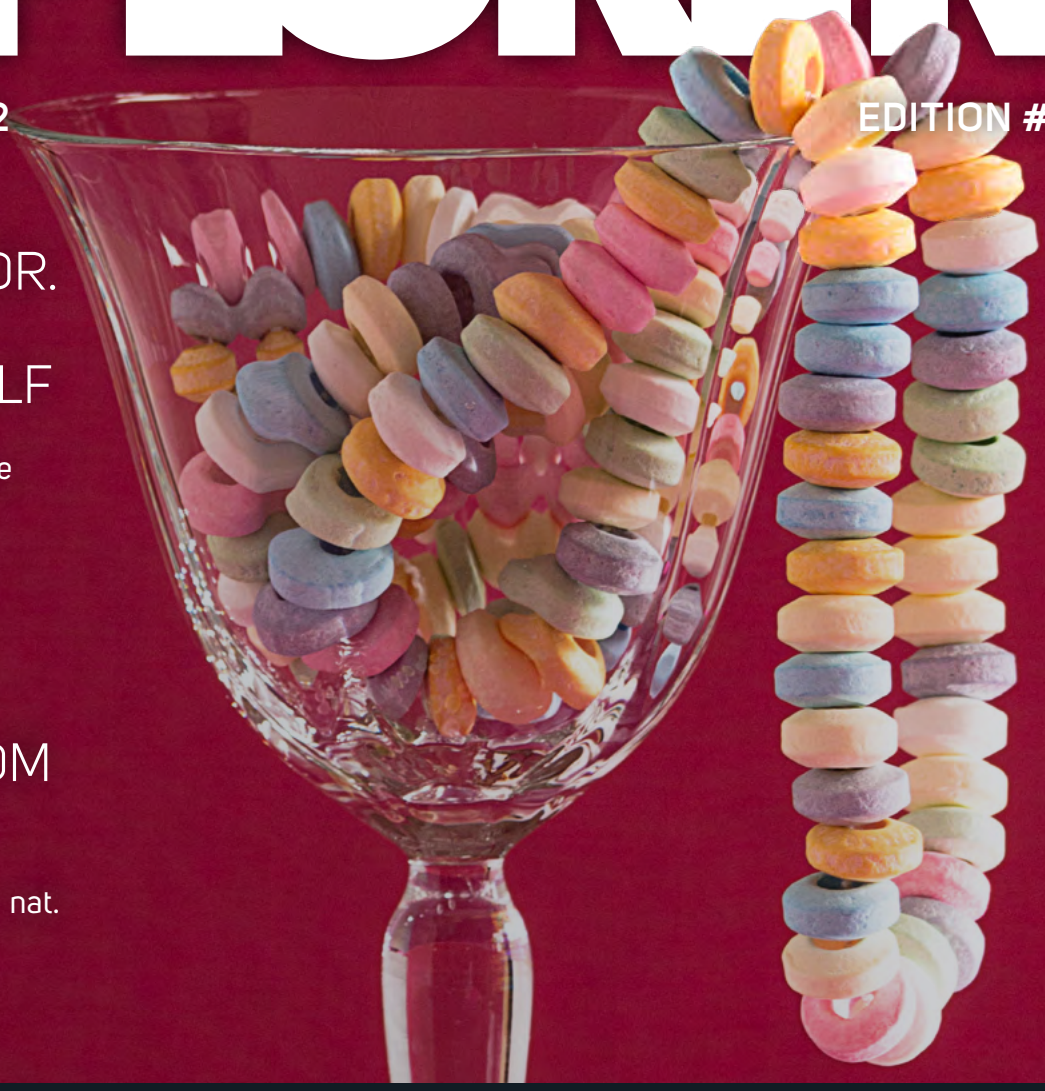
THE XPLORER

NOVEMBER 2022

EDITION # 2



INTERVIEW WITH PROF. DR. RER. NAT. MONIKA RAULF

CCDs in clinical practice

More on page 3

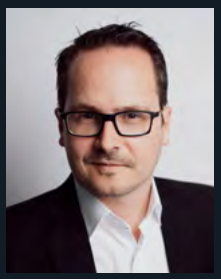
CCDs AND INSECT VENOM ALLERGY

Guest article by Dr. rer. nat. Irene Mittermann

More on page 11

CONTENTS

EDITORIAL



DEAR READERS,

welcome to the 2nd edition of THE XPLORER. Our first edition in June 2022 was about tropical fruits and their allergenic potential. In this second edition, our focus lies on CCDs (crossreactive carbohydrate determinats) and their influence on allergy diagnostics.

To shed light on this topic, we spoke to Prof. Dr. rer. nat. Monika Raulf, the head of the Competence Center of Allergology/Immunology at the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA) about the role of CCD-specific IgE antibodies in clinical practice.

Additionally, we dedicated another chapter to the diagnostic relevance of CCDs in allergy diagnosis, with a special focus on CCDs and insect venom allergy and how the blocking of CCD-specific antibodies increases the accuracy of in-vitro allergy diagnostics.

In the second part of THE XPLORER, we will explain some more about our newly opened e-learning platform, the MADx Academy, and give you a recap about the first ever MADx Distributor Convention, which took place in Vienna on September 5th and 6th, 2022. Thank you for your continued interest and support for this new project, and please enjoy the second issue of THE XPLORER!

Christian Harwanegg
CEO Macro Array Diagnostics

CCDs in clinical practice

Interview with Prof. Dr. rer. nat. Monika Raulf

How do you view the role of CCD-specific IgE antibodies in clinical practice?

Dr. Raulf: Since many allergens are glycoproteins, which are frequently found in pollen, especially grass pollen, in plant foods, but also in natural latex and woods as well as in insects, CCD-specific IgE antibodies are noticeably found in sera from patients with multiple sensitisations to plant allergens. The cross-reactive carbohydrates are present as side chains and thus relatively exposed in the glycoproteins and can thus easily act as immunogenic binding sites, i.e. IgE antibodies can easily bind there. This often makes it difficult to identify the allergens that are causative for severe symptoms. The detection of IgE antibodies against CCDs is on the one hand specific and correctly positive but does not correlate with the clinic.

Therefore, in my opinion, it is part of the diagnostic algorithm in polysensitised patients, for example with natural latex sensitisation without evidence of occupational natural latex contact, to test for anti-CCD antibodies in the serum. For example, we were able to prove that specific IgE against CCDs is only very rarely detectable in latex allergy sufferers from the healthcare sector. If, on the other hand, latex-specific IgE is found in polysensitised patients without detectable natural latex exposure, anti-CCD antibodies are also present in most cases.

> "CCD inhibition improves the specificity of allergy tests."

Specific IgE antibodies against CCDs could also be detected in employees from the wood processing industry with IgE antibodies against beech and pine wood, especially in all double-sensitised patients. This study and the further characterisation of the study group showed that in employees without allergic symptoms, the IgE binding was predominantly based on these carbohydrate structures. Based on this experience, it is part of our routine algorithm to also test CCDs, especially in the case of multiple IgE reactions to plant allergens.

Do you routinely test for the presence of CCD-specific IgE antibodies? If yes, how often do you detect CCD-positive sera? Which allergen sources are frequently CCD-positive in testing?

Dr. Raulf: Yes, we routinely test for CCD-specific IgE, especially in polysensitised patients, e.g. in cases of suspected wood dust sensitisation and in patients with positive IgE antibodies against natural latex if there is no occupational exposure. Among polysensitised patients, we often find that antibodies against CCDs are also present.

Which characteristics of IgE test systems have an influence on the level of CCD interference?

Dr. Raulf: Especially when using multi-allergens, strip tests and all assays in which naturally purified, especially food allergens are used, the CCD problem is still of great importance. For food allergen sources, the use of total extracts is still relevant and thus also the glycostructures contained therein, as component-resolved diagnostics are not fully available for these allergen sources, or not all allergens have been identified, isolated and are available yet.. One should therefore always bear in mind that differentiation between anti-CCD IgE and protein-specific or peptide-specific IgE is of great importance. To ensure the clinical relevance of the results of a blood allergy test, multiplex tests that also use extracts or allergen components isolated from the extracts should definitely integrate inhibition of anti-CCD-specific IgE antibodies. If the allergens are available in recombinant form, these are usually without CCD components and are to be preferred.

What do you see as the value of blocking CCD-specific IgE antibodies?

Dr. Raulf: For in-vitro diagnostics, natural glycoproteins such as bromelain, horseradish peroxidase (HRP) and ascorbate oxidase have proven to be screening tools, with HRP having the higher sensitivity. The glycoproteins mentioned above can be used to specifically detect anti-CCD IgE antibodies. They can also be used for inhibition, i.e. blocking of anti-CCD-specific IgE antibodies. However, the use of CCD tools alone does not allow to determine whether the IgE binding to a specific allergen source is based exclusively on CCDs or whether additional peptide epitopes are recognised or protein-specific IgE antibodies are involved.

The inhibition of CCD-specific IgE antibodies increases the specificity of an allergy test. Screening for CCD-specific IgE can be laborious and cannot always be performed in routine applications. Therefore, it may be advantageous to use test systems that automatically integrate this inhibition step. Recombinant allergens that are produced in E.coli usually do not comprise CCD epitopes and are to be preferred.

Another possibility to circumvent CCD interference is the use of molecular allergens – what experience have you had in this regard?

Dr. Raulf: The use of recombinantly produced allergens can circumvent this CCD problem. However, especially for occupational allergens, except for natural latex, there are only commercially available recombinant single allergens for very few allergen sources. In the context of elucidating sensitisation to natural latex, we have developed a diagnostic algorithm for serology using the available recombinant latex allergens as well as CCD tools. Thus, differentiation between clinically relevant allergy and sensitisation based on CCDs or other panallergens is possible, so that the patient can be given individual prevention and, if necessary, therapy recommendations.

ABOUT

PROF. DR. RER. NAT. MONIKA RAULF
studied chemistry and biology and obtained her doctorate at the Faculty of Biology before taking up a professorship at the Faculty of Medicine. Since 1990 she has been head of the Allergology/Immunology Competence Centre at the Institute for Prevention and Occupational Medicine of the DGUV, Institute of the Ruhr University Bochum (IPA).

> Polysensitised patients often have antibodies to CCDs.

A short introduction to CCDs

Most proteins in cells and tissues of all kinds of species are glycoproteins, i.e. proteins to which one or more sugar chains are covalently coupled.[1] [2] Due to the broad repertoire of monosaccharides and the numerous possibilities how they can be connected with each other, a vast number of such carbohydrate side chains exists. Strikingly, only a handful of distinct patterns were described to elicit a specific IgE-response in humans[3], either due to the presence of linking-patterns or of monosaccharides that are not found in humans.[4]

The most common sugar structures involved in IgE-responses are the "classical" cross-reactive carbohydrate determinants (CCDs) that are present in many different allergen sources both of plant- and animal origin.[4] Notably, IgE antibodies which bind to CCD structures are only occasionally involved in causing allergy symptoms.

Essentially, two concepts were proposed to explain this rare phenomenon: first, CCD-specific IgE was suggested to exhibit low affinity for its target structure and, therefore, should not be capable of inducing a stable cross-linking of IgE that is bound to effector cells which is required for activation of the latter. In contrast to this assumption, a study conducted with rabbit antisera showed high binding affinities[5] but, however, those results were not entirely conclusive for methodological reasons (affinity measurement based on polyclonal sera, controversial results obtained from different rodents). The second hypothesis assumed, that the distance between adjacent sugar-based IgE-binding sites on the surface of the glycoprotein is too large, that is incompatible with efficient and stable IgE cross-linking.[4] The understanding of those mechanisms is even further blurred by cases of patients showing a dicrepancy between CCD-induced basophil activation in-vitro.[6]

However, since CCD-specific IgE antibodies are detectable in more than 30% of pollen allergic subjects and even in 5% of the non-allergic population[7], they frequently lead to misleading results in serological allergy diagnostics, when allergen extracts, which frequently contain CCDs, or CCD-containing natural allergens are used. In this issue, methods to overcome this limitation are described.

The diagnostic relevance of CCDs

Cross-reactive carbohydrate determinants (CCDs) are sugar structures present in various pollen, fruits, cereals, nuts, spices, and insect venoms and can induce IgE production in up to 30% of allergic individuals, which can be regarded as a serious problem.[7] [8]

CCDs can be recognised by the human immune system as foreign and, in some individuals, may elicit the production of IgE antibodies.[9] IgE antibodies reactive with CCD epitopes are believed to have limited or no clinical significance partly due to their low avidity (total strength of multiple bindings between antibody and antigen) and marginal biological activity. In in-vitro diagnostic assays, CCD-reactive IgE antibodies neither predict the development of clinical symptoms upon allergen exposure nor are they associated with disease severity. [10] [11]

CCD reactivity, however, can impact the diagnostic accuracy of the quantitative measurement of IgE antibodies in a patient's serum analysis and thus has a major impact on the outcome of IgE testing. CCDs are a common cause of falsely elevated as well as false positive test results in in-vitro allergy diagnostic tests where glycosylated natural allergens or extracts are used. Such extracts could contain glycoproteins, which may react with patients' IgE. In the case of plant and insect venom allergens, the relevant epitope structure is an α-1,3-fucose on the Asn-linked sugar residue of so-called N-glycans. Due to their wide distribution, N-glycans carrying this epitope are known as CCDs.

The co-determination of IgE antibodies directed against CCD structures can give a misleading impression of clinically relevant polysensitisation and lead to inaccurate diagnosis and, subsequently recommendation of non-indicated immunotherapy. Remedies for sIgE based in-vitro diagnostics come in the form of non-glycosylated recombinant allergen components or of specific CCD inhibitors. The high potential of recombinant allergens is optimally realised in the context of molecular allergy diagnostics using allergen arrays with a total of nearly 300 allergens and extracts, whereas CCD inhibitors increase the specificity of conventional extract-based diagnosis. Reagents for the detection and the inhibition of the binding of CCD-specific IgE antibodies from plants and insects have been developed, whereas tools for galactose-containing cross-reactive carbohydrate determinants (GalCDs) of milk and meat lag behind.[12]

CROSS-REACTIVITY

Cross-reactive carbohydrate determinants as the name already suggests, are responsible for many different cross-reactions. In IgE cross-reactivity, antibodies bind to proteins, carbohydrates, or glycoproteins that have identical or similar antigenic properties. On the one hand, this can be due to a high degree of sequence similarity (linear epitopes) and on the other hand due to the similarity of the 3D structure (conformational epitopes).

"CLASSIC" CCDs

CCDs of the plant kingdom, arthropods and molluscs can be divided into two types, MMXF and MUXF. These are N-glycans with α-1,3-bound fucose, as they are found particularly in insects and in the entire plant kingdom – but not in mammals. In plants, an additional xylose at position 2 of the glycan backbone (β-1,2-linked xylose) can also represent an antigenic determinant.

Carbohydrates are present as side chains, so they are exposed and represent a binding site for IgE antibodies and are responsible for cross-reactions. Since plant allergens are mostly glycoproteins and occur frequently in pollen, especially grass pollen, as well as in plant foods and natural latex, IgE antibodies directed against CCDs are mainly found in sera from patients with multiple sensitisations to plant allergens. As CCD-specific IgE antibodies are largely clinically irrelevant, they can however complicate allergy diagnosis.

GALACTOSE-α-1,3-GALACTOSE (α-GAL)

Another cross-reactive carbohydrate determinant was found to be a potential cause of anaphylactic reactions to a recombinant glycoprotein drug (Cetuximab) containing α-1,3-galactose.[13] This galactose-containing determinant (GalCD, galactose containing cross-reactive carbohydrate determinant) was supposed as a trigger for delayed allergic reactions to red meat in several cases.[14] GalCDs are sugar structures on mammal cells and tissues, except in primates and humans who are not capable of producing the sugar residue naturally in the body. Thus, GalCDs may have clinical relevance in certain cases and can trigger meat allergies – possibly as a result of tick bites.[15]

At present, evidence is accumulating for red mammalian meat, tick bites and helminth infestations as the cause of sensitisation against GalCDs. American researchers assume that those affected have suffered bites from the American tick (Amblyomma americanum) beforehand. The carbohydrate is present in the saliva of the American tick and could thus explain the specific immune reaction against the molecule. However, the exact mechanism has remained unclear until now.

Allergies to α-Gal, unlike true meat allergy (allergy to the protein component of muscle meat), are limited to beef, lamb, venison, and pork. Offal contains a significantly higher concentration of α-Gal than pure muscle meat. However, the sugar molecule is not present in poultry or any kind of fish, therefore allergic reactions do not occur when poultry or fish are eaten.

Often, however, GalCDs probably cause false-positive results with milk and meat extracts.

Plants

MMX

MMXF3

MUXF3

Insects

MMF3F6

Mammals (excl. primates)

α-GAL

KEY

- N-Acetylglucosamine
- Galactose
- Fucose
- Mannose
- Xylose

THE IMPORTANCE OF CCDs IN ALLERGY DIAGNOSTICS

In literature, presence of anti-CCD antibodies is reported in up to 30% of allergic individuals. Therefore, it is essential to consider the possible presence of CCD-specific IgE antibodies in a patients' serum sample.

Adsorption of these sIgE CCD antibodies leads to results with higher clinical specificity, especially with regard to plants, plant food, molluscs, and insect venoms.[16] [17]

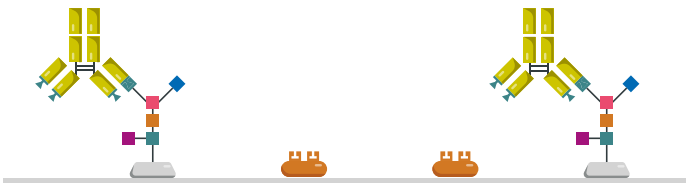
CCD-FREE ALLERGY DIAGNOSTICS WITH ALEX²

To eliminate CCD antibody interference in IgE testing, Macro Array Diagnostics integrated a CCD blocker which has the potential to adsorb CCD-specific antibodies. The CCD blocker is part of every ALEX² test kit without the need for an additional incubation step.

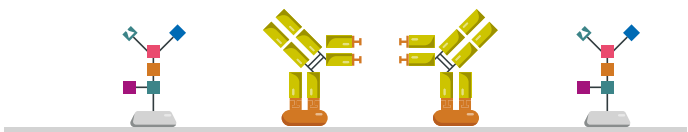
The CCD blocker is included in the serum diluent which is used in the first assay step. CCD-specific IgE is bound by the CCD blocker and, therefore, cannot combine with the CCD-structures present on the allergens. Thus, the CCD blocker prevents the binding of CCD-specific antibodies to CCDs on purified allergens (e.g. Cor a 11 from hazel nut) or CCD-containing allergen extracts (e.g. onion). This results in an IgE signal without CCD interference. Following a washing step, a color reaction is induced, and this results in the specific measurement of only allergen specific IgE antibodies. The important effect of CCD blocking becomes clear when comparing the results of an ALEX² test with and without CCD blocking. The test lacking the CCD blocking step generates several misleading test results. The ALEX² test performed with the CCD blocker eliminates misleading test results due to CCD interference, avoids clinically irrelevant results and is essential for accurate in-vitro allergy testing. This approach to IgE testing leads to a better patient management by avoiding overtreatment.

Watch video

False Positive



True Positive



KEY

- Allergen with CCDs
- Allergen without CCDs
- CCD-specific IgE antibody
- Protein-, peptide-specific IgE antibody

CCD case study

Eva, 19, from North-Western Europe

CLINICAL HISTORY

Allergic rhinitis caused by allergy to grass pollen and house dust mite has been observed since childhood. Furthermore, no allergies, other illnesses or regular medication are stated. Eva's mother has been advised to withhold peanuts and nuts during her pregnancy. Up to now Eva has avoided both peanuts and nuts, but traces of these foods were not avoided in the past. As a child she experienced atopic dermatitis, and she was treated with asthma medication in case of airway infections until the age of 12. No information on allergies in directly related persons could be collected.

PRESENT SITUATION (2019)

Last year Eva visited a general practitioner (GP) because of stomachache and diarrhoea after ingestion of an Asian dish. She asked the GP whether allergy to peanuts could be the cause of her symptoms. Specific IgE tests (singleplex) were ordered by the GP and these showed the following results:

Allergen source	IgE level [kU_A/L]
Almond	67.0
Hazelnut	33.2
Peanut	21.1
Coconut	6.6
Brazil nut	0.74

The patient was then referred to an allergologist, who repeated the in-vitro tests after one month (January 2019), with the same outcome.

Skin prick tests were performed and showed positive results for grass pollen and house dust mite. Prick-to-prick tests with fresh nuts – almond, brazil nut, cashew, hazelnut, macadamia, pecan nut, peanut and pistachio – showed negative results.

Provocation testing with peanut (open oral food challenge) also showed a negative result.

An ALEX test was requested to re-evaluate the specific IgE tests from December 2018 and January 2019.

INTERPRETATION (results on page 10)

- The skin prick test results were confirmed on the molecular level for grass pollen and house dust mite.
- The major grass pollen allergens Lol p 1 and Phl p 1 were positive, confirming a genuine sensitisation against grass pollen. AIT against grass pollen is indicated in the presence of corresponding symptoms.
- Pollen extract from rye showed a positive result due to cross-reactivity to timothy perennial rye grass components Phl p 1 and Lol p 1.
- The major house dust mite allergens Der f 1 and Der p 1 were positive, corresponding to a genuine sensitisation to house dust mite. In the presence of corresponding symptoms – AIT is indicated.
- On rare occasions Der p 5 is the only positive molecular allergen in mite sensitised patients, the implications for AIT prescription have not been researched yet.
- Ves v 5, one major component of wasp venom, was also positive. As the patient did not mention wasp allergy as an elicitor of symptoms, it can be assumed that the sensitisation is most likely clinically irrelevant.
- All tested plant foods, including possible triggers of strong allergic reactions, such as peanut or tree nuts, which were positive with the traditional test (singleplex) system, were negative.
- Previously avoided foods can be reintroduced into the patient's diet.

ALEX TEST RESULTS

Allergen, allergen source	Biochemical designation	Without CCD blocking [kU_A/L]	With CCD blocking (Standard protocol) [kU_A/L]
Phl p 1, Timothy grass	Beta-Expansin	9.03	9.02
Der p 1	Cystein Protease	14.16	13.69
Ves v 5, wasp	Antigen 5	0.36	0.51
Peanut	Extract	2.67	≤ 0.10
Lentil	Extract	2.67	≤ 0.10
Cor a 11, hazelnut	7S Globulin	3.27	≤ 0.10
Jug r 2, walnut	7S Globulin	9.83	≤ 0.10
Almond	Extract	2.36	≤ 0.10
Sunflower seed	Extract	9.36	≤ 0.10
Buckwheat	Extract	9.93	≤ 0.10
Garlic	Extract	10.51	≤ 0.10

Complete case study

SUMMARY

- The ALEX allergy test confirmed the results of the skin tests.
- Depending on the clinical symptoms, an indication for AIT against grass pollen and mite allergy can be given.
- The automatic CCD blocking of the ALEX test drastically reduced the number of clinically irrelevant results: **from 110 to 10.**
- CCD-specific IgE antibodies can have a strong influence on test results.
- Significantly better agreement of results with CCD blocking with the clinical picture.
- The interpretative effort of the physician was considerably reduced by blocking CCD-specific antibodies.

CCDs – a confounding factor in venom allergy

Venoms of honeybees and wasps can induce IgE-mediated hypersensitivity reactions in insect venom allergic patients which can manifest as local to severe systemic allergic reactions. Venom allergy is one of the most important causes of life-threatening anaphylactic reactions in adults. Severe reactions can occur immediately after a sting.[18] [19] The prevalence of large local reactions (LLR) is up to 26% in the adult population, where LLR are defined as swelling larger than 10 cm in diameter, lasting up to 48 hours. According to epidemiologic data between 3 and 8% of adults and 1% of children have a history of severe sting reactions. Severe allergic reactions to insect venoms affect different organs and include generalised swelling and itching, urticaria, angioedema, diarrhea, vomiting, chest tightness, shortness of breath with swelling of the throat and bronchospasm. Thus, the skin, the respiratory, gastrointestinal, and cardiovascular systems are affected. At least 40-100 fatal sting reactions are reported in the United States every year, and it is likely that additional deaths are not recognised and therefore not reported.[20] Unfortunately, there are no surrogate markers available to predict who will have a systemic reaction after a sting and whether it will be severe or not.[21] Insect venoms frequently causing severe allergic reactions belong to the order Hymenoptera and to the family of Apidae (Apis mellifera, honeybee), Vespidae (Vespula vulgaris, Vespula germanica, wasp, yellow jacket) or paper wasp (Polistes dominula).

BEE AND WASP VENOM ALLERGENS

Allergens present in Hymenoptera venoms may trigger a type I hypersensitivity reaction, mediated by allergen-specific Immunoglobulin E (IgE) antibodies, which is a risk factor for subsequent allergic reactions. Insect venom allergy occurs worldwide and is associated with several social restrictions and limitations of peoples' daily activities. Unfortunately, most of the patients have difficulties to correctly identify the stinging insect. Up to 50% of the patients with Hymenoptera allergy reveal double positive results in serological tests using bee and wasp venom extracts. This poses the problem of identifying the relevant venom for immunotherapy, especially in patients that show a severe reaction only with one venom.[22] Besides a thorough clinical history, testing for specific IgE-reactivity to purified natural or recombinant venom allergens has become an important diagnostic tool to identify the disease-causing insect. The identification of the insect venom causing the allergy reaction is essential for the selection of the right venom for a successful immunotherapy.

During the last 25 years, molecular biology techniques have allowed the identification and characterisation of bee and wasp venom allergens. For bee venom allergy, phospholipase A2 (Api m 1), hyaluronidase (Api m 2), and icarapin (Api m 10) are the most common allergens. Phospholipase A1 (Ves v 1) and Antigen 5 (Ves v 5) are important wasp venom allergens.[20] All the major bee venom allergens are highly immunogenic glycoproteins, consisting of a polypeptide (protein) chain with carbohydrate groups attached. Api m 1, Api m 2 and Api m 10 and also the wasp venom allergens Ves v 2 (hyaluronidase) and Ves v 3 (dipeptidyl peptidase IV) bear carbohydrates. It is important to know that the glycosylated allergens can bind IgE antibodies from allergic patients via protein and/or carbohydrate epitopes. In contrast to the major bee venom allergens, Ves v 1 and Ves v 5, the major wasp venom allergens, are not glycosylated.

N-GLYCOSYLATION

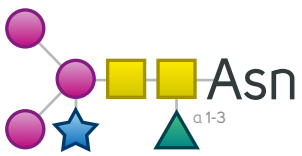
N-glycosylation is a post-translational modification of eukaryotic proteins, where oligosaccharide moieties are attached to the amino acid residues of asparagine (Asn, N). N-glycan branches can comprise different carbohydrate moieties, like mannose, fucose and xylose, depending on the organism or cell type. For example, fucose is present in the same manner on glycosylated proteins from pollen, plant-derived food, and insect venom.[23] For some proteins, N-glycosylation is necessary for the correct folding, for the function and half-life of the protein and can be critical for cell attachment or protein-ligand interactions.

CCDs

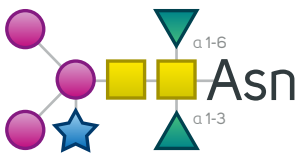
It was found that carbohydrate residues, especially α-1,3 fucose bound to the N-glycan core, are highly immunogenic and capable of eliciting an IgE immune response in about 20% of allergic patients. As glycosylated allergens from pollen, plant-derived food and insect venoms share the same carbohydrate moieties, the sugar residues comprise highly cross-reactive IgE epitopes and thus called cross-reactive carbohydrate determinants (CCD). CCDs can cause IgE-cross-reactivity between bee and wasp venom, but additionally between unrelated allergen sources like pollen, plant derived food, moulds, latex and even helminths and mites.[24] The clinical relevance and allergenic activity of carbohydrate-directed IgE is low, meaning that CCD-specific IgE does not cause symptoms. Therefore, CCD-specific IgE can lead to false positive and misleading in-vitro test results. Extracts, which are obtained from natural allergen sources, may contain glycosylated proteins, which are able to bind CCD-specific IgE. Using allergen extracts or glycosylated allergens, positive IgE-results can be based solely on the detection of CCD-specific IgE. Sera containing CCD-specific IgE often show IgE-reactivity to a multitude of extracts from pollen, vegetables, fruit, and insect venom and to purified natural glycosylated allergens. Based on these false-positive test results, patients may get wrong dietary advice and even unjustified immunotherapy.[25]

Especially in insect venom allergy the presence of CCDs plays an emerging role in allergy diagnosis. More than 50% of the patients show IgE double-positivity to bee and wasp venom extracts.[26] Therefore, it is challenging to dissect a true co-sensitisation to both venoms from cross-reactivity with homologous allergens in both venoms (e.g., Api m 2, Ves v 2) or cross-reactivity caused by IgE directed to clinically irrelevant carbohydrate epitopes. For example, patients allergic to grass pollen can mount an IgE response to N-glycans, which can also cross-react with bee venom allergens (e.g., Api m 1) even though the patients were not allergic to bee venom. Conversely, bee venom is a potent inducer of CCD-specific IgE, which can cross-react with pollen glycoproteins.

N-glycosylation of Api m 1 and Ves v 2



Api m 1 from bee venom



Ves v 2 from wasp venom

RELEVANT MONOSACCHARINES

- N-acetylglucosamine
- Mannose
- Fucose
- Xylose

IMPROVED ACCURACY THROUGH CCD BLOCKING

CCD-specific IgE can be determined in serum samples by using a natural glycosylated protein like bromelain from pineapple or horseradish peroxidase. This CCD-test shows the presence of carbohydrate-specific IgE, but by testing the same serum samples to glycosylated allergens or allergen extracts no statement can be made, if the sera contain solely CCD-specific IgE or additionally peptide-specific IgE.[25]

To circumvent the problems of CCDs, allergens were expressed as non-glycosylated proteins in E. coli, but it has been observed that in some cases, the non-glycosylated allergens show an incorrect folding and thus lower IgE reactivity as the natural glycosylated ones. To avoid false-positive test results caused by CCD-specific IgE, a CCD blocker can be used. The CCD blocker consists of a non-allergenic protein- or peptide backbone with several N-glycan chains attached. By adding a CCD-blocker to the serum sample, carbohydrate-specific IgE will bind to the CCD-blocker. In washing steps, the CCD-blocker and carbohydrate-specific IgE will be removed and only peptide-specific IgE will be detected in the test assay. This way, results remain positive for relevant allergens and "true sensitisations" can be assessed. Thus, CCD blockers are a useful tool to increase the test specificity and to reduce the number of false-positive test results.

SUMMARY

- Up to 50% of patients with Hymenoptera allergy show double positive results in serological tests with bee and wasp venom extracts.
- It is difficult to distinguish true co-sensitisation to both venoms from cross-reactivity with homologous allergens or cross-reactivity directed against CCDs.
- CCD blockers are a useful tool to increase the specificity of the test and to reduce the number of false-positive test results.

Status quo: life science companies in Europe

with Dr. Christian Harwanegg, CEO of MADx

How easy or how difficult is it to get funding as a life science company in Europe and Austria?

Christian Harwanegg: Life sciences are a very diversified industry. Companies under that label can produce anything from pharmaceuticals, implants, software, animal health products, etc. and then of course there are also diagnostics companies like MADx.

From my experience as CEO of MADx, I would say that it is pretty difficult to finance a diagnostics company for several reasons. On the one hand, the market is very clearly defined or at least conceived as clearly defined, there are usually pre-existing competitive situations and price structures, specific segments, and health insurance companies need to cover the costs in most developed economies. These limitations always exist. When I presented the MADx business model to various investors as an easy-to-implement and almost fool-proof business model, the following question often came up: "If it's so easy, and the market is so weak in innovation and characterised by monopolistic structures, why hasn't anyone else done it before?"

Assuming one receives funding, what problems can still arise?

Christian Harwanegg: There is a good public funding structure in Europe, but it requires certain financial resources to be available still. Small and medium-sized companies, for example, are funded up to 70%, but of course you still need the full 100%. There is not much ground to cover with friends and family funds for an IT start-up that only needs some laptops and servers. For a diagnostics company the price of basic laboratory equipment for a small team without any high-tech instruments costs a five-digit Euro sum. If you add special lab equipment or customised instrumentation developed for your specific needs, you quickly find yourself in the six to seven figure range.

Another difficulty is finding a suitable location that meets the requirements and regulations, offers space to grow over time and is affordable. There are far too few laboratory locations in Austria, and in Vienna they cost 25 to 30 euros per m². That is two to five times the price of a "normal" office space depending how far out you are willing to locate yourself. The accessibility of the location is also important to motivate qualified employees to work for you.

How long does it take on average for a company in the life-science sector to become profitable?

Christian Harwanegg: It depends mostly on how inert the market is in which you are operating. The classic cycle regarding medical devices starts with product development and product registration with the necessary approvals. The approval process can be complex, accompanied by external and clinical studies, which can cost a lot of time and money. Once you can legally sell, you need to find a partner to market and distribute the product within the promising markets, unless you want to hire experienced sales staff right away.

For an investor, that's not interesting, because: From the idea to the prototype, until the product has a certain market maturity, you must calculate three to five years at best in the European market. If you want to gain a foothold in the USA or China, it takes an additional three to five years longer and there is an additional effort due to regulatory processes that you need to go through. Typically these countries have higher entry barriers than Europe.

After entering the market, however, you have to convince the market to buy your product. For medical professionals switching involves effort which often has to be paid for by a company. Decisions are made by a complex consortium of physicians, administrators, IT staff, legal and purchasing managers. The product performance must be shown by company external independent users, preferably by scientific publications in peer-reviewed journals - which can take another two to three years.

So, from the investor's perspective, five to eight years easily pass in which the company has to be supported - without knowing whether the product will really be a commercial success. Investors in the industry today are interested in business models that are highly scalable at an infinitely fast rate. An example would be business models that are highly scalable via digitalisation and can be rolled out in many countries without major legal and technical hurdles. In contrast, in diagnostics and medical technology and pharmaceutical products, there is a certain market limitation as there is only a limited number of patients who are in need of a certain product (or can afford it) and only a certain amount of the money pie is available from a total cake of health care spendings.

> "Investors want business models that can be scaled quickly and infinitely."

What difficulties are present in the European market?

Christian Harwanegg: Many innovative and valuable companies are founded and funded in Europe, either by European programs or local funding and subsidies. One of the big problems from my perspective is that often companies which have been subsidised for years or decades by public money in Europe, are being sold to US or Chinese conglomerates as soon as they are profitable, which then stop innovation, transfer production to emerging or third world countries, and skim off the profits – to buy more companies.

In countries like China and Russia, public contracts are generally awarded to domestic manufacturers. Only if there is no other manufacturer does the contract go to a foreign manufacturer. While these countries follow the "Me first" principle, the European market is much more open. For example, any American or Chinese manufacturer can immediately launch and sell its CE-marked product on the European market. For a European diagnostics provider to enter the Chinese market, most of the time a local manufacturing entity must be established. In countries like Russia, a single diagnostic registration can cost five to six digit sums in Euros. Looking at the US, the FDA has closed applications for diagnostic products for more than two years now – meaning any new product that was launched in 2020 in Europe will not likely hit the US market before 2026.

What are the prerequisites for achieving long-term success as a diagnostics company?

Christian Harwanegg: First and foremost, the product must work and meet the basic requirements for the intended use. In the case of MADx, we want to offer every patient a correct and complete allergy test that is, at best, also more economical than competing products in the industry. This ensures market expansion and long-term success.

But even here there are some unnatural barriers that have grown historically. For example, calls for tenders only happen at certain periodic intervals. A potential customer who awarded a tender one year ago is thus simply not accessible to the manufacturer for three, five, or even seven years. Tender criteria are often written up by customer groups which have been fed by the dominating providers and don't intend to use the best product but lock the specs for a certain manufacturer.

In diagnostics, longer-term contracts are usually made with customers; 5 year contracts are not uncommon. This ensures availability and price stability for both sides. The manufacturer can secure his production volume and calculate better, while the customer has the security of knowing that there will be no massive price increases for a certain time period and that they will not have to worry much about external influences such as inflation or currency exchange rates.

If you had one wish to secure the long-term success of MADx, what would it be?

Christian Harwanegg: I would like to see more flexibility and foresight in the health care systems. A strategy needs to be defined on how to better deal with the diagnosis of health problems or diseases in the long term.

We live in an economic and political system where decisions are made on a short term (1 to 4 years) basis and the focus is on short-term cost-cutting or profit maximisation. Instead, we should be asking how best to ensure the health of the population in the long term.

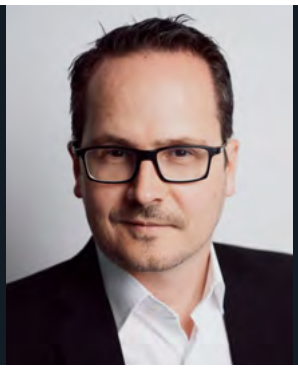
A health initiative is a long-term project from which no decision maker benefits immediately. On the contrary, it takes a whole generation of doctors, studies and cohorts who will no longer experience the praise and recognition for these initiatives themselves. It is difficult to prove from a health economics perspective that a health policy measure has had a clear positive effect in terms of population growth, climate change and lifestyle.

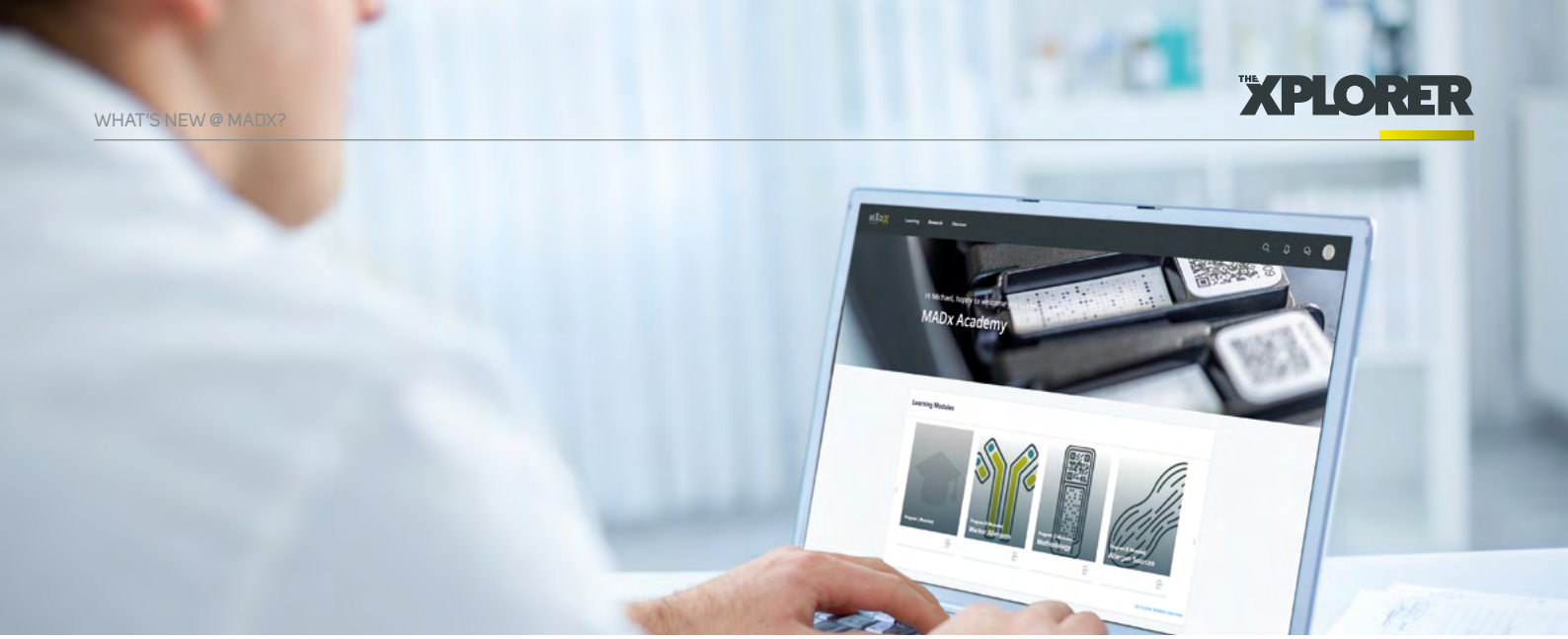
That is why, from a company perspective, it is necessary to be competitive in the short term, but also following a clear long-term vision that might span decades.

ABOUT

DR. CHRISTIAN HARWANEGG
studied Molecular Genetics at the University of Vienna, Austria. He joined a team of entrepreneurs in 1999 and graduated with a PhD in 2003. He has spent his entire professional education and career working in the development of all aspects of allergy testing in a multiplexed setup.

> "Health systems need more flexibility and foresight."





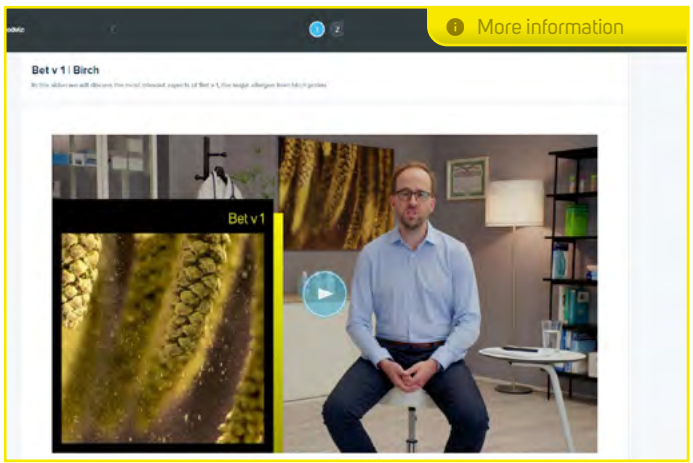
E-learning, events and studies

INTRODUCING THE MADX ACADEMY

We already announced the launch of the MADx Academy in the previous issue of THE XPLORER – now it is online, and the first users are already using the platform.

The MADx Academy is a learning platform that will introduce distributors and physicians into the MADx universe and deepen the understanding of not only our technology and products, but also allergology on a wider scale.

So what can users expect from the MADx Academy? It is a platform that aims to teach aspects of allergology in an easy-to-follow short video format. Every learning program (e.g., "allergen sources") comes with different modules (e.g., "latex"). Every module comes with an exam that can be taken to revise the content and claim a digital certificate if all answers are correct. Furthermore, we will present exciting case studies on the platform. Our aim is to broaden the horizon in the field of molecular allergy diagnostics and thus be one step ahead of the game.



MADx ACADEMY

MADX DISTRIBUTOR CONVENTION 2022

After two rather unusual years, it was finally time to get together again, exchange ideas in person and get an outlook on the years to come. Therefore, on September 5th and 6th, 2022, we hosted the first global MADx Distributor Convention. We were able to welcome participants from five continents and spent two wonderful days together in the heart of the beautiful city of Vienna.

Some highlights of this event included:

- Panel discussion regarding "Future of the allergologist | Economic and social impact of allergy | Future of digital patient care" with Prof. Eva Untersmayer-Elsenhuber, Dr. Paul Scheidegger, Prof. Petra Ziegelmayer and MADx CEO Dr. Christian Harwanegg
- Trainings for technology and marketing & sales
- Distributor of the Year award ceremony, with our winners from BioVendor (Czech Republic & Slovakia) , DASIT S.p.A (Italy) and ALPCO (USA)
- News and announcements
- Complimentary leisure activities in Vienna
- Great Austrian food at the restaurant Labstelle
- A lot of exciting conversations in between
- And more!

The team at MADx was excited to welcome so many distributors from all over the world at this event and is already looking forward to hosting it again in the future.

We want to thank all distributors for their participation and their warm feedback for the first ever MADx Distributor Convention! We are proud of the partnerships we have built thus far and are excited to work together to become the global #1 in multiplex allergy testing.

Watch video

Highest revenue ratio 2021 – BioVendor

Newcomer of the Year 2022 – ALPCO

First Ever Distributor – DASIT

ALEX² featured in new global fish study

In September, the first comprehensive multinational fish study featuring ALEX² with the title "Identification of Potentially Tolerated Fish Species by Multiplex IgE Testing of a Multinational Fish-Allergic Patient Cohort" was published.

For this study, sera from 263 fish-allergic patients from Austria, China, Denmark, Luxembourg, Norway, and Spain were tested using a research version of our ALEX² multiplex IgE quantification assay.

The results of this study show how using the ALEX² technology will improve precision of fish allergy diagnostics and help to identify tolerated fish for each patient, thereby avoiding unnecessary dietary restrictions.

The full abstract of this study is available via Pubmed, ScienceDirect and ResearchGate.

INDEX

1 Mari A. IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. Int Arch Allergy Immunol 2002; 129:286–295 https://doi.org/10.1159/ 000067591 PMID: 12483033 14.

2 Holzweber F, Svehla E, Fellner W, Dalik T, STubler S, Hemmer W, et al. Inhibition of IgE binding to cross-reactive carbohydrate determinants enhances diagnostic selectivity. Allergy 2013; 68:1269–1277 https://doi.org/10.1111/all.12229 PMID: 24107260

3 Platts-Mills TA, Hilger C, Jappe U, et al. Carbohydrate epitopes currently recognized as targets for IgE antibodies. Allergy. 2021;76(8):2383-2394.

4 Homann A, Schramm G, Jappe U. Glycans and glycan-specific IgE in clinical and molecular allergology: Sensitization, diagnostics, and clinical symptoms. J Allergy Clin Immunol. 2017;140(2):356-368..

5 Jin C, Bencurova M, Borth N, et al. Immunoglobulin G specifically binding plant N-glycans with high affinity could be generated in rabbits but not in mice. Glycobiology. 2006;16(4):349-357.

6 Mari A, Ooievaar-de Heer P, Scala E, et al. Evaluation by double-blind placebo-controlled oral challenge of the clinical relevance of IgE antibodies against plant glycans. Allergy. 2008;63(7):891-896.

7 Mari A. IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. Int Arch Allergy Immunol. 2002;129(4):286-295.

8 Holzweber F, Svehla E, Fellner W, Dalik T, STubler S, Hemmer W, et al. Inhibition of IgE binding to cross-reactive carbohydrate determinants enhances diagnostic selectivity. Allergy 2013; 68:1269–1277 https://doi.org/10.1111/all.12229 PMID: 24107260

9 Hemmer W. Human IgE antibodies against cross-reactive carbohydrate determinants. In: Kosma P, Muller-Loennies S (Eds.), Anticarbohydrate Antibodies. From Molecular Basis to Clinical Application. Springer Wien, 2012, pp. 181–202

10 Ahrezem O, Ibanez MD, Lopez-Torrejon G, Sanchez-Monge R, Sastre J, Lombardero M, et al. Orange germin-like glycoprotein Cit s 1: an equival allergen. Int Arch Allergy Immunol 2006; 139:96–103 https:// doi.org/10.1159/000090384 PMID: 16357490 11.

11 Malandain H, Giroux F, Cano Y. The influence of carbohydrate structures present in common allergen sources on specific IgE results. Eur Ann Allergy Clin Immunol 2007; 39:216–220. PMID: 18236996 12. Altmann F. Coping with cross-reactive carbohydrate determinants in allergy diagnosis. Allergo J Int 2016; 25:98–105 https://doi.org/10.1007/s40629-016-0115-3 PMID: 27656353

12 Altmann F. Coping with cross-reactive carbohydrate determinants in allergy diagnosis. Allergo J Int. 2016;25(4):98-105. doi: 10.1007/s40629-016-0115-3. Epub 2016 Jun 25. PMID: 27656353; PMCID: PMC5016538.

13 Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, Murphy BA, Satinover SM, Hosen J, Mauro D, Slebos RJ, Zhou Q, Gold D, Hatley T, Hicklin DJ, Platts-Mills TA. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. N Engl J Med. 2008 Mar 13;358(11):1109-17. doi: 10.1056/NEJMoa074943. PMID: 18337601; PMCID: PMC2361129.

14 Jappe U, Minge S, Kreft B, Ludwig A, Przybilla B, Walker A, Varga R, Seidel P, Biedermann T, Anemüller W, Kromminga A, Ruëff F, Merk H, Wagner N, Treudler R, Worm M, Waldmann I, Saloga J, Becker WM, Goldmann T, Platts-Mills TA, Homann A. Meat allergy associated with

galactosyl-α-(1,3)-galactose (α-Gal)-Closing diagnostic gaps by anti-α-Gal IgE immune profiling. Allergy. 2018 Jan;73(1):93-105. doi: 10.1111/all.13238. Epub 2017 Aug 15. PMID: 28670695; PMCID: PMC7254904.

15 Commins SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS, Kocan KM, Fahy JV, Nganga LW, Ronmark E, Cooper PJ, Platts-Mills TA. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-α-1,3-galactose. J Allergy Clin Immunol. 2011 May;127(5):1286-93.e6. doi: 10.1016/j.jaci.2011.02.019. Epub 2011 Mar 31. PMID: 21453959; PMCID: PMC3085643.

16 Sinson E, Ocampo C, Liao C, Nguyen S, Dinh L, Rodems K, et al. Cross-reactive carbohydrate determinant interference in cellulose-based IgE allergy tests utilizing recombinant allergen components. PLoS One 2020;15:e0231344.

17 Hemmer W, Altmann F, Holzweber F, Gruber C, Wantke F, Wohrl S. ImmunoCAP cellulose displays cross-reactive carbohydrate determinant (CCD) epitopes and can cause false-positive test results in patients with high anti-CCD IgE antibody levels. J Allergy Clin Immunol 2018;141:372–81.

18 Worm M., Moneret-Vautrin A., Scherer K., Lang R., Fernandez-Rivas M., Cardona V., Kowalski M.L., Jutel M., Poziomkowska-Gesicka I., Papadopoulos N.G., Beyer K., Mustakov T., Christoff G., Bilo M.B., Muraro A., Hourihane J.O., Grabenhenrich L.B. First European data from the network of severe allergic reactions (NORA). Allergy, 2014,69:1397-1404.

19 Dhami S., Zaman H., Varga E.M., Sturm G.J., Muraro A., Akdis C.A., Antolín-Amérigo D., Bilò M.B., Bokanovic D., Calderon M.A., Cichocka-Jarosz E., Oude Elberink J.N., Gawlik R., Jakob T., Kosnik M., Lange J., Mingomataj E., Mitsias D.I., Mosbech H., Ollert M., Pfaar O., Pitsios C., Pravettoni V., Roberts G., Ruëff F., Sin B.A., Asaria M., Netuveli G., Sheikh A. Allergen immunotherapy for insect venom allergy: a systematic review and meta-analysis. Allergy, 2017,72:342-365.

20 Golden DBK. Insect sting anaphylaxis. Immunol Allergy Clin North Am 2007;27:261-72.

21 Gattinger P., Lupinek C., Kalogiros L., Silar M., Zidarn M., Korosec P., Koessler C., Novak N., Valenta R, Mittermann I. The culprit insect but not severity of allergic reactions to bee and wasp venom can be determined by molecular diagnosis. PLoS One, 2018, Jun 25;13(6):e0199250. doi: 10.1371/journal.pone.0199250.

22 Eberlein B., Krischan L., Darsow U., Ollert M., Ring J. Double positivity to bee and wasp venom: Improved diagnostic procedure by recombinant-allergen based IgE-testing and basophil activation test including data about cross-reactive carbohydrate determinants. J Allergy Clin Immunol, 2012,130:155-61.

23 Platts-Mills TA., Hilger C., Jappe U., Van Hage M., Gadermaier G., Spillner E., Lidholm J., Keshavarz B., Aalberse RC., Van Ree R., Goodman RE., Pomes A. Carbohydrate epitopes currently recognized as targets for IgE antibodies. Allergy, 2021,76:2383-2394.

24 Gattinger P., Mittermann I., Lupinek C., Hofer G., Keller W., Bidovec-Stojkovic U., Korosec P., Koessler C., Novak N., Valenta R. Recombinant glycoproteins resembling carbohydrate-specific IgE epitopes from plants, venom and mites. EbioMedicine, 2019,39:33-43.

25 Aberer W., Holzweber F., Hemmer W., Koch L., Bokanovic D., Fellner W, Altmann F. Inhibition of cross-reactive carbohydrate determinantes (CCDs) enhances the accuracy of in vitro allergy diagnosis, Allergologie select, 2017,2:141-149.

26 Müller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honeybee and Vespula venom by estimation of IgE antibodies to species-specific major allergens Api m 1 and Ves v 5. Allergy, 2009;64:543-8.

Copyright

Publisher
Macro Array Diagnostics GmbH
Lemböckgasse 59/Top 4, A-1230 Vienna

Scientific content
Mag. Peter Forstenlechner,
Dr. Christian Lupinek,
Dr. Irene Mittermann,
Dr. Sandra Wieser

Non-scientific content/Editing/ English translation
Tanja Judmann, MA

Brand/Design
Mag. Barbara Hamza