulted from more accurate and better targeted SIT prescription[55].

In a study of 746 patients who received SIT with birch or grass pollen, a third of patients experienced no improvement, or only moderate improvement in their condition(37). When investigators retrospectively examined the patients' IgE sensitization profiles using CRD, they found that 73% of those who were sensitized to major allergens reported good or very good improvement, compared with only 16% of those sensitized exclusively to minor allergens[37]. Other studies have also described heterogeneity in patients' IgE sensitization profiles to a given pollen in local populations, indicating that some patients are sensitized to both major and minor components and some to only minor components[45, 56]. Currently, SIT is performed using natural allergen extracts[1, 42, 57]. Although most commercial extracts are standardized for major allergen content, amounts of minor allergenic components can be very low or variable[58, 59]. Therefore, it is possible that patients sensitized only to minor components may not receive sufficient amounts of allergen for a successful treatment outcome[1, 37].

Several studies set out to assess whether data from CRD is a useful compliment to standard techniques for diagnosis and management of pollen allergy. These studies were carried out in Italy and Spain, where the abundance of grass, tree and weed pollens and frequently overlapping pollen seasons can make identification of the sensitizing allergen difficult[38, 60].

Letran and co-workers compared treatment decisions for SIT based on SPTs with *Olea europaea*, *Phleum pratense*, palm profilin, and peach peel extract with those based on CRD using nOle e 1, rPhl p 1-5b, rPhl p 12, rPhl p 7, and rPru p 3 in children and adults with seasonal pollen allergy. SPT-based SIT prescriptions were changed in 55% of patients when component level data from CRD became available. The most common change was from SIT with grass + olive pollen to grass pollen only; thus CRD was able to show that the double sensitization indicated by the SPT was not clinically relevant. In more than a third of patients who had their SIT prescription changed from grass + olive pollen to grass pollen, CRD revealed IgE binding to cross-reactive Phl p 12 and/or Phl p 7. The authors concluded that MA improved the selection of suitable patients for SIT in an area with overlapping pollen seasons[61].

Sastre and colleagues compared the SIT prescription based on results of SPT and MA diagnosis in Spanish adults with pollinosis and reported disagreement in 54% of patients. Disagreement levels ranged from 40% for a positive SPT to *Platanus* extract and positivity to the specific Pla a 1 and/or Pla a 2 allergens, to 16% for a positive SPT to grass extract and positivity to the specific
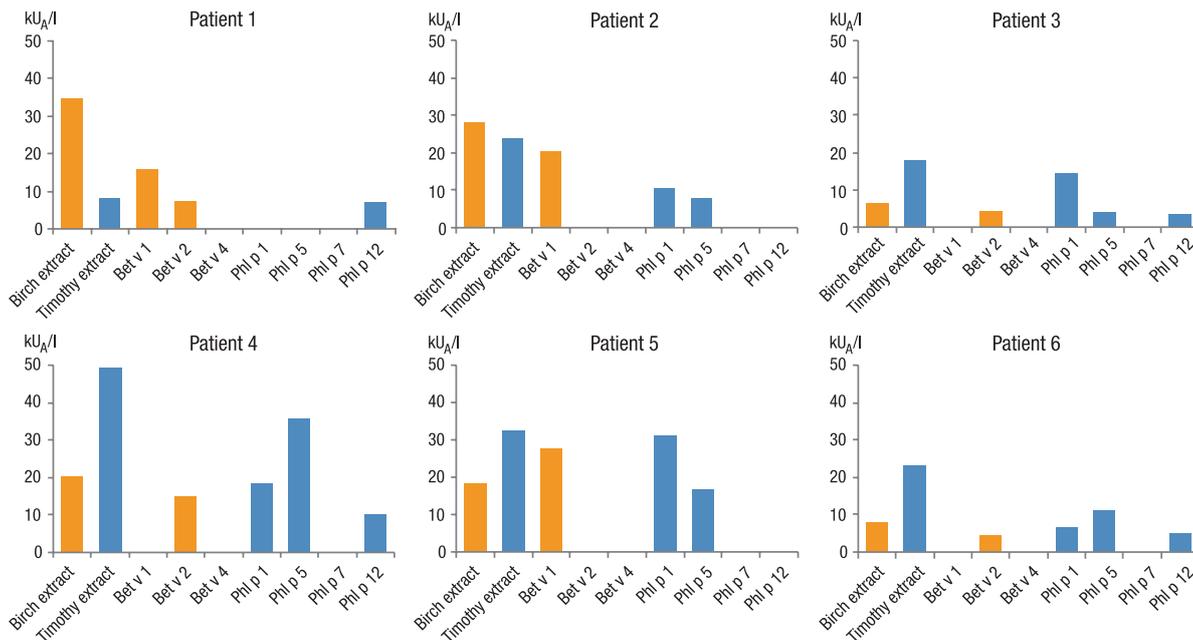
Figure 2. *MA reveals variations in patient sensitization profiles to birch (Betula verrucosa) and timothy (Phleum pratense) pollen. Levels of specific IgE antibodies to birch and timothy pollen extracts and components are shown in six pollen-allergic patients. Patient 1 is sensitized to the major birch allergen Bet v 1; cross-reactive binding of IgE to the profilins Bet v 2 and Phl p 12 is responsible for the low level of positivity to timothy extract. Patients 3, 4 and 6 are sensitized to the major timothy allergens Phl p 1 and Phl p 5; positivity to birch extract results from cross-reactive IgE binding to Bet v 2. Patients 2 and 5 are co-sensitized to the major allergens for both birch and timothy.*

Phl p 1 and/or Phl p 5 allergens, with SPT indicating false positive sensitizations. The investigators cited sensitization to cross-reactive components, such as profilin or polcalcin, as one possible reason for the poor agreement between methodologies and concluded that CRD was useful in facilitating accurate prescription of pollen immunotherapy, at least in areas of complex pollen sensitization[55].

Passalaqua assessed the utility of component-level data from an allergen microarray in patients who were prescribed SIT using standard methodology (clinical history, SPT, IgE assay) and found that MA provided additional relevant information on IgE cross-reactions in about 70% of patients. CRD revealed an absence of sensitization to profilins in a relevant proportion of patients with grass sensitization, indicating genuine poly-sensitization. The investigators assessed the relevance of the additional information as 'remarkable' in enabling a more confident diagnostic approach and management of disease in at least 90% of cases[39].

In an observational study that used MA to investigate the IgE sensitization profiles to Phleum pratense in Italian children with grass pollen allergy diagnosed by SPT, only 4% of patients had a sensitization profile that matched a molecularly designed SIT preparation containing Phl p 1, Phl p 2, Phl p 5a, Phl p 5b and Phl p 6. The investigators concluded that immunization was likely to be underpowered, due to sensitization to all components in

the SIT preparation and to additional ones; overpowered, due to sensitization to only some of the molecules contained in the SIT preparation; or both underpowered and overpowered in around two thirds of the population. In around 5% of patients, immunization would be unrelated to their sensitization, with a potential risk of developing new sensitizations to unrelated allergens in the SIT preparation[45].

Implications of MA for allergy diagnosis and treatment

The studies summarized above illustrate that by identifying the sensitizing allergens, CRD can complement results of SPT and extract-based specific IgE tests and can help the physician decide which sensitization to treat and to select a SIT extract from the most appropriate source(s) for the patient. In several studies, MA data revealed that poly-sensitization indicated by the SPT was, in fact, due to sensitization to cross-reactive components, which resulted in a change of the SIT prescription from multi- to mono-allergen immunotherapy. This has important implications for treatment, as recent reviews indicate that more supporting data is required to validate the efficacy of multi-allergen immunotherapy in poly-sensitized patients in routine clinical practice[62-64]. It is also possible that inclusion of additional allergen sources in the SIT preparation, which may not be responsible for the patient's symptoms, may dilute the concentration of relevant allergens to below that required for clinical effectiveness[65].

By eliminating false positive results caused by sensitization to cross-reactive components, CRD reduced the prescription of multi-allergen SIT in individuals with primary sensitization to only one allergen source, minimizing patients' exposure to high concentrations of unrelated allergens. Component-resolved immunotherapy (CRIT) with only clinically relevant purified or recombinant allergens has been proposed as a means of avoiding new sensitizations to components in crude SIT extracts[57]. CRIT with a mixture of recombinant Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, and Phl p 6 was shown to be safe and effective in grass pollen allergy in randomized, double-blind, placebo-controlled studies[66, 67]; however, recombinant allergens are currently not approved for immunotherapy in clinical practice[68].

MA can be used to monitor the efficacy of SIT

Current practice guidelines recommend that the patient's response to SIT be evaluated on a regular basis, with a decision about continuation of effective treatment made after 3 to 5 years of treatment[69]. However, less that 20% of patients who start SIT continue treatment for the minimum required duration of 3 years[70], with lack of effectiveness cited as one main reason for patients' discontinuation of sublingual immunotherapy (SLIT)[71, 72].

The efficacy of SIT treatment can be followed by monitoring the levels of allergen specific IgG4 and IgE antibodies (Figure 3). One of the mechanisms of action in SIT appears to be a block of IgE-mediated reactions by IgG4 antibodies[73-76]. Effective treatment with appropriate immunotherapy is accompanied by a significant increase in allergen-specific IgG4, which can be used as an objective marker of an immune response to therapy[77].

Significantly reduced symptom scores and, in most cases, a decrease in specific IgE antibody levels are seen in the later stages of treatment[73, 78, 79]. MA enables a detailed, quantitative follow-up of IgE and IgG4 responses to specific marker allergen components[56, 73, 74, 78, 80, 81] that facilitates evaluation of the immune response to treatment and adjustment of therapy, and may help improve compliance. The efficacy of SIT and the level and persistence of the increase in IgG4 antibodies is dependant on the sensitizing allergen dose; if the dose is too low, the therapy is less likely to be successful[74, 81]. It is therefore important that allergen extracts used for SIT contain sufficient amounts of all the allergen components that the patient is sensitized to. MA can also be used to evaluate the allergen content of an extract. In one study, MA was used to indirectly evaluate the components present in an extract of Phleum pratense by measuring increases in specific IgG4 in patients undergoing SIT. IgG4 levels to all allergen components except rPhl p 12 increased; suggesting that Phl p 12 was underrepresented in the extract[81].
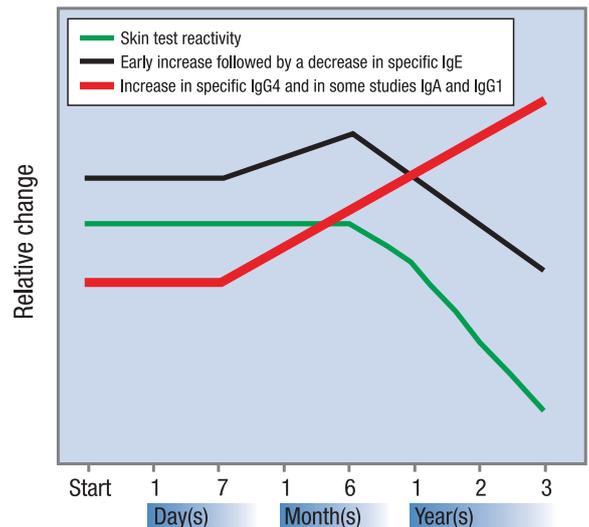
Figure 3. Typical immunological changes during the course of effective SIT treatment. A decrease in skin-prick test reactivity and improvement of clinical symptoms during SIT are accompanied by an increase in circulating levels of specific IgG4 and a late decrease in specific IgE. Using MA, changes in IgG4 and IgE can be used to monitor the efficacy of SIT treatment. Adapted from Akdis, 2011[73].

Pollen-food syndrome or primary allergy?

Pollen allergic individuals commonly react to various plant-derived foods, such as fruits, nuts and vegetables. MA helps to resolve whether these reactions are caused by IgE cross-reactions (pollen-food syndrome)[82-84], or by primary sensitization to plant food allergens, enabling the risk associated with the reactions to be assessed.

Conclusions

MA enhances the precision of allergy diagnostic testing. Routine use of CRD to compliment traditional diagnostic techniques could increase the success rate of SIT by guiding the physician in selecting candidates who are sensitized to the specific allergen components in commercial SIT preparations and who are most likely to benefit from treatment. In patients with an inconsistent allergenic case history who display poly-sensitization in allergen extract-based tests, CRD can distinguish true poly-sensitization from IgE cross-reactivity to cross-reactive allergen components, enabling the physician to select the most appropriate SIT vaccine or vaccines for the patient. CRD can also be used to monitor the efficacy of, and compliance with, SIT treatment. Improved diagnosis, treatment and patient monitoring could increase the overall success rate of SIT, which brings important benefits given the high cost and long duration of the treatment.

List of abbreviations

CCD	Carbohydrate cross-reactive determinant
CRD	Component-resolved diagnosis
CRIT	Component-resolved immunotherapy
IgE	Immunoglobulin E
IgG4	Immunoglobulin G4
IL–10	Interleukin 10
MA	Molecular allergology
SCIT	Subcutaneous immunotherapy
SIT	Specific immunotherapy
SLIT	Sublingual immunotherapy
SPT	Skin prick test
TGF–ß	Transforming growth factor-ß
Th1	T-helper type 1
Th2	T-helper type 2

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