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Multiplex component-based allergen microarray in recent clinical studies

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Keywords: IgE, ImmunoCAP ISAC, CRD, multiplex, microarray, allergen components, allergic disease, asthma, rhinitis, food allergy, eczema

Abbreviations:

AD: atopic dermatitis

AR: allergic rhinitis

CCDs: cross-reactive carbohydrate determinants

CRD: component resolved diagnosis

EoE: eosinophilic esophagitis

FeNO: Fraction of exhaled nitric oxide

IA: idiopathic anaphylaxis

IgE: Immunoglobulin E

ISAC : ImmunoCAP ISAC® 112 or earlier versions of this microarray

LTP: Lipid transfer protein

OAS: oral allergy syndrome

SPT: skin prick testing

sIgE: specific IgE

SIT: specific immunotherapy

tlgE:total IgE

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Background

Due to the high prevalence of allergic diseases globally [1] there are increasing demands in clinical practice for managing these IgE-mediated conditions [2, 3]. Traditionally, diagnosis relied on clinical history and sensitization that was demonstrated through an allergy test based on allergen extracts [4]. During the last decade component-resolved diagnostics has been introduced into the field of clinical allergology, providing information that cannot be obtained from extract-based tests. Component-resolved data facilitate more precise diagnosis of allergic diseases and identify sensitizations attributable to cross-reactivity [5-8]. Furthermore it assists risk assessment in clinical practice as sensitization to some allergenic molecules is related to persistence of clinical symptoms and systemic rather than local reactions [9]. The information may also aid the clinician in prescription of specific immunotherapy (SIT) in patients with complex symptoms and sensitization patterns, [5, 9] and in giving advice on targeted exposure reduction or on the need to perform food challenges [10].

The detection of IgE antibodies against allergenic molecules is feasible either using singleplex (one assay per sample) or multiplex (multiple assays per sample) measurement platforms [9]. The most commonly used and documented microarray for IgE antibody measurement is the ImmunoCAP® ISAC. Another microarray platform (Microtest®) recently became available and shows comparable results to ISAC according to the manufacturers, a finding that needs to be further evaluated [11].

The ISAC platform combines biochip technology and purified natural and recombinant allergen components in a miniaturized assay [12-14] and gives semiquantitative results (ISAC standardized units/ISU). An earlier version of the microarray could simultaneously analyse sensitization to 103 allergen components [15, 16]; 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, as well as markers of sensitization to cross-reactive carbohydrate determinants (CCDs). In addition to the CE-marked ISAC microarray, customized biochips with additional components have been produced for special purposes, for example the MeDALL biochip [17-19].

One disadvantage with the commercially available multiplexed immunoassay is the pre-defined set of allergen components. Some test results might therefore be irrelevant for the actual needs of their patients, and in addition, this rigidity does not allow testing for the most novel allergen components. A new and more 'flexible' multiplex system based on naked fluorescent micro-beads that is customized to test for specific IgE to allergen components has been shown to give comparable results to ISAC [20]. These arrays could represent alternatives in allergy diagnosis in the future, but to date there is very little data on their performance.

Peptide microarrays used for IgE epitope mapping are additional tools in diagnosing and predicting allergies. Although this method is still under development and evaluation [21] epitope mapping has the potential to reveal important information on antigen structure and the unique response of individual patients and could be applied for manufacturing safer immunotherapeutic agents [22-24].

The performance, both technical and clinical, of ISAC has been validated in several studies [9, 15-17, 25-29]. This microarray offers several potential advantages over singleplex component tests by visualizing the patient's sensitization profile against a broad panel of allergen components. The assay requires only 30 microliters of serum or plasma favouring its use in pediatric patients, because such a minute volume of serum can be obtained from a capillary blood sample. Furthermore, the microarray enables investigation of allergic patients with a complex symptomatology, such as severe eczema, unstable asthma, and chronic urticaria in a more cost-efficient manner than singleplex tests [4, 9].

This review gives an overview of recent studies outlining the clinical applications of multiplexing in aiding the diagnosis and risk assessment of allergic patients, as well as its usefulness in studies of allergic diseases (Table 1).

Microarray for broad sensitization profiling

The detailed IgE antibody profile generated with a component based microarray makes ImmunoCAP ISAC well suited for use in multi-sensitized patients. Applied on patients with inhalation allergies it has helped in pinpointing geographical heterogeneity in specific sensitizations and elucidating cross-reactive sensitizations [30-33]. This is of importance in the clinical practice for risk assessment and further treatment of these patients. For example,

effective immunotherapy treatment targeting Bet v 1 will not benefit birch sensitized individuals without sensitization to the major pollen allergen Bet v 1 [34], which can be a considerable group in some regions [35]. Similarly, in southern Spain, sensitization to minor allergens from olive tree (Ole e 7 and Ole e 9) [36] is more common than sensitization to the major allergen Ole e 1 leading to unsatisfactory results when using therapy targeting Ole e 1. Immunotherapy with single recombinant molecules is still under development, but CRD improves also extract based immunotherapy through improved identification of the primary sensitizing source which supports correct selection of extract to use in allergen immunotherapy.

The microarray technique can contribute to a better characterization of pollen sensitized patients as the majority of these are not monosensitized [37]. In regions with overlapping pollen seasons, sensitizations due to cross reactivity can be revealed allowing polysensitized patients to get clinically relevant treatment. Besides its use in pollen allergic patients, the microarray technique elucidates the sensitization profile against food allergen components, which enables making a risk prognosis of future reactions [38, 39]. Geographical and age-related differences in sensitization patterns against food allergens that are revealed can also be of clinical importance.

Additionally, 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 allergens, and more than half were sensitized to lipid transfer proteins (LTPs), while 8% were sensitized to seed storage proteins [29]. In another study, the microarray provided additional relevant information on IgE cross-reactions in around 70% of polysensitized patients with respiratory symptoms, enabling a more confident diagnosis and therapeutic approach [40]. 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 to improve disease management [40].

Other studies have identified profilin as a severe food allergen in allergic patients overexposed to grass pollen [41], and showed that lupine sensitization in bakers asthma may be caused by cross-reactivity with LTP, profilin and CCDs in wheat flour [42]. 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 IgE profile from

ISAC testing indicated that the patients who did not experience OAS were the ones with broader sensitization to several allergen components, including perennial allergens such as mite, cat, dog and molds and these patients were also more frequently sensitized to profilins [43]. After examining children with seasonal allergic rhinitis and oral allergy syndrome Ludman et al [44] suggested that the pattern of pan-allergen sensitization changes from PR10 to nsLTP with increasing age.

Poly-sensitization to components is associated with prevalence of asthma

The relationship between multiple IgE sensitizations and asthma has been amply demonstrated and lately this has also been studied using microarray technique [45, 46]. In a Swedish study within the ECHRS program, the prevalence of asthma, FENO levels, and bronchial responsiveness were shown to increase with the number of sensitizations to perennial, pollen, and food allergens (Figure 1) [47]. In the same study asthma sensitization against perennial allergens had the strongest association with asthma, FeNO levels and bronchial responsiveness, while sensitization against food allergens was independently related to FeNO. Among perennial allergens sensitization to cat or/and dog components was more related to systemic and local eosinophilic inflammation, bronchial responsiveness and asthma needing to be treated [48]. Furthermore, co-sensitization to food allergens strengthened the association to asthma prevalence and airway inflammation in pollen-sensitized individuals.

Microarray data helped to refine the severity assessment in Swedish children allergic to furry animals [49, 50]. 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 secretoglobulin (Fel d 1) from furry animals was shown to be more prevalent than among controlled mild-to-moderate asthmatics. Furthermore, children with problematic 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. A more evolved IgE antibody profile including multisensitization against lipocalin and sensitization against Equ c 1, a major horse allergen component, was associated with more severe disease indicating molecular spreading of sensitization to allergen components. In American children asthma was shown to be associated with sensitization to lipocalins and nsLTP while rhinitis was associated with specific IgE antibodies to PR-10 proteins and specific grass components [51].

The levels of specific IgE against animal components are related to disease manifestations. In a population-based study, asthma and rhinitis in schoolchildren was shown to be associated with higher levels of sensitization to animal components [52]. Sensitization to several components from the same allergen source in cat- and dog-sensitized children associated strongly with asthma pinpointing the relation of a more complex sensitization profile with the likelihood of asthma [52]. In that study the majority of cat- and dog-sensitized children were sensitized to more than one cat or dog components respectively (Figure 2). Sensitization to both cat extract and any cat component but not sensitization to only extract was independently associated with more bronchial responsiveness and higher FeNO [53]. Results from studies on the BAMSE cohort where the MEDALL customized version of the ISAC chip was used, show that sensitization to Fel d 1 and Can f 1 in childhood and polysensitization to either cat or dog allergen molecules predict cat and dog allergy cross-sectionally and longitudinally better than IgE to cat or dog extract [54].

Atopic dermatitis

Patients with atopic dermatitis (AD) have a particularly high risk of food allergy [55] and often have high total IgE (tIgE) levels. High tIgE levels do not seem to affect measurements using ISAC [56] and the absence of non-specific binding leads to a very good specificity. Microarray technique has been validated for use in patients with AD [26] where it can improve the detection of sIgE antibodies. Testing with only a standard panel of allergens can result in that some allergen-specific sensitizations remain undetected in individuals with high tIgE levels, patients in which the applicability of wide range microarray based testing has been shown. [57]. The usefulness 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 [57]. Sensitization to profilins, tropomyosins, and PR-10 proteins were possibly indicative of more severe disease in children [57], whereas in adults, sIgE to cow's milk allergen components (but not to PR-10 components) was predictive of severe AD [58]. 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 strong indications to exclude atopic involvement in the disease examining both primary and secondary allergic response with just a single test. Microarray testing can be much more comprehensive than skin prick testing (SPT) or singleplex sIgE tests and can follow up the evolution of IgE sensitization in AD during atopic march but its results have to be interpreted by allergists with experience in the field of molecular allergology [59].

Eosinophilic esophagitis

Children with eosinophilic esophagitis (EoE) are very often poly-sensitized to both aeroallergens and foods [60, 61]; however, the relationship between symptoms and sensitization is not straightforward. The role of pollen-food allergy syndrome in EoE has been investigated using microarray testing [62]. Whereas children were more frequently sensitized to food allergens [63], the majority of adults were sensitized to aeroallergens like grass and tree pollen, house dust mite and furry animal [64]. In adults, food sensitizations were often caused by PR-10 [63] or profilin [64] cross-reactivity following primary sensitization to pollen [65]. Elimination diets guided by the results of ISAC and pollen-specific immunotherapy in cases where high levels of IgE responses were detected, led to significant improvement of EoE symptoms after 1 year of therapy [62].

Investigating clinical allergy phenotypes

The detailed sIgE profile revealed by component resolved diagnosis (CRD) can differ between individuals displaying the same sensitization to allergen extract, and CRD may therefore be helpful in differentiating phenotypes of clinical allergies [66]. Using ImmunoCAP ISAC, individual risk assessments in for example food and latex allergies becomes feasible using only a very limited amount of serum, and several studies have shown that results from microarray testing are comparable to those of single analyte assay [9]. Despite the good correlation between these two techniques, the measurements of microarray technique have to be confirmed with singleplex technique before provocation in the case of risk assessment as they present only a semiquantitative estimation of IgE levels [9].

For instance, in patients suspected of peanut allergy, 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. Most patients with clinical reactions are sensitized to Ara h 2 and Ara h 6 [38, 39, 67, 68]. The sensitization profiles 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 [38, 68] and Ara h 3 [38], more prevalent among patients with a history of anaphylaxis [39]. The component based test results reveal that some patients sensitized to complete peanut extract solely show IgE reactivity to cross reactive molecules such as Ara h 8, profilin or CCDs [39].

Testing patients against a combination of latex components Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 on the microarray can give a better predictive value than latex extract for symptomatic latex allergy. Sensitization to any one of these components indicated latex allergy with virtually no false-positive results; sensitization to latex profilin (Hev b 8) and/or CCDs only, enabled discrimination between mere sensitization and genuine allergy [69, 70]. Most patients mono-sensitized to Hev b 8 did not suffer any clinical symptoms to latex [70]. However microarray technique can be slightly less sensitive in picking up sensitization to CCD as compared to singleplex measurement to these carbohydrate [69].

Discovering unexpected sensitizations

Microarray testing is a useful tool to identify unexpected causes of allergic symptoms. An interesting example of this is the use of retrospective microarray 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 also 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 microarray technique [71].

Although the broad panel of allergen components on the ISAC chip has the potential to reveal unanticipated sensitizations it has to be acknowledged that this technique is less sensitive than ImmunoCAP method [9]. However this is not necessarily disadvantageous for the clinical practice as the high sensitivity of singleplex testing enables detection of low-affinity antibodies that may have little to no clinical relevance [9].

Furthermore, the revealing of unforeseen sensitizations to potentially dangerous molecules can be a disadvantage as the interpretation of such asymptomatic sensitizations is problematic or sometimes impossible [9, 72]. The difficulties in understanding these results are more evident in sensitization to food allergens and Hymenoptera venoms rather than respiratory allergens as they can cause severe allergic reactions on reexposure presenting the clinician with the dilemma how to manage this unanticipated information.

Causes of co-factor-dependent food allergy

Clinical history, which is the cornerstone in allergy diagnostics, sometimes fails to identify the culprit trigger in cases of co-factor-dependent food allergy. Using IgE testing with microarray in a retrospectively study of 74 patient cases, Cardona and colleagues observed that underlying sensitizations to plant-derived foods, mainly vegetables and cereals, accounted for the reactions with 92% of patients sensitized to LTPs (Pru p 3) [73]. In a further study on Spanish patients with a complex clinical history and multiple sensitizations to plant-foods and pollens it was demonstrated that a broad spectrum of clinical reactions can be explained by LTP sensitizations [74]. Similarly, in an Italian cohort having suffered from food-dependent, exercise-induced anaphylaxis, microarray data showed that almost 80% were sensitized to Pru p 3 while the prevalence of sIgE to profilin, PR-10, tropomyosin and gliadin were lower [75]. However, components other than LTP can be responsible for symptoms in co-factor-dependent food allergy, 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 analysis offered by microarray testing can identify unforeseen sensitizations that present a risk of severe reactions, providing additional, clinically important information that is not generated from routine allergen extract or singleplex IgE testing. In a British study, ISAC testing identified hitherto unknown sensitizations in up to 20% of patients with IA, that were highly likely to cause anaphylaxis (Figure 3). In the majority of cases, sensitizations to wheat omega-5-gliadin and shrimp components, were demonstrated [76].

Work-up of specific immunotherapy patients

Immunotherapy based on recombinant allergens is effective but not superior to traditional extract based vaccines [66]. In addition to a series of technical and financial factors, this make it difficult to achieve patient-tailored immunotherapy in daily clinical practice [77, 78]. However, specific and cross-reactive components present on the ISAC microarray can identify true co- or multi-sensitization. By this, it would be easier to select the patient-appropriate allergen source(s) to use leading to improved specificity of the SIT prescription [9]. In an area of Spain with overlapping pollen seasons, microarray data used in a study demonstrated that sensitization to cross-reactive components of timothy grass and olive tree

were causing clinically irrelevant, positive SPT results. Component level data modified the physicians' SPT-based SIT prescription in more than half of the study population [79]. In another area of Spain with similarly complex pollen exposure, ISAC testing changed the SIT prescription in 54% of the study population by revealing false negative extract test results, most commonly to plane (40%) and grass extract (16%) [80]. These studies stress the importance of CRD in choosing appropriate immunotherapy to each patient and broad range testing through microarray technique can become in the future a requirement before immunotherapy due to the high diversity of geographically-related IgE profiles.

Specific pollen components present on the ISAC microarray, e.g. Art v 1, Amb a 1, Par j 2, Bet v 1, Ole e 1, Cry j1, Cup a 1, Phl p 1 and Phl p 5, allow for detection of genuine sensitization in most patients suffering from pollen allergies. Together with clinical history, which is still the most important milestone in the diagnostic algorithm, microarray technique enables the clinician to improve selection of patients for SIT prescriptions even in the most complex cases of multi-sensitization [81].

Changes in sIgE levels to specific allergen components can potentially be used as a surrogate markers for the clinical effectiveness of SIT despite the fact that ISAC as a semi-quantitative method may not be the best alternative to follow levels of IgE [9]. Singleplex and multiplex technique present discordance in their measurements of allergen component-specific IgE levels during up dosing of SIT as they increase according the first method and seem to decrease according the latter [82]. This perceived decrease in allergen component-specific IgE levels as measured by microarray methods can however be used to monitor the blocking effect of induced IgG4 antibodies as it seems that increasing levels of IgG4 suppress the apparent IgE levels on ISAC during immunotherapy. However according to many previous studies [83] the rise of levels of IgG4 and the decrease of specific IgE do not always correlate with clinical efficacy. 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 using ISAC. 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 [84].

Investigating the allergic march and assessing the risk for symptomatic allergy

The broad range of allergen components on the ISAC microarray and the minimally invasive blood sampling required makes this microarray highly suitable for investigating the evolution of allergic sensitizations from infancy to adulthood as performing a large number of SPT or sIgE assays is not always possible.

Testing for sIgE sensitization with microarray technique 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 allergen components at 8 and 16 years of age were predictive of the presence and severity of birch pollen AR and reported OAS at age 16. A high level of sIgE to Bet v 1 or sensitization to many PR-10 proteins at 4 years of age was associated with increased incidence and persistence of AR up to age 16 [18]. IgE sensitization to cat and timothy components had higher clinical value as they associated independently with new-onset of rhinitis and asthma, compared with traditional extract-based methods [53].

Similarly, in a longitudinal study on the German MAS-90 birth cohort, preclinical sensitization to grass components at 3 years of age predicted the onset of seasonal AR at age 12 [85].

Prospective studies using microarray IgE testing (both standard ISAC chip and the MEDALL version) have shown spreading of IgE sensitization to other components of the same allergen source during childhood [17, 86]. The IgE response to grass pollen components preceded allergic symptoms by several years, starting as a asymptomatic mono- or oligo-sensitization, continued with increasing number of sensitizations to allergen components during the preclinical and early stages of AR. This development of different IgE reactivities is known as molecular spreading [85] (Figure 4). Microarray data showed that the order of sensitization to grass pollen components in children typically started with mono-sensitization to Phl p 1, spreading to Phl p 4 and Phl p 5; followed by Phl p 2, Phl p 6, and Phl p 11; and finally to the highly cross-reactive Phl p 12 and Phl p 7 [85].

Analysis of microarray data from the Manchester Asthma and Allergy Study identified sIgE patterns that could predict different allergic diseases [87]. Sensitizations to dust mite, pollen and furry animal allergen components were associated with asthma, sIgE to pollen and dust mite with rhino-conjunctivitis and to egg/milk allergens with eczema. In the same cohort [88]

allergen components to which patients showed an IgE response fell into three major groups. Children sensitized to components from plant allergens were more than 12 times more likely to have pollen allergy, but not asthma or wheeze; those positive to components of predominantly dust mite allergens were 3 times more likely to have asthma and twice as likely to have pollen allergy; and positivity to a broad but specific range of protein families, was most strongly associated with asthma. Thus, different patterns of IgE reactivities to various proteins families were associated with different clinical symptoms.

A retrospective comparison of 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 [86]. This highlights the usefulness of early broad sIgE 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. The clinical evolution of allergic diseases related to the evolution of IgE sensitization beginning with IgE reactivity to milk and egg components in early childhood, sensitizations to plant allergens occurring at a somewhat later age, and sIgE to cross-reacting allergens appearing after 6 years of age [89].

Limitations of microarrays

Despite all its advantages, molecular allergology remains a third-line (or second-line for experienced users) testing according to current clinical practice [90]. Thorough clinical history followed by allergen extract-based testing is still the cornerstone of diagnosing the majority of allergic patients [90]. Extract-based tests are still used as first line tests as not all allergens of the extract sources are represented on the microarray. Not all allergenic sources are equally represented by allergen components on the chip e.g. some specific components from hazelnut and mites are missing [91] so the addition of certain proteins, e.g. 2S albumins from more nuts, seeds and legumes would be desirable. However in most cases the existing components allow an accurate risk assessment and disease management [92]. IgE-reactivities to cross-reactive allergens of the same protein family but from different sources may be used and extrapolated to indicate sensitization also to those missing on the chip.

Other limitations of the microarrays are that multiplexed component testing today is semi-quantitative, has greater coefficient of variation and is less sensitive than quantitative methods, and consequently is less appropriate for monitoring changes in sensitization. However the lower sensitivity is not always disadvantageous as it probably detects sensitization levels of higher clinical significance [53].

Multiplexed component testing is more expensive than SPT [11] and should be used for the appropriate indications. The added clinical value is limited in cases of recent convincing clinical reactions, in younger children or in monosensitized subjects where traditional extract-based tests can give sufficient information [93]. Further, the amount of additional information generated by multiplexed component testing requires a certain experience for interpretation of results. By revealing sensitizations to allergens that are not routinely tested for, reactions previously categorized as idiopathic can be explained, but the microarray testing can also identify clinically non-relevant sensitizations.

Conclusions

The usefulness of ISAC has been validated in a wide spectrum of allergic diseases like asthma, allergic rhinoconjunctivitis, atopic dermatitis, eosinophilic esophagitis, food allergy and anaphylaxis. Although more and larger studies are needed, the existing evidence supports the use of this technique which gives allergists 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. Component resolved data can better help to select relevant allergens for SIT compared with extract testing and may help to follow the response to immunotherapy treatment. In investigational studies, microarray technique has provided valuable insight into the allergic march and molecular spreading in the preclinical stages of allergic diseases, indicating that the likelihood of developing symptomatic allergy is associated with specific profiles of sensitization to allergen components.

In summary, multiplex assays have found their way into routine allergy diagnostics due to their ability to aid in improved risk assessment, selection of appropriate allergens for immunotherapy and detection of unknown sensitizations. Multiplex component testing is especially suitable for patients with complex symptomatology.

Conflict of interest:

M.P. Borres, A. Kober and M. Berthold are employees of Thermo Fisher Scientific

Table 1. List of selected epidemiological and clinical publications using ImmunoCAP ISAC/microarray platform.

Broad sensitization profiling		
	AD	Fung Ann All Imm 2013 [94], Ott JEADV 2014[57], Gray PAI 2014[55], Rockman CTA 2014 [58], Mari Cur Op All Imm 2011 [59], Choi Ann Derm 2014 [26], Gray PAI 2014 [55]
	Asthma or rhinitis	Patelis JACI 2012 [47], Nordlund Allergy 2012 [49], Konradsen PAI 2014 [50], Bjerg PAI 2015 [52], Patelis CEA 2015 [53], Simpson JACI 2015 [46], Custovic JACI 2015 [45]
	EOE	van Rhijn Allergy 2013 [65], Erwin Ann Allergy Asthma Immunol 2010 [63], Simon Allergy 2010 [64], Armentia Allerg Immun 2015 [62]
Investigating clinical allergy phenotypes	Peanut allergy	Ackerbauer Int Arch All Imm 2015 [39], Ciprandi Allergy 2015 [38], Klemans CEA 2013 [67], Hong Allergy 2012

		[68]
	Latex allergy	Ebo CEA 2010 [43], Schuler CTA 2013 [70]
Discovering unexpected sensitizations		
	Explanation in difficult cases	Gassner N Enj J Med 2013 [95]
	Causes of co-factor-dependent food allergy	Cardona Allergy 2012 [73], Pascal CEA 2012 [74], Romano CEA 2012 [75]
	Idiopathic anaphylaxis	Heaps CEI 2014 [76]
Work-up of specific immunotherapy patients		Letran Ann All Asth Imm 2013 [79], Sastre Allergy 2012 [80], Schmid JACI 2015 [96], Van gasse Clin chim Acta 2015 [97], Asero Eur ann all cli imm 2012 [81], Wollmann JACI 2015 [98]
Allergic march and risk for symptomatic allergy		Asarnoj JACI 2015 [54], Westman JACI 2015 [99], Hatzler JACI 2012 [85], Onell, Hjalle, Borres 2012 [86]

Figure legends

Figure 1. Increasing prevalence of asthma with increasing number of sensitizations to perennial, pollen, and food allergens as measured with ImmunoCAP ISAC (Figure re-drawn from ref 41, Patelis et al) (asterisk indicates $p < 0.05$ when compared with the group without any allergic sensitization, no adjustments of p values for multiple comparisons were made)

Figure 2: Venn diagrams of children sensitized (≥ 0.3 ISU) to the main allergen components from cat (A) and dog (B). (Figure re-drawn from ref 46, Bjerg et al)

Figure 3: The ISAC array contributes to improving the diagnosis in 20% of patients with idiopathic anaphylaxis according Heaps et al. (Figure re-drawn from ref 70, Heaps et al).

Figure 4. A simplified figure of molecular spreading showing the increasing number and complexity of sensitization against timothy components over time in children from the MAS-90 cohort with allergic rhinitis. IgE antibodies can be detected several years before disease on-set.

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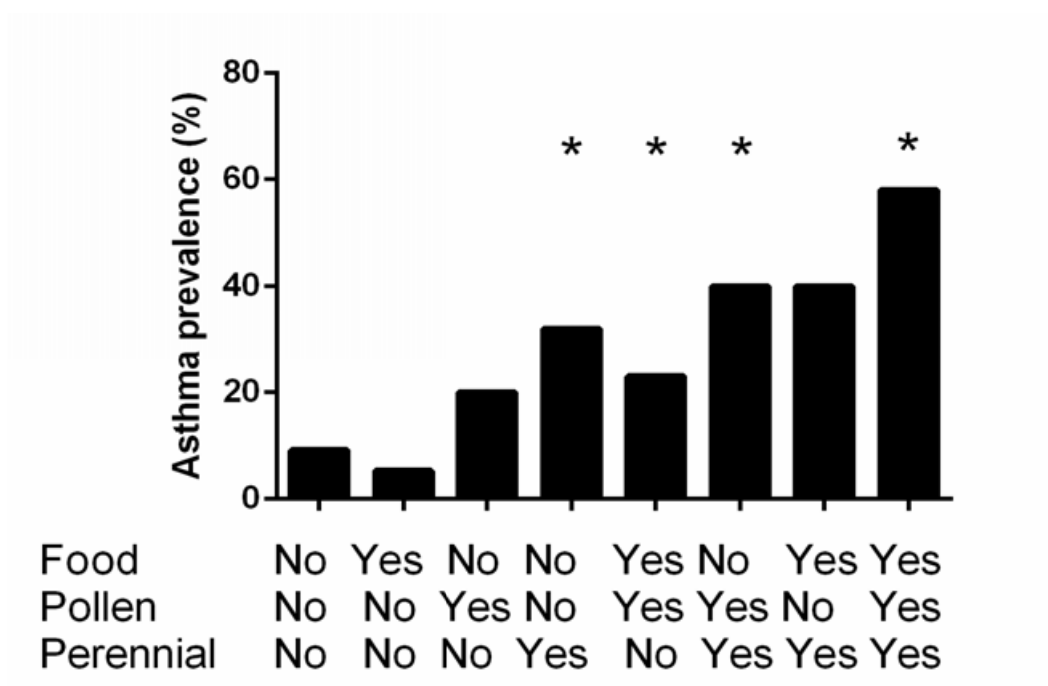
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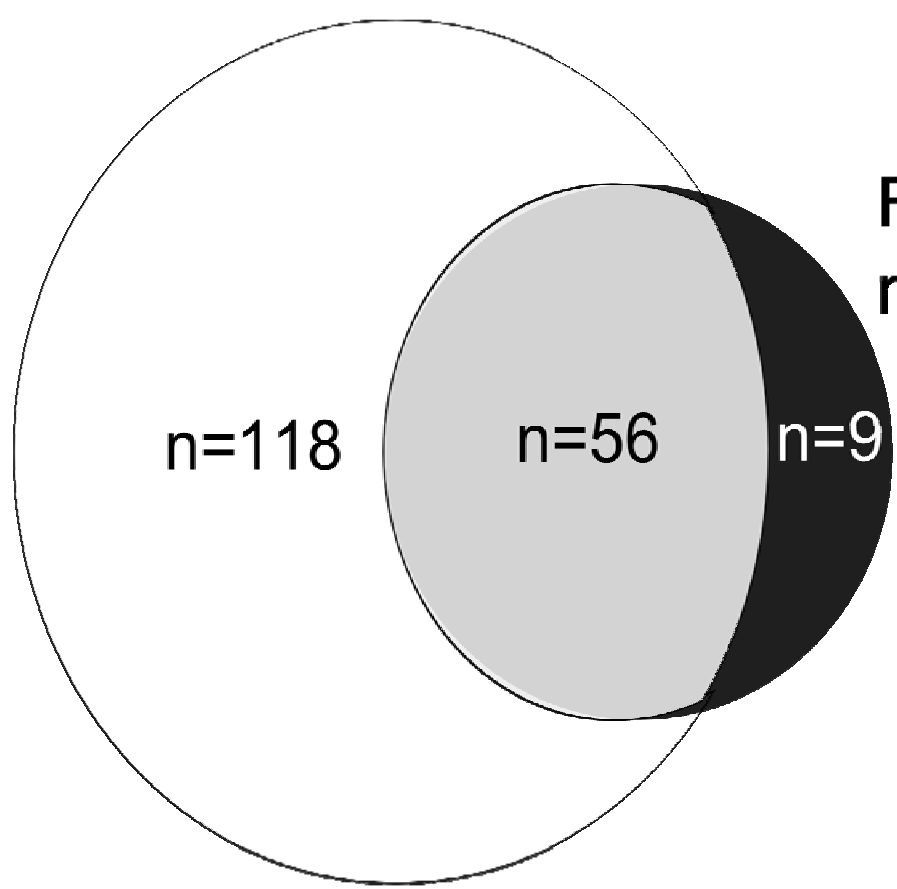
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Fel d 1
n=174



Fel d 4
n=65

A

