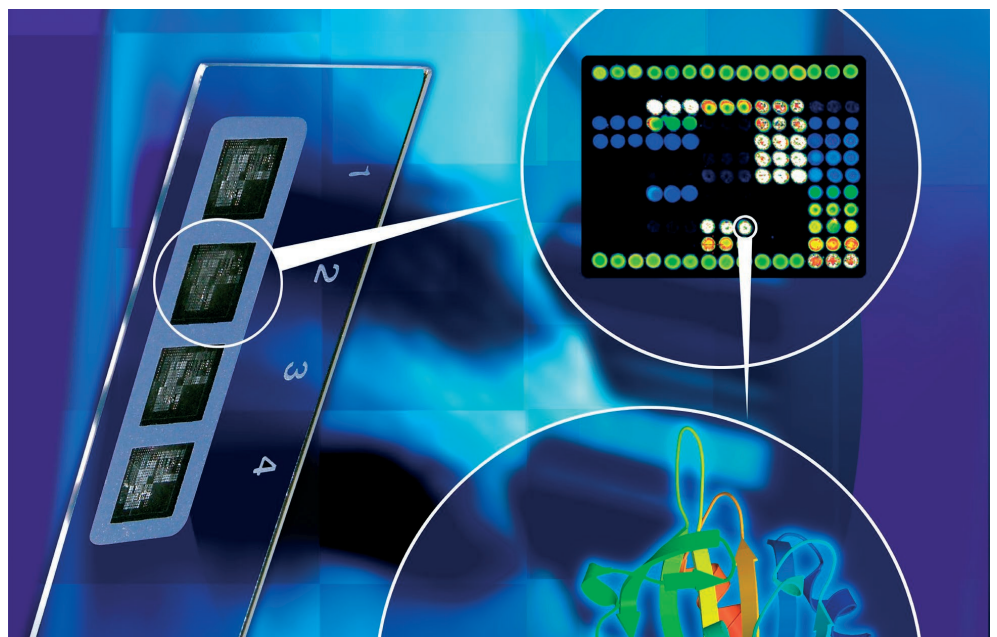


Journal

ImmunoDiagnostics

Utility of broad and detailed sensitization profiling

In this issue we present an overview of the many clinical applications of ImmunoCAP ISAC demonstrated since the microarray was first introduced more than a decade ago. The broad sensitization profile of a multitude of allergen components generated in one go helps the clinician to improve the diagnosis and risk assessment of allergic patients and helps in decisions on immunotherapy treatment. The ISAC platform has, and will continue to contribute considerably to the understanding of allergic diseases.



3 Clinical benefits of IgE antibody profiling with ImmunoCAP® ISAC

Multiplexed Allergen Components



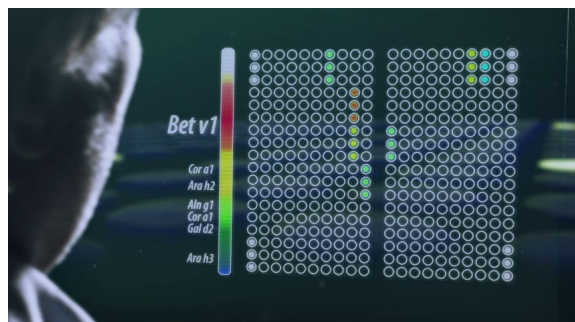
The use of component resolved diagnosis is clinical practice has increased considerably during the last years and will in the near future represent a standard tool for the allergist, as predicted in the consensus document on

Molecular Allergy diagnosis, published by WAO-ARIA GAL²EN (see ref 2).

The microarray has evolved over the years from the first prototypes carrying around 50 allergenic proteins up to now over a hundred recombinant and native allergen components. Since its introduction on the market an increasing number of allergist and other specialists make use of the broad and detailed sensitization profiles gained with only a droplet of blood. ISAC has been shown to provide clinically relevant results that facilitate accurate diagnosis and optimize patient management, and in particular multi-sensitized patients and patients with an inconsistent clinical history gain from the use of ISAC.

ImmunoCAP ISAC has also been widely used for the study of allergic diseases; studies demonstrating geographical diversity of sensitizations that correlate with different exposures, the molecular spreading of sensitizations within allergens and over different allergens with time during e.g. childhood have all used the microarray.

This collection of highlights of the literature on ImmunoCAP ISAC is by no means a complete list of references - there are many more out there!



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Clinical benefits of IgE antibody profiling with ImmunoCAP ISAC

Measuring specific IgE antibodies (sIgE) against allergen components provides valuable information on the clinical significance of sensitizations that cannot be obtained from allergen extracts. This component-resolved data facilitates diagnosis of allergic diseases and assists risk assessment in clinical practice. The information may also aid the clinician in prescription of specific immunotherapy (SIT) in patients with complex symptoms and sensitization patterns^(1, 2).

ImmunoCAP ISAC

The ImmunoCAP ISAC platform combines biochip technology and purified natural and recombinant allergen components in a miniaturized assay⁽³⁻⁵⁾. The results provide a snapshot of the patient's sensitization pattern, revealing both specific and cross-reactive sensitizations. An earlier version of the microarray contained 103 allergen components^(6, 7); the latest generation ISAC microarray contains 112 specific and cross-reactive allergen components including risk markers for food allergy, specific markers of pollen, mite, animal, mould, crustacean, and insect venoms, and markers of sensitization to cross-reactive carbohydrate determinants (CCDs) (Fig 1). In addition to the regular ISAC microarray, custom biochips with additional components have been produced for special purposes, for example the MeDALL biochip⁽⁸⁻¹⁰⁾.

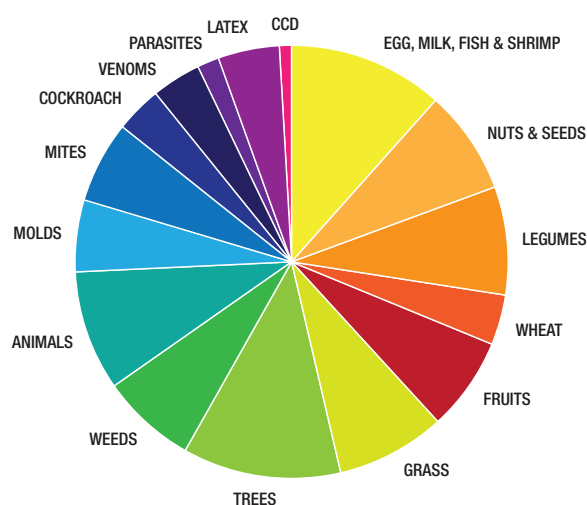


Figure 1. Representation by allergen source of the components on ImmunoCAP ISAC 112. The microarray includes species specific components as well as the most relevant cross-reacting components chosen to provide information on hundreds of allergen sources.

“ Poly-sensitized pediatric and adult patients in whom sensitization to cross-reacting allergens is suspected are most suited for ISAC testing, especially when both food and airborne allergens are involved.”

Consensus document on Molecular-based Allergy Diagnostics⁽²⁾
 Canonica et al, WAO - ARIA - GA²LEN

The performance, both technical and clinical, of ISAC has been validated in several studies^(2, 6-8, 11-15); the microarray offers several advantages over singleplex ImmunoCAP component tests, visualizing the patient's sensitization profile in a cost-efficient manner by simultaneous testing against a broad panel of allergen components. The Xplain software is available to aid in the interpretation of the 112 component results, making the ISAC information more accessible to clinicians.

This review gives an overview of clinical applications of the ISAC platform in the diagnosis and risk assessment of allergic patients, as well as its usefulness in studies of allergic diseases.

ISAC for broad sensitization profiling

The comprehensive IgE antibody profile generated with ISAC makes the microarray particularly well suited for studying component-specific IgE responses in multi-sensitized patients. Studies that used ISAC in patients with inhalation allergies have pinpointed geographical differences in specific sensitizations and helped elucidate cross-reactive sensitizations⁽¹⁶⁻¹⁹⁾. As an example, frequent unexpected sensitizations to cypress, olive and plane tree (Cup a 1, Cry j 1, Ole e 1, Pla a 1, and Pla a 2) but low frequency of sensitization to panallergens were shown in a central European population sensitized to pollen⁽¹⁹⁾. Some of these results were likely due to cross-reactive carbohydrate epitopes on these native purified components. In these situations the Xplain software may help to interpret the possible impact of antibodies to CCDs. In contrast with these findings, patients with rhinitis and asthma from northwest Italy showed frequent co-sensitization to panallergens, mainly profilins and PR-10⁽¹⁷⁾.

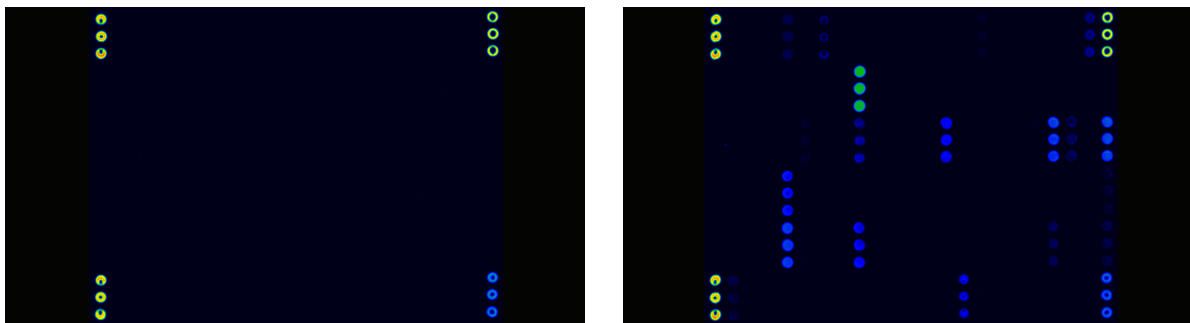


Figure 2. ISAC is as valuable to rule out sensitizations, as it is to profile multi-sensitized patients. To the left a microarray from a non-sensitized patient and to the right a chip from a multi sensitized patient.

ISAC has shown that food sensitizations are common in patients with respiratory allergies and vice versa. In poly-sensitized Italian patients with respiratory symptoms, the majority (58%) had sIgE to food-specific allergens, and more than half were sensitized to lipid transfer proteins (LTPs), while 8% were sensitized to seed storage proteins⁽¹⁵⁾. ISAC provided additional relevant information on IgE cross-reactions in around 70% of poly-sensitized patients with respiratory symptoms, enabling a more confident diagnosis and therapeutic approach⁽²⁰⁾. In over 90% of these cases, information such as sIgE to components possibly associated with food allergy, unexpected sensitization to profilins, LTPs, and potential triggers of oral allergy syndrome (OAS) were judged to be of special clinical relevance and improved disease management⁽²⁰⁾.

Other studies identified profilin as a severe food allergen in allergic patients overexposed to grass pollen⁽²¹⁾, and showed that lupine sensitization in bakers may be caused by cross-reactivity with LTP, profilin and CCDs in wheat flour⁽²²⁾. A Belgian study showed no significant differences in PR-10 sensitization profiles between birch pollen-allergic patients with and without OAS symptoms. Interestingly, however, the broad profile from ISAC testing indicated that the patients who did not experience OAS were the ones with a broader sensitization to several allergen components, including perennial allergens such as mite, cat, dog and molds and were also more frequently sensitized to profilins⁽²³⁾.

Poly-sensitizations and asthma risk

The relationship between multiple IgE sensitizations and asthma has been studied with ISAC. Prevalence of asthma, FENO levels, and bronchial responsiveness increased with the number of sensitizations to perennial, pollen, and food allergens, with co-sensitization to food allergens signaling an increased risk of asthma and airway inflammation in pollen-sensitized individuals⁽²⁴⁾. Microarray data refined the severity assessment in Swedish children who were allergic to furry animals. In severely asthmatic schoolchildren, multi-

sensitization to lipocalins (Mus m 1, Equ c 1, Fel d 4, Can f 1 and 2), kallikrein (Can f 5) and secretoglobin (Fel d 1) from furry animals was associated with increased bronchial inflammation, suggesting that sensitization to several lipocalins from cat, horse and mouse could aggravate asthma symptoms, with a higher number of sensitizing lipocalins increasing the likelihood for severe asthma⁽²⁵⁾. Children with severe asthma were more likely to be multi-sensitized to three or more lipocalin components, and to have higher sIgE levels to cat, dog and horse and a more complex IgE antibody profile indicating molecular spreading of sensitization to allergen components⁽²⁶⁾. In a population-based study, asthma in schoolchildren was strongly associated with co-sensitization to Fel d 1/Fel d 4 and Can f 1/Can f 2/ Can f 5 in cat- and dog-sensitized children, respectively⁽²⁷⁾.

Atopic dermatitis

Patients with atopic dermatitis (AD) have a particularly high risk of food allergy⁽²⁸⁾ and often have high total IgE (tIgE) levels, which sometimes make it difficult to distinguish between specific and non-specific sensitizations. ISAC has been validated in patients with AD⁽¹²⁾ and can improve detection of sIgE antibodies, as high tIgE levels do not affect these measurements.

ISAC-detected sIgE antibodies to milk and egg components and to peanut storage protein components (Ara h 1, Ara h 2, Ara h 3, Ara h 6) correlated well with patient-reported food reactions, and the odds of symptomatic AD increased significantly with the number of detectable components to a particular food⁽²⁹⁾. The number of sIgE sensitizations paralleled tIgE levels, indicating that allergen-specific sensitizations may go undetected in atopic individuals with high tIgE titers if a wide range of allergen components is not tested⁽³⁰⁾. The importance of broad testing was underlined by microarray data showing that patients with AD were frequently sensitized to allergens not routinely included in currently established sIgE screening panels, such as *S. aureus* exotoxins, *A. alternata*, and hazelnut allergens⁽³⁰⁾. Sensitization to

profilins, tropomyosins, and PR-10 proteins were possibly indicative of more severe disease in children⁽³⁰⁾, whereas in adults, sIgE to cow's milk allergen components, but not PR-10, was predictive of severe AD⁽³¹⁾. Given the large number of potential triggers or worsening factors in AD, negative results for the whole array of allergens on the ISAC microarray give good indications to exclude atopic involvement in the disease. Such comprehensive testing cannot easily be achieved with skin prick testing (SPT) or singleplex sIgE tests⁽³²⁾.

Eosinophilic esophagitis

Children with eosinophilic esophagitis (EoE) are very often poly-sensitized to both aeroallergens and foods^(33, 34); however, the relationship between symptoms and sensitization is not straightforward. The role of pollen-food allergy syndrome in EoE has been investigated using ISAC⁽³⁵⁾. Whereas children were more frequently sensitized to food allergens⁽³⁶⁾, adults were most commonly

sensitized to aeroallergens, notably profilins⁽³⁷⁾. In adults, food sensitizations were often caused by PR-10 cross-reactivity following primary sensitization to birch pollen⁽³⁸⁾.

Investigating clinical allergy phenotypes

Several studies have shown that results from ISAC are comparable to those of single analyte assays⁽³⁹⁻⁴³⁾. ISAC can be integrated with traditional sIgE measurements, and by the improved resolution of sensitizations can aid in patient risk assessment by reducing the need for challenge testing and informing on the degree of risk in a challenge situation.

The six peanut components present on the ISAC microarray (Ara h 1–3, Ara h 6, Ara h 8, and Ara h 9) could distinguish the majority of peanut-allergic patients from non-atopic controls⁽³⁹⁾. Most patients with clinical

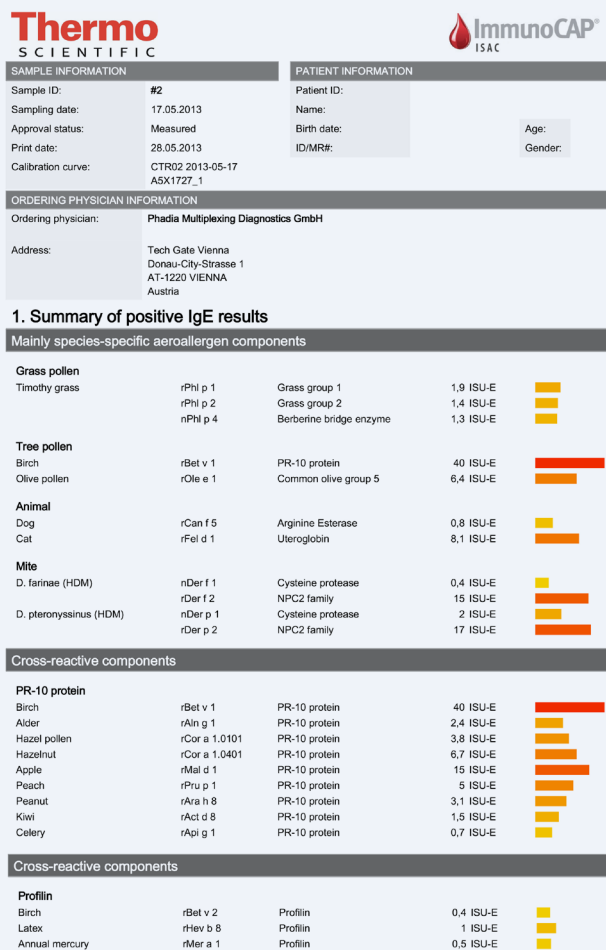
Case description:

Robert, a man from northern Europe and in his mid-thirties, suffers from rather severe hay fever during spring and early summer, but he does not have asthma. When he eats fruit such as apples, pears, plums and cherries he gets swollen lips and throat, although he considers himself fortunate not to react to peanuts, which he loves.

Many years back he started to react with runny nose and sneezing in the presence of cats and when he found himself in humid indoor environments. Lately, he has started sneezing in his home also during the winter, and has tried to understand what the cause of this reaction is.

Robert uses anti-histamines during spring, but now that his reactions appear also in winter he consults an allergist, who decides to run an ISAC test. The results show that he has primary sensitizations to both indoor allergens – mite, cats and dogs – and to birch and grass pollen. His broad cross-reactive sensitization to PR-10 proteins explains his pollen-food reactions. He is however, not sensitized to any storage proteins from nuts or seeds, neither to LTP proteins, which indicate a low level of risk for severe reactions.

Surprisingly, the data reveals sIgE to Ole e 1, a specific allergen in olive pollen. This is rather unusual living in Britain, but could be a cross-reaction from a primary ash sensitization. When discussing his test results with the allergist, he suddenly understands why he gets allergic reactions at home – his wife has a number of small olive plants that she brings indoors during winter!



reactions were sensitized to Ara h 2 and Ara h 6^(39, 40, 44, 45). The sensitization profile indicated the probability of a severe allergic reaction, with sIgE to Ara h 1, Ara h 2, Ara h 3 and Ara h 6, in particular Ara h 2^(44, 45) and Ara h 3⁽⁴⁵⁾, more prevalent among patients with a history of anaphylaxis⁽³⁹⁾. The microarray revealed that some patients sensitized to complete peanut extract had no IgE reactivity to the genuine peanut components but were sensitized to Ara h 8, profilin or CCDs⁽³⁹⁾.

sIgE to the latex components Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 on the ISAC microarray was indicative of symptomatic latex allergy. Sensitization to any one of these components indicated latex allergy with almost no false-positive results; sensitization to latex profilin (Hev b 8) and/or CCDs only, enabled discrimination between mere sensitization and genuine allergy^(42, 46). Most patients mono-sensitized to Hev b 8 did not suffer any clinical symptoms to latex⁽⁴⁶⁾.

Discovering unexpected sensitizations

ISAC is a useful tool to identify unexpected causes of allergic symptoms. An interesting example of this is the use of retrospective ISAC testing to reveal the cause of winter rhinoconjunctivitis in a group of Swiss children. In 1986, the children had tested negative for perennial allergens and were asymptomatic; analysis of sera taken at this early timepoint showed that none of the children were sensitized to the main alder tree allergen Aln g 1. However, by 2006, all those who developed winter rhinoconjunctivitis were sensitized to Aln g 1, but not to other tree pollens. This coincided with the planting of a Japanese variant of alder trees in the local area where the children passed on their

way to school. These trees had a winter pollination season, which explained the unusual seasonal symptoms; this exposure/symptom connection would have been difficult to reveal without ISAC⁽⁴⁷⁾.

Causes of co-factor-dependent food allergy

In many cases of co-factor-dependent food allergy (CDFA) it is challenging to identify the underlying sensitization from the clinical history. Using ISAC to retrospectively study 74 cases of CDFA in northern Spain, Cardona and colleagues observed that 92% of patients were sensitized to LTPs (Pru p 3)⁽⁴⁸⁾. The importance of LTPs and absence of sensitization to other plant-food panallergens in CDFA was also demonstrated by ISAC in patients from the same region who had a complex clinical history and multiple sensitizations to plant-foods and pollens⁽⁴⁹⁾. Similarly, in an Italian cohort with food-dependent, exercise-induced anaphylaxis, microarray data showed that almost 80% were sensitized to Pru p 3⁽⁵⁰⁾. Nonetheless, allergenic components other than LTP can be responsible for symptoms in CDFA, highlighting the importance of complete screening to thoroughly evaluate different sensitizations and help avoid episodes of anaphylaxis.

Idiopathic anaphylaxis

Allergen avoidance is not an option in idiopathic anaphylaxis (IA), since the triggering allergen(s) is unknown. However, the broad screening offered by ISAC can identify unforeseen sensitizations that present a risk of severe reactions, providing additional, clinically important information that is not available from routine allergy work-up. In a UK study, ISAC identified hitherto unknown sensitizations that were highly likely to cause anaphylaxis,

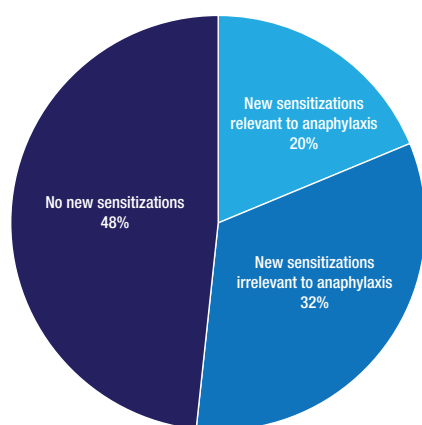


Figure 3a. Heaps et al showed that the microarray results could help identify anaphylaxis trigger in 22/110 of patients. In 32 % of the cases new sensitizations were identified although not thought to contribute to the anaphylaxis. Redrawn from⁵².

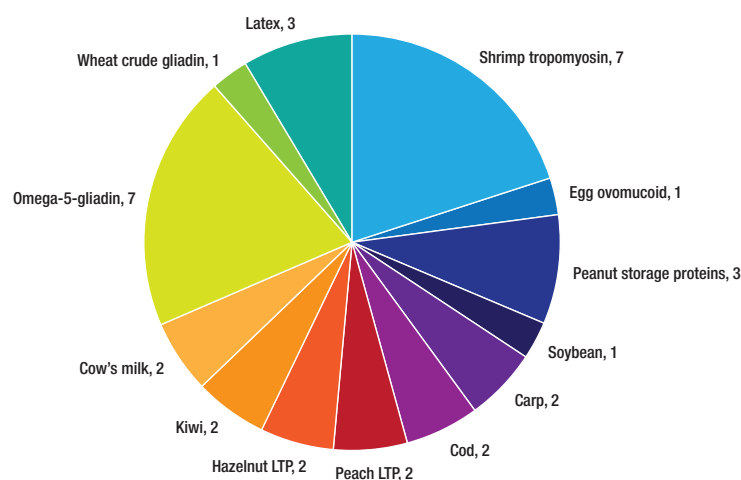


Figure 3b. Distribution of novel specific sensitizations considered highly relevant to the anaphylactic event in 22 patients. Number of patients positive for each component shown. Redrawn from⁵².



most commonly to wheat omega-5-gliadin and shrimp components, in up to 30% of patients with IA^(51, 52). (Fig 3). Completely negative ISAC tests have also been used to rule out IgE-mediated etiology in some patients with IA (personal communication Prof. Moneret-Vautrin).

Work-up of specific immunotherapy patients

Many pollen-allergic patients are multi-sensitized - the wide range of specific and cross-reactive components on the ISAC chip can identify true co- or multi-sensitization and improve the specificity of the SIT prescription⁽²⁾. In an area of Spain with overlapping pollen seasons, microarray data identified sensitizations to cross-reactive components of timothy grass and olive tree that were responsible for clinically irrelevant SPT results. This component level data modified the SPT-based SIT prescription in more than half of the study population⁽⁵³⁾. In another area of Spain with complex pollen exposure, ISAC testing improved SIT prescription in 54% of the study population by revealing false negative extract test results, most commonly to plane (40%) and grass extract (16%), that were identified by positivity to Pla a 1 and/or Pla a 2, and Phl p 1 and/or Phl p 5, respectively⁽⁵⁴⁾.

Using only 8 allergen components, (Art v 1, Amb a 1, Par j 2, Bet v 1, Ole e 1, Cup a 1, Phl p 1 and Phl p 5), all of which are present on the ISAC microarray, it is possible to detect genuine sensitization to several main pollen species. Together with clinical history, diagnostic work-up with ISAC therefore enables the clinician to improve SIT prescriptions even in the most complex cases of multi-sensitization⁽⁵⁵⁾.

Changes in sIgE levels to specific allergen components can be used as a surrogate marker for the clinical effects of SIT. In one study, a decrease in ISAC-measured sIgE to Bet v 1 in patients receiving birch pollen SIT was associated with a strong increase in Bet v 1-specific IgG. This apparent decrease in sIgE to Bet v 1 could be explained

by the blocking of IgE binding by therapy-induced Bet v 1 sIgG antibodies. Decrease of ISAC sIgE binding was a predictor of clinical improvement and was useful to monitor the development of allergen-specific IgG responses to specific and cross-reactive allergens during SIT⁽⁵⁶⁾.

Improving the understanding of allergic diseases

The broad range of allergens on the ISAC microarray make it highly suitable for investigating the evolution of allergic sensitizations in order to understand the development of symptomatic allergy. In many allergies, first sensitizations occur during infancy - the minimally invasive blood sampling required for ISAC make it well suited for use in young children, where a large number of SPT or sIgE assays are not possible.

Investigating the allergic march and assessing the risk for symptomatic allergy

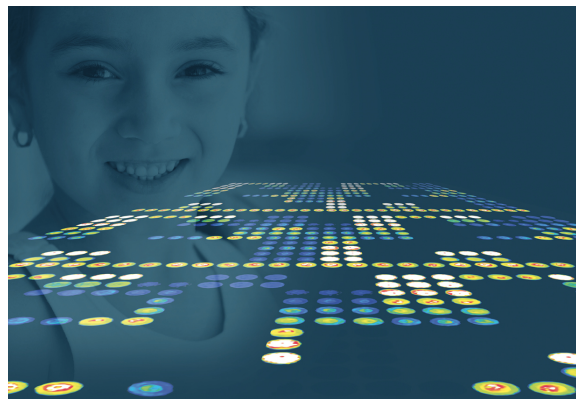
Testing for sIgE sensitization at the preclinical stage may predict the onset of seasonal allergic rhinitis (AR). Data obtained from the Swedish BAMSE cohort showed that IgE reactivity to PR-10 proteins at 8 and 16 years of age could predict the presence and severity of birch pollen AR at age 16. A high level of sIgE to Bet v 1 or sensitivity to many PR-10 proteins at 4 years of age was associated with increased incidence and persistence of AR up to age 16⁽⁹⁾. Sensitization to grass components at 3 years of age predicted the onset of seasonal AR at age 12⁽⁵⁷⁾.

Prospective studies using ISAC have shown spreading of IgE sensitization to other components of the same allergen source during childhood^(8, 58). The IgE response to grass pollen preceded allergic symptoms by several years, starting as a weak mono- or oligo-sensitization that increased in complexity during the preclinical and early stages of AR⁽⁵⁷⁾. Microarray data showed that the order of sensitization to grass pollen components in children

“ One of the most important implications of molecular-based allergy diagnosis is its ability to distinguish genuine sensitization from sensitization due to cross-reactivity. This information helps the clinician to determine whether a single, a few closely related, or several widely different allergen sources need to be considered.”

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Canonica et al, WAO - ARIA - GA²LEN



typically started with mono-sensitization to Phl p 1, spreading to Phl p 4 and Phl p 5; then Phl p 2, Phl p 6, and Phl p 11; and finally to Phl p 12 and Phl p 7⁽⁵⁷⁾.

Analysis of microarray data from the Manchester Asthma and Allergy Study identified sIgE patterns that could predict different allergic diseases⁽⁵⁹⁾. Allergen components to which patients showed an IgE response fell into three major groups. Children sensitized to components in group 1, comprising plant allergens, were more than 12 times more likely to have pollen allergy, but not asthma or wheeze; those positive to group 2 components, comprising predominantly dust mite allergens, were 3 times more likely to have asthma and twice as likely to have pollen allergy; and positivity to group 3, containing components from a broad range of protein families, was most strongly associated with asthma⁽⁶⁰⁾.

A retrospective comparison of ISAC sIgE profiles with clinical history, SPT results, and diagnosed conditions from infancy through to adulthood showed that sIgE to peanut, soy, fish, nuts, wheat, and mite components could be detected well before clinical reactions were reported, and an increase in tIgE was associated with molecular spreading in nearly all children evaluated⁽⁵⁸⁾.

This highlights the usefulness of early broad profile testing. Melioli reported that the sum of sIgE to components on the microarray closely paralleled the levels of sIgE to the corresponding allergen extracts and tIgE, with the evolution of sensitizations closely reflecting the clinical characteristics of the allergic march. Milk and egg were the most frequent sensitizations in early childhood; sensitizations to plant allergens occurred later, with sIgE to cross-reacting allergens appearing after 6 years of age⁶¹.

Conclusions

The usefulness of ISAC has been demonstrated in a wide range of allergic diseases. The technique gives clinicians a broad picture of a patient's sensitization profile from a single test, and provides information on specific and cross-reactive sensitizations that facilitate diagnosis, risk assessment, and disease management. The broad range of allergens on the microarray can reveal unexpected sensitizations to allergens that are not routinely tested and can underlie symptoms or carry a risk of severe reactions. Component resolved data can improve the selection of patients and relevant allergens for SIT compared with extract testing and can indicate the response to treatment. In investigational studies, ISAC has provided valuable insight into the allergic march and molecular spreading in the preclinical stages of allergic diseases, indicating likelihood of developing symptomatic allergy. The small amount of serum required and the large amount of information gleaned from a single test makes it well suited for use in young children as well as in adults.

ISAC provides clinically relevant test results that facilitate accurate diagnosis and optimize patient management, particularly when the patient has an inconsistent clinical history, is multi-sensitized, or shows an unsatisfactory response to SIT.

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