

NEW LABORATORY METHODS OF DIAGNOSING ALLERGIC DISEASE - PART II - WHERE ARE WE GOING?

Cathy van Rooyen, MBChB, MMed(Path)(Viro), FRCPath.

Correspondence

Allergy, Immunology and Virology Pathologist, Ampath Pathology, National Reference Laboratory (Immunology) and Allergy Clinic.

Email: vrooyenc@ampath.co.za

ABSTRACT

This is the second part of the article and deals with recent improvements in allergy diagnostics and touches on new testing methods that are in development.

IMMUNOCAP IgE TO ALLERGEN COMPONENTS

Natural allergen sources may contain many different proteins, but only a few of them are allergenic. The identification of these allergenic proteins has led to the development of a new concept in allergy diagnosis, namely component-resolved diagnostics or MD. This allows the clinician to identify potential disease-eliciting molecules, predict cross-reactivity, severity of reactions and probability of the development of tolerance. Knowledge of different protein families and their physical properties (e.g. sensitivity to heat and digestion) can be used to advise patients on appropriate dietary avoidance measures.^{3,4,22}

The allergen component names include their scientific acronym and number (e.g. Ara h 2 means the second

allergen from *Arachis hypogaea* or peanut).³

ISAC MICROARRAY TEST

The ISAC test is a multiplex microarray chip (Figure 1) in which IgE is detected to multiple recombinant allergen components. The current ISAC microchip is a miniaturised immunoassay platform using only 20ul of serum to measure specific IgE to 112 different recombinant allergen components. This test should not be used as a screening test in patients with a history indicating a low suspicion of allergy, but should be used as a diagnostic tool in patients with suspected allergen cross-reactivity such as combined food and pollen allergies or in patients with multiple allergies.

Figure 1a-c . An example of an ISAC 'chip' and the machine used

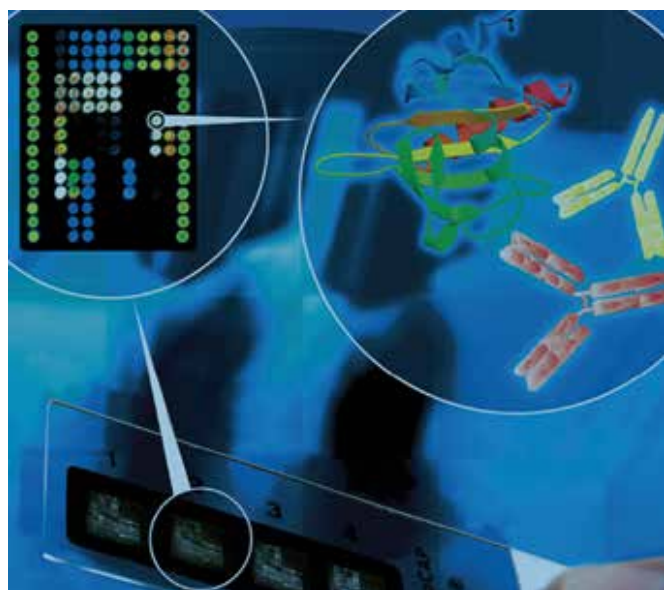


Figure 1a

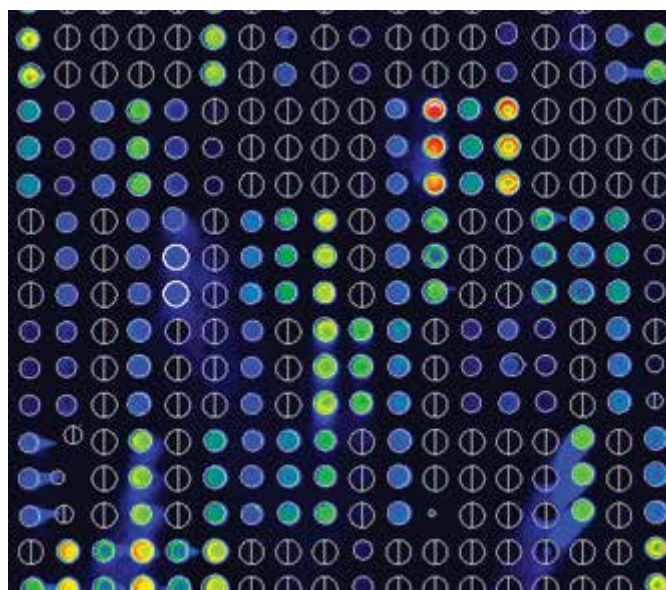


Figure 1b



Figure 1c

CLINICAL VALUE OF IgE TESTING TO ALLERGEN COMPONENTS

One of the major advantages of MD is the ability to distinguish between primary species specific sensitisation and cross-reactivity to proteins with similar protein structures, which may help to evaluate the risk of reaction upon exposure to different allergens. Protein structure and stability to heat and digestion may affect tolerance to raw or cooked foods and the severity of clinical reactions. This knowledge can be used to individualise patient management by including advice on targeted allergen exposure reduction, selection of suitable allergens for specific immunotherapy or the need to perform food challenges.^{3,4,22,26}

POLLEN ALLERGY AND CROSS-REACTIVE FOOD ALLERGY

Patients with pollen allergies are often sensitised to cross-reactive components that occur in pollens, as well as foods of plant origin. The most common cross-reactive components are cross-reactive carbohydrate determinants (CCD), profilins, Proteinase-10 (PR-10) and lipid transfer proteins (LTP).^{3,4,22,23} Clinical relevance and severity of reactions can be predicted, e.g. IgE to CCD is the least likely to cause symptoms. The likelihood of symptomatic allergy increases with IgE to profilin, then PR-10 and LTP is the most likely to induce clinically relevant reactions.^{3,4,22,24,25} Profilin and PR-10 are also heat labile, so patients with this allergy may be able to tolerate cooked food, while being symptomatic to raw food. Knowledge of protein localisation may also contribute to patient management, as PR-10 is mainly localised to the pulp of fruit and LTP to the peel. Patients with LTP allergy may sometimes be able to tolerate peeled fruit.^{3,4,22} As cross-reactivity may have a substantial impact on patient management, there should be a greater awareness when interpreting allergy tests regarding potential cross-reactivity between pollens and food of plant origin. A typical pointer to potential cross-reactivity is when pollens and several foods of plant origin are positive on IgE-

mediated allergy tests, especially positive reactions to a combination of wheat, peanut and soy.

NUT AND SEED ALLERGY

Storage proteins are the dominant allergens in nuts, seeds, fruit stones and kernels. The main storage proteins are designated according to molecular weight and are grouped in 7/8S and 11S globulins and 2S albumins. These proteins are very stable to heat and digestion, therefore patients also react to cooked and processed foods. Sensitisation to storage proteins is regarded as an important risk factor for severe systemic reactions, particularly if sensitisation to more than one storage protein in a particular allergenic source is identified. The 2S albumin seems to be the dominant allergen with the highest risk for severe systemic reactions in tree nut, seed and peanut allergies.^{3,4,23,24}

Peanut contains 32 known proteins, of which 18 have been identified as potential allergens. Five of these are currently offered as components on ImmunoCap testing (Ara h 1, 2, 3, 8 and 9) and six on the ISAC (Ara h 1, 2, 3, 6, 8 and 9). Many individuals sensitised to peanut may be tolerant to peanut. This may often be seen due to sensitisation to cross-reactive components, e.g. CCD, profilin, PR-10 or LTP.

Ara h 1, 2 and 3 are all major allergens, are associated with primary sensitisation to peanut and are all seed storage proteins. Ara h 2 sensitisation in particular, is considered a risk marker for severe allergic reactions. A recent study indicated that a cutoff of 1.63 kU/ml had a specificity of 100% and a sensitivity of >70%, which greatly decreased the need for peanut challenges.²⁷ Ara h 8 is a PR-10 protein and if a patient is symptomatic due to IgE to Ara h 8, symptoms most commonly are restricted to oral allergy syndrome (OAS). Ara h 9 is a LTP and is often associated with systemic and more severe reactions in addition to OAS, although many patients with Ara h 9 sensitisation may be asymptomatic.^{3,22} In a study performed at Ampath Laboratories on 100 peanut sensitised patients from various regions in South Africa, Ara h 9 sensitisation was the most common allergen component sensitisation (38%) (Unpublished data). However, data is lacking to explain the primary sensitiser or the clinical symptoms, associated with this sensitisation.

WHEAT ALLERGY

A positive result to wheat-flour extract does not always correlate with clinical symptoms, as cross-reactivity with grass-pollen is common. This is a particular problem in South Africa, where incorrect diagnosis of wheat allergy occurs frequently. The wheat component Tri a 19 omega-5 gliadin is associated with true wheat allergy and is an important risk marker for immediate reactions to wheat in children and for exercise-induced anaphylaxis after wheat ingestion in adults.^{3,22}

SOY ALLERGY

The main soy allergens are Gly m 5 and Gly m 6, seed storage proteins. These allergens indicate primary sensitisation to soy and are also high-risk markers for severe allergic reactions to soy. Pollen-sensitised individuals may also react to Gly m 4, the PR-10 in soy. These patients may have severe OAS or even systemic reactions. Most commercial soy extracts contain low levels of Gly m 4, therefore pollen sensitised patients with a suspicion of soy allergy should be tested separately to Gly m 4.^{3,22,23}

MILK ALLERGY

The major allergens are casein (Bos d 8), alpha-lactalbumin (Bos d 4) and beta-lactoglobulin (Bos d 5), although allergies to minor proteins like bovine serum albumin (BSA)(Bos d 6) and lactoferrin (Bos d lactoferrin) have been reported. Casein is the most important and abundant allergen in milk and hard cheese and is heat stable. Patients with high levels of IgE to casein are at risk for severe reactions and are less likely to outgrow their milk allergy.^{3,22,28} Please note that there is a significant homology between casein of different species and patients with casein reactivity have a high risk of reacting to the milk of other animal species.²⁸ Whey proteins (alpha-lactalbumin and beta-lactoglobulin) are heat labile and patients reacting to these proteins may often tolerate heated or fermented milk products. BSA is a serum albumin which is a main protein in mammalian blood and is an important allergen involved in milk, meat and epithelia allergy.²⁹ Sensitised patients may react to different meats (beef, lamb and pork), epithelia (cat and dog) and cow's milk.^{3,22,28}

EGG ALLERGY

Egg white is the most important source of egg allergy and contains 23 different proteins. The most important allergens are ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin/conalbumin (Gal d 3) and lysozyme (Gal d 4). Although ovomucoid comprises of only 10% of the total egg white protein, it has been shown to be the dominant allergen and is allergenic in minute amounts.^{3,22,28,30} This protein is very stable to heat and digestion, therefore allergic patients cannot tolerate egg in baked products. High levels of IgE to Gal d 1 are also associated with persistent egg allergy. On the contrary, absence or low levels of IgE antibodies to Gal d 1 are associated with an increased probability of tolerance to ingestion of cooked egg. This may guide clinicians when to perform a cooked egg challenge.^{30,31} Gal d 5 is present as the protein egg livetin in egg yolk and in chicken as chicken serum albumin and may cause "bird-egg syndrome", where patients may react to egg yolk, chicken meat and feathers.³²

FISH ALLERGY

Cod (Gad c 1) and Carp (Cyp c 1) parvalbumins are markers for fish sensitisation in general. There is a high degree of cross-reactivity between parvalbumins from different

fish species, therefore clinically sensitised patients are often recommended to avoid all fish species. However, the difference in parvalbumin content in some species may explain tolerance in parvalbumin sensitised individuals to some species, e.g. tuna, mackerel or swordfish.³³ Parvalbumin is a very stable protein, which may explain reactions to cooked fish as well as cooking vapor.^{3,22,28}

True shellfish allergy is best indicated by the shrimp allergen Pen m 2, an arginine kinase.³⁴ Pen a 1 is a tropomyosin and a major allergen in shrimp. This protein is very heat stable and cross-reacts with other tropomyosins, muscle proteins found in crustaceans (shrimp, lobster, crab), arachnids (house dust mite), insects (cockroach) and mollusks (squid).^{3,22,28,35}

IMPLICATIONS FOR ALLERGEN IMMUNOTHERAPY

Allergen components can be of value in selecting patients for bee venom immunotherapy. The success of allergen immunotherapy is dependent on appropriate patient and specific immunotherapy vaccine selection.

Patients frequently test positive to both bee and wasp venom. This may be due to CCD cross-reactivity or dual sensitisation. Honeybee phospholipase A2 (Api m 1) can be used to indicate a true honeybee allergy and wasp phospholipase A1 (Ves v 1) and wasp antigen 5 (Ves v 5) to indicate true wasp allergy.³⁶

Cross-reactions occur commonly between furry animals, therefore the primary sensitiser should be identified before choosing appropriate allergen immunotherapy. Cat uteroglobin (Fel d 1) is the major cat allergen component indicating primary sensitisation to cat and can be used as a specific marker that cat allergen immunotherapy is likely to be of clinical value. Cat Fel d 2 (cat serum albumin) is likely to cross-react with most other mammalian albumins and Fel d 4, a lipocalin, with horse, dog and cow, therefore sensitisation to these components should not be used as motivation for allergen immunotherapy. The dog allergens Can f 1 and Can f 2 are lipocalins and are associated with primary sensitisation to dog. Can f 1 can be used as a specific marker indicating likelihood of successful allergen immunotherapy to dog. Can f 5, a prostatic allergen secreted by male dogs only, is also an indicator of primary sensitiser to male dogs. Equ c 1, a horse lipocalin, is the major allergen in horse dander, but results should be interpreted in correlation with the clinical history, as there is some cross-reactivity with mouse and cat lipocalin.^{3,22,28}

Specific markers indicating the likelihood of success of grass pollen immunotherapy is Cyn d 1 or grass group 1 allergen for Bermuda grass allergy and Phl p 1 (grass group 1) and

Phl p 5 (grass group 5) for Timothy grass allergy. There is significant similarity between the group 1 and group 5 grass pollen allergen components in other grass species, especially those belonging to the *Pooidae* subfamily, e.g. Rye grass. Patients sensitised to Phl p 1 only and not to any of the other allergen components in Timothy grass, are probably sensitised to Bermuda grass and/or Maize pollen and not to Timothy or Rye grass.^{3,26}

THE FUTURE IN IgE MEDIATED ALLERGY DIAGNOSTICS

CUSTOMISED MULTIPLEX MICROARRAY CHIPS

The possibility of creating customised microarray profiles suited to specific diagnostic applications should become available in future. Options should also be made available to combine complete allergen extracts with allergen components, to enable the use of microarray tests for screening purposes in selected patients. As new and clinically relevant allergen components are discovered, they will be added to the current diagnostic repertoire.

ALLERGEN EPITOPE PROFILING

In each allergen component, there are commonly several different epitopes. An epitope is a three dimensional binding site for a specific antibody. Antibodies may be directed to linear epitopes (not destroyed by heating or processing) or conformational epitopes (destroyed by heating or processing). Patients that recognise a greater number of epitopes are more likely to have clinical symptoms after allergen exposure and are less likely to develop tolerance or outgrow their allergy.^{11,12} The use of IgE epitope mapping of allergens, using microarray based immunoassays, will probably be the next step in IgE mediated allergy test development.

BASOPHIL MEDIATED ALLERGY

Basophils have IgE receptors on their cell surfaces, therefore they may be activated either directly or via specific IgE in the patient's serum. While protein allergens are usually required for IgE binding, basophils may also be activated directly by small molecular weight allergens.^{5,6} Foods, colourants, preservatives and drugs often induce non-IgE mediated basophil activation. Basophil mediated allergy may include an immediate or a delayed allergic response. Prominent symptoms are rhinitis, asthma, gastrointestinal symptoms and urticaria.

Basophil mediated allergy can be measured by a basophil activation test (BAT) or the commercial equivalent Cellular Allergen Stimulation Test (CAST®). The first generation CAST® test was a CAST®-ELISA, where sulfidoleukotriene release by activated basophils was measured by ELISA technology.³⁷ This assay was very slow and time-consuming

and had to be performed within 4 hours of venopuncture, making it impractical for routine laboratory diagnostics. The next generation assays use flow-cytometry (flow-CAST® or other in-house flow-cytometry based BATs) to identify particular basophilic activation markers after stimulation by a particular allergen. These assays are more suited to routine use, as specimens can be processed for up to 24 hours after collection (Figure 2). A wide range of commercial allergens are available, which include foods, colourants, preservatives, venoms, latex and drugs. If commercial drug allergens are not available, it is possible to use actual drugs as allergens, provided there is no cellular toxicity. Different dilutions of the drugs are used and results are correlated with patient controls. This can currently only be performed at the Immunology department of Ampath's National Reference Laboratory (NRL). Several screening tests are available, most notably food and inhalant screens and even drug allergy screens, e.g. a general anaesthetic allergy screen.



Figure 2. Flow cytometry for CAST testing

The overall sensitivity and specificity of BAT in classical inhalant allergy and latex allergy equals or exceeds 90%.^{5,38} Studies in patients with food allergy have varied, due to the diversity of food allergens available. In individual patients, BAT has confirmed the diagnosis of primary food allergy, such as anaphylaxis, to multiple different food allergens. In two specific studies of patients with apple allergy and carrot, celery and hazelnut allergy, the sensitivity of BAT was shown to be 85-90% and the specificity 80-90%.³⁸ Drug allergy diagnosis is very tricky, due to the broad spectrum of different drugs and metabolites and the involvement of unknown epitopes and haptens. Studies using BAT in the diagnosis of allergies to neuromuscular blocking agents have shown approximate sensitivities of 65-80% and specificities of 93-100%.^{39,40} Studies on aspirin sensitivity have shown approximate sensitivities of 55-60% with specificities exceeding 90%.⁴¹ Here it should be kept in mind that aspirin-induced respiratory and cutaneous

reactions are often pseudo-allergic reactions that result from inhibition of cyclo-oxygenase-1 (COX-1), with subsequent depletion of prostaglandin E2 and unrestrained synthesis of leukotrienes. These reactions cannot be diagnosed by BAT. Sensitivity of the BAT for the diagnosis of allergy to beta-lactam antibiotics have been estimated at approximately 55%, with a specificity exceeding 90%. Sensitivity can be improved by testing more widely for different metabolites and side-chains and combining testing methods. In a multicenter study aimed at evaluating assays for the diagnosis of beta-lactam antibiotic allergy, a diagnostic algorithm was developed combining skin tests, specific IgE, BAT and controlled challenge and this resulted in a test sensitivity of 92%.^{18,42}

THE FUTURE

COMPONENT TESTING ON BAT

Component testing on BAT has recently become available with a limited number of allergen components commercially available for testing, namely CCD and profilin for pollen cross-reactivity, peanut storage proteins Ara h 1, 2 and 6 and the milk components casein, alpha-lactalbumin and beta-lactoglobulin. The use of components may help to distinguish between clinically relevant and irrelevant IgE results, diagnose clinically relevant allergy and the development of tolerance more accurately and to select and monitor specific component-resolved immunotherapy.^{43,44}

T-CELL MEDIATED ALLERGY

T-cell mediated reactions are delayed allergic reactions and can typically be classified as a type IV Gel and Coombs hypersensitivity reaction.⁵ Patients most often present with skin reactions such as maculopapular rashes, ocular symptoms, respiratory symptoms, oral lichen planus and loosening of dental and orthopaedic metal prostheses, but other symptoms may also occur, depending on the type of exposure.⁴⁵⁻⁴⁹ The most common allergens causing T-cell mediated allergy are drugs, metals, latex and food.⁵⁰

PATCH TESTING: THE ATOPY PATCH TEST

The atopy patch test can be used to elicit delayed type hypersensitivity reactions on the skin of sensitised subjects. Patch testing is the gold standard for the diagnosis of contact allergies, but is only recently being evaluated for food and drug allergies. There isn't good standardisation of protocols for allergen concentration and composition yet.^{51,52} Generally the patient skin is exposed to the occluded allergen for 48-72 hours and reactions are interpreted at 72 hours.

LYMPHOCYTE PROLIFERATION ASSAYS

Lymphocyte proliferation assays can be used to diagnose T-cell mediated allergy. Classic lymphocyte proliferation

assays measure lymphocyte proliferation after stimulation with allergens for 5-6 days. After 5-6 days of culture, radioactive thymidine is added and the radioactivity of the cellular DNA is measured four hours later. A modified version of the classic lymphocyte proliferation assay is the MELISA test. The specificity of this assay is increased by the depletion of contaminating monocytes, which may lead to false positive reactions, as well as by morphological examination of lymphocytes to confirm lymphoblast transformation. The MELISA test is only available in South Africa at Ampath laboratories. Specimens need to be processed within 24 hours of collection, as viable lymphocytes are needed. This test can be performed to a wide range of commercially available allergens including all allergens available for CAST® testing, as well as a wide range of metal allergens.⁵³

The T-cell proliferation test most widely used worldwide is the beryllium lymphocyte proliferation test, used for occupational screening of workers with Beryllium exposure. The sensitivity of this assay is approximately 68% and the specificity approximately 70%.^{54,55} In a study performed on patients with suspected drug-induced exanthems, the sensitivity of T-cell proliferation assays were shown to be 75%.⁵⁶ A further study revealed a sensitivity of 78% and specificity of 85% of the lymphocyte proliferation assay in the diagnosis of drug allergy. These results are somewhat biased by the high number of penicillin allergies and cannot be generalised to all other drug allergies.⁵⁷ Lymphocyte proliferation tests have also been used to diagnose food allergy, most notably reactions to cow's milk and peanut.⁵⁸⁻⁶⁰

THE FUTURE

Flow-cytometric assays to measure lymphocyte proliferation are being developed to replace current lymphocyte proliferation assays reliant on radioactive labelling. The advantages of flow-cytometric assays are that specific cell populations can be measured, which may increase specificity. An ongoing evaluation of flow-cytometric T-cell proliferation assays are currently being performed at the Immunology Department of Ampath NRL. Promising results have been shown, specifically in the diagnosis of latex allergy, where a pilot study using 20 patients indicated a sensitivity of 95% and specificity of 80% (Unpublished data). The use of allergen components, instead of crude allergen extracts, may also prove to be useful in distinguishing between sensitisation and clinical allergy.⁵⁸

UNVALIDATED DIAGNOSTIC TESTS

Technological advances or new tests are not always of value and unfortunately there are many techniques available that have not been validated scientifically or that cannot identify allergy by means of a known immunological mechanism. These tests are frequently promoted directly

to the public and are usually not offered by conventional diagnostic laboratories. These tests include, but are not limited to, provocation-neutralisation testing, hair analysis, Vega testing, kinesiography, iridology, auriculo-cardiac reflex, leucocytotoxic testing, ALCAT testing and Immunopro IgG mediated allergy testing. There are many publications reviewing these tests,⁶¹⁻⁶³ including a position statement by the Allergy Society of South Africa (ALLSA).⁶⁴

CONCLUSION

The clinical history should always be the first starting point in allergy diagnosis. The mechanism of pathogenesis should be considered when allergy tests are requested, as well as the most appropriate allergen selection. Initial or screening tests should be used to make the initial diagnosis and identify the offending allergens. More specialised tests should be used to predict the severity of allergy, relevant cross-reactivity versus primary sensitisation and the likelihood of allergy resolution. Clinicians should be aware of the different testing modalities available and their uses and limitations. As the field of allergy diagnostics is rapidly expanding and becoming more technologically advanced, clinicians may need to rely more on advice on testing and interpretation from specialists in the field.

DECLARATION OF CONFLICT OF INTEREST

The author is employed by Ampath Pathologists, a provider of diagnostic testing for allergy.

REFERENCES

- Johansson SGO. Raised levels of a new immunoglobulin class (IgND) in asthma. *Lancet* 1967;2:951.
- Adkinson Jr NF, Bochner BS, Busse WW, Holgate ST, Lemanske Jr RF, Simons FER, editors. *Middleton's Allergy Principles & Practice*, Philadelphia : Elsevier 2009;7(2):1255.
- Sastie J. Molecular diagnosis in allergy. *Clin Exp Allergy* 2010;40:1442-60.
- Harwanegg C, Hiller R. Protein microarrays in diagnosing IgE-mediated diseases: spotting allergy at the molecular level. *Expert Rev Mol Diagn* 2004;4(4):89-98.
- Adkinson Jr NF, Bochner BS, Busse WW, Holgate ST, Lemanske Jr RF, Simons FER. *Middleton's Allergy Principles & Practice*, USA 2009;7(1):14-15.
- Ebo DG, Sainte-Laudy J, Bridts CH, et al. Flow-assisted allergy diagnosis: current applications and future perspectives. *Allergy* 2006;61:1028-1039.
- Stejskal VDB, Olin RG, Forsbeck M. The lymphocyte transformation test for diagnosis of drug-induced occupational allergy. *J Allergy Clin Immunol* 1986;77:26-411.
- Stejskal V, Danersund A, Lindvall A, et al. Metal-specific lymphocytes: Biomarkers of sensitivity in man. *Neuroendocrinol Lett* 1999;20:289-298.
- Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+ CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004;199(12):1679-1688.
- Savilahti EM, Karinen S, Salo HM, et al. Combined T regulatory cell and Th2 expression profile identifies children with cow's milk allergy. *Clin Immunol* 2010;136:16-20.
- HA Sampson. Improving in-vitro tests for the diagnosis of food hypersensitivity. *Curr Opin Allergy Clin Immunol* 2002;2:257.
- Beyer K, Ellman-Grunther L, Järvinen KM, et al. Measurement of peptide-specific IgE as an additional tool in identifying patients with clinical reactivity to peanuts. *J Allergy Clin Immunol* 2003;112:202.
- Pagani M, Antico A, Cilia M, Calabro D, Pto S, Pecora S, Burastero SE. Comparison of different diagnostic products for skin prick testing. *Eur Ann Allergy Clin Immunol* 2009;41(1):23-31.
- Focke M, Marth K, Flicker S, Valenta R. Heterogeneity of commercial timothy grass pollen extracts. *Clin Exp Allergy* 2008;38:1400-1408.
- Spickett G. *Oxford Handbook of Clinical Immunology and Allergy*. Oxford University Press, 2013;3:550-3551.
- Adkinson Jr NF, Bochner BS, Busse WW, Holgate ST, Lemanske Jr RF, Simons FER. *Middleton's Allergy Principles & Practice*, Philadelphia : Elsevier 2009;7(2):1272-1275.
- Ortolani C, Ispano M, Pastorello EA, et al. Comparison of results of skin prick tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. *J Allergy Clin Immunol* 1989;83:683.
- De Weck AL, Sanz ML, Gamboa PM, et al and members of ENDA. Diagnosis of Immediate-Type β -lactam allergy in vitro by flow-cytometric basophil activation test and sulfidoleukotriene production: a multicenter study. *J Investig Allergol Clin Immunol* 2009;19(2):91-109.
- Solensky R. Allergy to penicillins. www.uptodate.com. (accessed 31/01/2013).
- Eriksson NE. Allergy screening with Phadiatop and CAP Phadiatop in combination with a questionnaire in adults with asthma and rhinitis. *Allergy* 1990;45(4):285-92.
- Wall K, Norén M, van Rooyen C. Phadiatop – an atopy test with unique allergen composition relevant for most geographical regions. *J Allergy Clin Immunol* 2014; in press.
- Borres MP, Ebisawa M, Eigenmann PA. Use of allergen components begins a new era in paediatric allergology. *Pediatr Allergy Immunol* 2011;22:454-461.
- Hauser M, Egger M, Wallner M, Wopfner N, Schmidt G, Ferreira F. Molecular properties of plant food allergens: a current classification into protein families. *Open Immunol J* 2008;1:1-12.
- Pascal M, Munoz-Cano R, Reina Z, et al. Lipid transfer protein syndrome: clinical pattern, cofactor effect and profile of molecular sensitization to plant-foods and pollens. *Clin Exp Allergy* 2012;42:1529-1539.
- Ebo DG, Hagendorens MM, Bridts CH, De Clerck LS, Stevens WJ. Sensitization to cross-reactive carbohydrate determinants and the ubiquitous protein profilin: mimickers of allergy. *Clin Exp Allergy* 2004;34:137-144.
- Sastre J, Landivar ME, Ruiz-Garcia M, Adregnette-Rosigno MV, Mahillo I. How molecular diagnosis can change allergen-specific immunotherapy prescription in a complex pollen area. *Eur J Allergy Clin Immunol* 2012;67(5):11-709.
- Eller E, Bindslev-Jensen C. Clinical value of component-resolved diagnostics in peanut-allergic patients. *Allergy* 2013;68:190-194.
- Ferreira F, Hawranek T, Gruber P, Wopfner N, Mari A. Allergic cross-reactivity: from gene to the clinic. *Allergy* 2004;59:243-267.
- Wal JM. Structure and function of milk allergens. *Allergy* 2001;56(suppl 67):8-35.
- Dean T, Heine RG. Egg allergy in childhood: an update. *Curr Opin Allergy Clin Immunol* 2009;9(3):50-244.
- Haneda Y, Kando N, Masahiro Y, et al. Ovomucoid IgE is a better marker than egg white-specific IgE to diagnose boiled egg allergy. *J Allergy Clin Immunol* 2012;129(6):1681-2.
- Quirce S, Maranon F, Umpierrez A, de Las Heras M, Fernandez-Caldas E, Sastre J. Chicken serum albumin (Gal d 5) is a partially heat-labile inhalant and food allergen implicated in the bird-egg syndrome. *Allergy* 2001;56(8):62-754.
- Van Do T, Elsayed S, Florvaag E, Hordvik I, Endresen C. Allergy to fish parvalbumins: studies on the cross-reactivity of allergens from 9 commonly consumed fish. *J Allergy Clin Immunol* 2005;116(6):6-1320.
- Yu C-J, Lin Y-F, Chiang B-L, Chow L-P. Proteomics and Immunological analysis of a novel shrimp allergen, Pen m2. *J Immunol* 2003;170:445-453.
- Ayuso R, Lehrer SB, Reese G. Identification of continuous, allergenic regions of the major shrimp allergen pen a 1 (tropomyosin). *Int Arch Allergy Immunol* 2002;127(1):27-37.
- Müller U, Schmid-Grendelmeier P, Hausmann O, Helbling A. IgE to recombinant allergens Api m1, Ves v 1, and Ves v 5 distinguish double sensitization from cross reaction in venom allergy. *Allergy* 2012;67:1069-1073.
- Moneret-vautrin DA, Sainte-Laudy J, Kanny G, Fremont S. Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. *Ann Allergy Asthma Immunol* 1999;82:33-40.
- Erdmann SM, Heussen N, Moll-Slodowy S, Merk HF, Sachs B. CD63 expression on basophils as a tool for the diagnosis of pollen-associated food allergy: sensitivity and specificity. *Clin Exp Allergy* 2003;33:607-614.
- Sudheer PS, Hall JE, Read GF, Rowbottom AW, Williams PE. Flow cytometric investigation of peri-anaesthetic anaphylaxis using CD63 and CD203c. *Anaesthesia* 2005;60:251-256.
- Abuaf N, Rajoely B, Ghazouani E, Levy DA, Pecquet C, Chabane H. Validation of a flow cytometric assay detecting in vitro basophil activation for the diagnosis of muscle relaxant allergy. *J Allergy Clin Immunol* 1999;104(2 pt 1):411-218.
- Camboa P, Sanz ML, Caballero MR, Urrutia I, Antepara I, Esparza R. The flow-cytometric determination of basophil activation induced by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) is useful for in vitro diagnosis of the NSAID hypersensitivity syndrome. *Clin Exp Allergy* 2004;34:1448-1457.
- Eberlein B, León SI, Darsow U, Rüeff F, Benhrendt H, Ring J. A new basophil activation test using CD63 and CCR3 in allergy to antibiotics. *Clin Exp Allergy* 2010;40:411-418.
- Erdmann SM, Sachs B, Schmidt A, et al. In vitro analysis of birch-pollen-associated food allergy by use of recombinant allergens in the basophil activation test. *Int Arch Allergy Immunol* 2005;136:230-238.
- Wallowitz ML, Chen RY, Tzen JTC, Teuber SS. Ses i 6, the sesame 11S globulin, can activate basophils and shows cross-reactivity with walnut in vitro. *Clin Exp Allergy* 2007;37:929-938.

45. Merrit K, Rodrigo JJ. Immune response to synthetic materials. Sensitization of patients receiving orthopaedic implants. *Clin Orthop* 1996;326:71-9.
46. Deutman R, Mulder TJ, Brian R, Nater JP. Metal sensitivity before and after total hip arthroplasty. *J Bone Joint Surg Am* 1977;59(7):862-5.
47. Elves MW, Wilson JN, Scales JT, Kemp HBS. Incidence of metal sensitivity in patients with total joint replacements. *Br Med J* 1975;4(5993):8-376.
48. Waterman AH, Schrik JJ. Allergy in hip arthroplasty. *Contact Dermatitis* 1985;13(5):294-301.
49. Thomas P, Braathen LR, Dörig M, Auböck J, Nestle F, Werfel T, Willert HG. Increased metal allergy in patients with failed metal-on-metal hip arthroplasty and peri-implant T-lymphocytic inflammation. *Allergy* 2009;64(8):1157-1165.
50. Hari Y, Frutig-Schnyder K, Hurni M, et al. T cell involvement in cutaneous drug eruptions. *Allergy* 2001;56:1398-1408.
51. Niggemann B, Reibel S, Wahn U. The atopy patch test (APT) – a useful tool for the diagnosis of food allergy in children with atopic dermatitis. *Allergy* 2000;55:281-285.
52. De Boissieu D, Wageut JC, Dupont C. The atopy patch tests for detection of cow's milk allergy with digestive symptoms. *J Pediatr* 2003;142(2):203-205.
53. Valentine-Thon E, Schiwara H. Validity of MELISA for metal sensitivity testing. *Neuroendocrinol Lett* 2003;24(1-2):57-64.
54. Stange AW, Furman FJ, Duane EH. The Beryllium Lymphocyte Proliferation test: relevant issues in beryllium health surveillance. *Am J Indust Med* 2004;46:453-462.
55. Middleton DC, Lewin MD, Kowalski PJ, Cox SS, Kleinbaum D. The BeLPT: Algorithms and implications. *Am J Indust Med* 2006;49:36-44.
56. Sachs B, Erdmann J, Malte Baron M, Neis T, Masaoudi, Merk HF. Determination of interleukin-5 secretion from drug-specific activated ex vivo peripheral blood mononuclear cells as a test system for the in vitro detection of drug sensitization. *Clin Exp Allergy* 2002;32:736-744.
57. Nyfeler B, Pichler WJ. The lymphocyte transformation test for the diagnosis of drug allergy: sensitivity and specificity. *Clin Exp Allergy* 1997;27:175-181.
58. Flinterman AE, Pasmans SGMA, den Hartog Jager CF, Hoekstra MO, Bruijnzeel-Koomen CAFM, Knol EF, van Hoffen E. T cell responses to major peanut allergens in children with and without peanut allergy. *Clin Exp Allergy* 2010;40:590-597.
59. Motrich RD, Gottero C, Rezzonico C Jr, Rezzonico C, Riera CM, Rivero V. Cow's milk stimulated lymphocyte proliferation and TNF alpha secretion in hypersensitivity to cow's milk protein. *Clin Immunol* 2003;109:203-211.
60. Shek LPC, Bardina L, Castro R, Sampson HA, Beyer K. Humoral and cellular responses to cow milk proteins in patients with milk-induced IgE-mediated and non-IgE-mediated disorders. *Allergy* 2005;60:912-919.
61. Condemi JJ. Unproved diagnostic and therapeutic techniques. In: Metcalfe DD, Sampson HA, Simon RA, eds. *Food Allergy: Adverse reactions to foods and food additives*. London: Blackwell Scientific, 1997;2.
62. James JM. Unproven diagnostic and therapeutic techniques. *Curr Allergy Asthma Rep* 2002;2:87-91.
63. Potter PC, Mullineux J, Weinberg EG. The ALCAT test – inappropriate in testing for food allergy in clinical practice. *S Afr Med J* 1992;81:384.
64. Position Statement, ALCAT and IgG Allergy & Intolerance Tests. www.allergy.org (accessed 21 November 2013).

new Omnair® Nasal Spray for Allergic Rhinitis

Takeda, is pleased to announce the introduction of a NEW Nasal Spray, Omnair®.

Omnair® Nasal Spray (50 µg ciclesonide) is indicated for:

-  The treatment of nasal symptoms associated with seasonal allergic rhinitis in adults and children 6 years of age and older.
-  The treatment of nasal symptoms associated with perennial allergic rhinitis in adults and children 12 years of age and older.

Omnair® Nasal Spray (120 puff metered spray), is a topical glucocorticosteroid (ciclesonide) delivered as a hypotonic suspension that has anti-inflammatory properties. Ciclesonide is a pro-drug that is enzymatically hydrolysed to a pharmacologically active metabolite des-ciclesonide following intranasal application.

Des-ciclesonide has anti-inflammatory activity with affinity for the glucocorticoid receptor that is 120 times higher than the parent compound.

Omnair® Nasal Spray (50 µg ciclesonide) has less than 1 % systemic availability with 99 % protein binding.



omnair®
(ciclesonide) Nasal Spray, 50 µg



Omnair® once daily dosage

**2 sprays (50 µg/spray)
in each nostril once daily.***

* SAR > 6 Years; PAR > 12 Years

53 OMNIAIR® Nasal Spray, Reg. No. 43/21.5.1/0008. Each nasal spray is a metered-dose, manual pump spray formulation containing a hypotonic aqueous suspension of ciclesonide. Each puff (actuation) of the pump delivers 50 micrograms of ciclesonide in a volume of 70 microlitres from the nasal actuator. Each bottle contains: 7.9 g of suspension/bottle (10 ml bottle) and 13.5 g of suspension/bottle (15 ml bottle). Preservatives: Contains potassium sorbate 0.12 % and edetate sodium 0.01 %. For full prescribing information refer to the approved package insert.
TAKEDA (Pty) Ltd. Reg. No. 1982/011215/07. 1 Libertas Road, Corner Main Road and Sloane Street, Bryanston 2191. Tel: 0861 TAKEDA (0861 825 332). Fax: 0861 TAKE SA (0861 825 372).
OMN2014-02-003

