

# Allergens for Diagnosis and Treatment of Allergic Diseases

Allergy and Immunology Section of Slovene Medical Society  
and  
University Clinic of Respiratory and Allergic Diseases  
Golnik, Slovenia

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Medical Faculty of Ljubljana



# Allergens for Diagnosis and Treatment of Allergic Diseases

## Programme

### 1. Immunotherapy

Chairmen: Asist. Peter Kecelj, dr.med., Dr. Peter Korošec, univ.dipl.biol.

9:00 – 9:10 Doc.dr. Mitja Košnik, dr.med.: Traps in Allergy Diagnosis and Therapy

9:10 – 9:30 Luka Camlek, dr.med.: Specific Immunotherapy for Pollen and the Fate of Cross Sensitivity

9:30 – 9:45 Asist. Nisera Bajrovič, dr.med.: Unusual Indication for Specific Immunotherapy

9:45 – 10:00 Andreja Peternelj, univ.dipl.biol.: Sensitivity of Basophils and Protection of Patients After the End of Venom Immunotherapy

10:00 – 10:30 Coffee break

10:30 – 10:50 Doc.dr.sc. Asja Stipič-Marković, prim.dr.: Peptide Immunotherapy

10:50 – 11:40 Prof.dr. Rudolf Valenta: Recombinant Allergens for Diagnosis and Therapy of Allergy

11:40 – 12:10 Prof.dr. Herbert Riechelmann: Modified Extracts for Allergen Immunotherapy

12:10 – 13:30 Lunch (sponsor HAL)

### 2. Accuracy of Diagnostic Methods in Allergy

Chairmen: Prof.dr. Alojz Ihan, dr.med., Prim. Aleksander Brunčko, dr.med.

13:30 – 13:45 Dr. Branko Pevec: Different Allergenicity of Different Apples in Birch-sensitive Patients with Oral Allergy Syndrome: in vivo study

13:45 – 14:00 Asist. Peter Kecelj, dr.med.: Laser Pletysmography for Allergen Testing

14:00 – 14:15 Znan.sod.dr. Branka Wraber, univ.dipl.biol., Vesna Vodušek, dr.med., Petja Šušteršič: Gray zone specific IgE: sensibilization alert or misleading data?

14:15 – 14:30 Asist. Mihaela Zidarn, dr.med.: Value of sIgE for Screening in Antibiotic Reactions

14:30 – 14:45 Dr. Peter Korošec, univ.dipl.biol.: Cellular In Vitro Tests for Allergy Diagnosis

14:45 – 15:00 Karmen Česen, dipl.MS: Quality control of skin prick testing

15:00 – 15:30 Satellite symposium (Glaxo Smith Kline)

15:30 – 15:45 Other topics

## Organisers:

Allergy and Immunology Section of Slovene Medical Society

University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia

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## **Pitfalls in allergy diagnosis and treatment**

Mitja Košnik

*University Clinic of Respiratory and Allergic Diseases, GOLNIK - SLOVENIA*

### **Results of IgE tests are important for**

- ❖ Confirmation of diagnosis (allergy)
- ❖ Allergen avoidance measurements
- ❖ Selection of allergen for immunotherapy

**Quite often results of tests are unreliable.** Good allergen extract is a prerequisite for correct allergy diagnosis.

Mistakes, that can produce false negative allergy tests:

- ❖ Denaturation of allergen during extraction
- ❖ Extracts made from raw foodstuff but foods eaten boiled
- ❖ Influence of extraction procedure (enzymatic digestion)

Mistakes, that can produce false positive results

- ❖ Crossreactivity (particularly in carbohydrate epitopes)
- ❖ Contamination of allergen extracts

### **Unappropriate interpretation of allergy tests**

- ❖ Interpretation of positive allergy tests: positive allergy test is not equivalent to allergic disease
- ❖ There is no correlation between concentration of sIgE and severity of disease.
- ❖ Often immunotherapy is directed to treat allergy tests results instead of patient.

### **Conclusion**

- ❖ Allergy tests are not completely reliable
- ❖ Do not treat allergy tests. Treat patients!

## Specific pollen immunotherapy and destiny of cross reactivity

Luka Camlek

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Allergenic proteins originate from a variety of sources and induce immune system to produce high affinity immunoglobulin E (IgE). The allergen cross-reactivity occurs when IgE antibodies originally raised against one allergen bind a protein from different source. The interaction with such homologous protein can trigger allergic reaction. Cross-reactivity should be used to describe clinical features showing the reactivity to a source without previous exposure. Other terms should be used to differentiate different pathogenetic mechanisms, for example co-recognition when we are unable to differentiate the sensitizing protein and co-sensitization which underlies the presence of IgE toward epitopes that are not shared between allergens. Cross reactivity usually, but not always reflects phylogenetic relation between organisms. Phylogenetically related proteins have high homology in their primary structure which results in homologous 3D structure and thus potential cross-reactivity. Some proteins from phylogenetically unrelated organisms can be cross-reactive, as for example in syndromes associated with birch, mugwort and ragweed pollen.

The structural characteristics of proteins are major determinants of cross reactivity. IgE cross-reactions appear because of shared structures at primary and tertiary structure of proteins. Cross-reactivity requires more than 70% sequence identity and is seldom present when proteins have less than 50% homology.

Since we use allergen extracts, which are mixtures of allergens we must define the term of major allergen. The major allergen is an allergen which contributes more than 20% of allergenic activity in more than 20 % of sensitized patients. This requires testing with extracts from which the allergen in question has been selectively removed and it is usually not done in clinical routine.

The allergen data bank contains more than 400 allergens and 200 isoallergens. Most of them can be grouped into a small number of structural protein families regardless of their source. There are several groups of cross-reactive proteins in pollen:

- Fagales pollen – group 1 (PR10)
- Profilins
- Pollen calcium binding proteins - polcalcins
- Oleaceae pollen – group1
- Thaumatin like proteins
- Grass pollen – group 1 ( $\beta$  expansin)
- Grass pollen – group 5
- Ragweed pollen (pectate lyase)
- Compositae pollen – group1 (PR-1)
- Cross reactive carbohydrate determinants (CCD)

### Pollen cross-reactivity

#### 1. Grass pollen interrelationships

There are some examples of grass pollen cross reactivity:

- Pooideae: strong cross reactivity based on homology of group 1,2/3 and 5 major allergens, possible unique allergens in timothy and sweet vernal
- Chloridoideae: cross reactivity between members, lack of group 3 and 5 allergens accounts for little cross reactivity with Pooideae.
- Panicoideae: lack of group 3 and 5 allergens accounts for little cross reactivity with Pooideae, more cross reactivity with Pooideae than Chloridoideae.
- Juncaceae, Cyperaceae, Typhaceae, Areceae: cross reactivity within families

#### 2. Tree and weed pollen interrelationships

- Coniferales: Strong cross reactivity within Cupressaceae based on group 1 and 2 major allergens

- **Amaranthaceae:** Strong cross reactivity between amaranths, strong cross reactivity between *Atriplex* species.
- **Fagales:** Strong cross reactivity between Betulaceae members based on group 1 and 2 allergens. Fairly strong cross reactivity between Betilaceae and Fagalaceae members based on group 1 and 2 allergens.
- **Oleaceae:** strong cross reactivity between Betulaceae members base on group 1 allergens, group 3 calcium binding proteins cross react.
- **Asteraceae:** strong cross reactivity between ragweeds, strong cross reactivity between *Artemisia* species, minor to little cross reactivity between ragweeds and mugwort, marshelder or cocklebur.

### **Pollen associated food intolerance (OAS)**

Up to 80% of birch pollen allergic patients suffer from the so called oral allergy syndrome (OAS). They have immediate itching in the mouth and throat as well as local edema after eating a variety of fruit, nuts and vegetables. The cause for this condition is cross-reactivity between aeroallergens, which are initial source of sensitization, and ingested allergens. The list of involved foods is rapidly expanding. A significant proportion of patients (8,7 %) with OAS also react with systemic symptoms. Definition of allergens has led to important conclusions: the symptoms related to Bet v1 related proteins tend to be mild, whereas the correlation with lipid transfer proteins seems to be associated with more severe symptoms. Patients reacting to commercial extracts are more likely to experience severe reactions than patients reacting to fresh food only.

### **Specific pollen immunotherapy**

Specific pollen immunotherapy is usually done with mixture of allergens to which the patient is sensitized. It is not usual clinical practice to specifically determine the major allergen and use its purified form in immunotherapy. The results of immunotherapy are usually very satisfying, but there is a constant danger of sensitizing the patient to previously nonreacting allergen. We can abolish the patient's symptoms to the sensitizing allergen and to its cross-reacting allergens. The second result of immunotherapy is that we only abolish patients' symptoms to the sensitizing allergen and the cross reactivity remains unchanged. The worst result of immunotherapy is that we cause new sensitization with the mixture of allergens that we usually use for immunotherapy.

There are some important steps how to prevent such unwanted results of immunotherapy. First step is very precise determination of sensitizing allergen. After the specific allergen is isolated we have several options. One option is recombinant allergens. This technique enables isolation of specific allergen without unwanted and potentially sensitizing proteins. In this technique it is very important to choose the right expression vector to prevent CCD cross-reactivity.

The second option is peptide immunotherapy. Here we use only part of sensitizing protein and so minimize possibilities for new sensitizations.

To maximize the benefit of specific immunotherapy we need precise and reliable diagnostic procedures and isolated sensitizing allergens for immunotherapy to minimize side effects and unwanted phenomena of immunotherapy. After all, we suppose to make people feel better, not worse.

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## Basophil sensitivity and protection of patients after insect venom immunotherapy

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### Introduction

Venom immunotherapy (VIT) provides widespread protection against systemic anaphylactic reactions after a sting of the respective insect. In spite of the high efficiency of venom immunotherapy, the molecular and cellular basis of the desensitisation progress remains poorly understood. There are three possible mechanisms of immunotherapy, namely at antibody (Ab) level (induction of specific IgG or blocking Ab; reduction of specific IgE Ab), at T-cell level (shift from Th2 to Th1; induction of regulatory T cells (IL-10); T-cell anergy) and at effector cells level (reactivity of basophils, mast cells and/or eosinophils) [1].

We focused on the effector cells level i.e. on basophil responsiveness, as basophils play an important role in the pathogenesis of allergic anaphylactic reactions. The aim of this study is to evaluate whether there is any correlation between basophil response to allergen-specific stimulation and the occurrence of allergic reactions in patients who were stung after the VIT was stopped and to evaluate whether there is any difference between basophil response before and at different time points during VIT. As a method to measure basophil response we choose basophil activation test (BAT). BAT is a very specific and sensitive cellular *in vitro* method for the quantitative determination of the degranulation of basophilic granulocytes in blood with flow cytometer for Hymenoptera venom hypersensitivity. The general concept of these *in vitro* tests is to mimic *in vivo* the contact between allergens and circulating basophils. CD63 surface expression on the surface of basophils was measured with flow cytometer. The surface marker CD63 is a glycoprotein of 53kDa and is located inside the histamin containing granule membrane in resting basophils and is expressed on their surface after activation [2].

### Methods

**Subjects** were patients allergic to honey bee or wasp venom. Venous blood from patients in first group was sampled after immunotherapy was ended (n = 29) and blood from patients in the second group was sampled three times – before VIT, 1 month and 1 year after beginning of VIT (n = 9). Peripheral blood was obtained from subjects after informed consent was given.

**BAT:** Blood aliquots were incubated with stimulation buffer and serial concentrations of venom, anti-FcεRI mAb, fMLP (L-formyl-L-methionyl-L-leucyl-L-phenylalanine) or stimulation buffer only.

Thereupon cells were incubated with mAb in cold water which stopped degranulation. Thereafter, samples were subjected to erythrocyte lysis with lysing solution, washed, resuspended in fix buffer, and analyzed by means of flow cytometry.

**Specific IgE:** Honey bee or wasp venom specific IgE (sIgE) from patient's serum were measured with FEIA Cap system.

**Basophil sensitivity ratio:** a percentage ratio between basophil CD63 response at 0.1 and 1 µg/ml of venom dilutions [3].

### Results

CD63 basophil response in patients stung after VIT with no reactions and those with reactions was significantly different at 0,1µg/ml of venom concentration, but not at 1 and 0,01µg/ml. We observed no significant differences in sIgE values between these two subgroups of patients.

We could not find any difference in CD63 basophil response after 1 month of VIT, however CD63 basophil response has significantly decreased after 1 year of VIT in approximately half of patients. On the basis of those observations we divide patients in two subgroups. One subgroup has lower basophil sensitivity ratio and markedly decreased basophil response after 1 year of VIT and the other subgroup has higher basophil sensitivity ratio and no differences in basophil response after 1 year of VIT. We also observed reduction in sIgE values in the subgroup with lower basophil sensitivity ratio.

## Conclusions

Our results suggest that there is a difference at the level of basophil responsiveness in patients during and after VIT. However further studies with more patients are needed to reveal the mechanism and cause for these differences.

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## Peptide immunotherapy

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A major goal of clinical immunology is to develop new strategies that will induce a state of immune tolerance by selectively blocking pathogenic immune responses while maintaining protective one. SIT in allergy means, the administration of progressively increasing doses of an allergen to ameliorate clinical allergic disease. But, the efficacy of current protein based immunotherapy is limited, in large part, by the small amounts of allergen that can be given safely without causing severe allergic reactions. The rationale for using short peptides (peptide immunotherapy, PIT) is to reduce the potential for these side effects while retaining the beneficial effect of peptide T-cell epitopes to modify T-cell responses. The reduced ability of peptides to induce mast cell activation is likely due to inability of this short molecule to cross link of IgE affixed to high-affinity FcεRI on mast cells. Focusing the immune response towards defined T cell epitopes of whole allergen is an alternative in which high molar doses of T cell epitope can be delivered over a shorter time period. However, the major challenge of a peptide-based vaccine is the identification of immunodominant epitopes that could bind to several HLA alleles covering the majority of a genetically distinct population. Also, the potential barrier is the apparent complexity of allergen-specific T-cell response in terms of dominant epitopes in humans, unpredictability of early and late allergic reactions and the dose difficulties associated with the standardization of the protein level.

With the aid of algorithms that predict binding to multiple HLA classes II alleles with high avidity sequences of potential immunodominant epitopes from causative allergen could be selected. Peptide can often partially mimic epitopes but usually with substantial loss of affinity. The affinity of small peptide is lower because it has little intrinsic structure and major loss of entropy upon antibody binding. Pooling of additional promiscuous epitope may increase responsiveness and coverage. To date, clinical trials of PIT have been performed in two allergies: cat and bee venom. Relatively long peptides of 27 and 35 amino acids of the major cat allergen Fel d 1 were used in PIT and resulted in the induction of tolerance in IL-4 producing cells. In the other trials, PIT of bee venom allergy was performed with a mixture of short peptides that directly represent T-cell epitopes (17, 12, 11 aminoacids) of the bee venom major allergen, phospholipase A2. The studies showed modulation of the immune response against the whole allergen (induction of T cell tolerance and a decrease in the specific IgE:IgG4 ratio).

Although PIT is theoretically attractive, it is important to note that serum IgE in allergic individuals may sometimes bind to relatively short linear epitopes of protein allergens and induce IgE-mediated early phase undesirable reactions.

## From allergen genes to allergy vaccines

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Immunoglobulin E (IgE)-mediated allergy affects more than 25% of the population in industrialized countries. During the last years the cDNAs coding for most of the relevant disease-eliciting allergens have been isolated and expressed as recombinant allergens. Based on recombinant allergens it has become possible to reconstruct the epitope complexity of the most common allergen sources and novel diagnostic tests have been developed which allow the dissection of patients reactivity profiles down to the single molecules. Furthermore it has become possible to develop by recombinant DNA technology a new type of allergy vaccines with reduced allergenic activity. The engineering of hypoallergenic derivatives of the major birch pollen allergen, Bet v 1, by genetic engineering and the vaccination of birch pollen allergic patients (n=124) in a double-blind, placebo-controlled study will be reported. Active treatment induced protective IgG antibodies which inhibited allergen-induced release of inflammatory mediators. Furthermore a reduction of cutaneous sensitivity as well as an improvement of symptoms in actively treated patients was observed. Most important, rises of allergen-specific IgE induced by seasonal birch pollen exposure were significantly reduced in vaccinated patients. Thus, a novel allergy vaccine based on genetically engineered allergen derivatives was developed which not only ameliorated allergic reactions, but also reduced the IgE production underlying the disease. According to this proof of concept study it can be envisioned, that it will be possible to develop therapeutic and prophylactic vaccines against the most common forms of IgE-mediated allergies.

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## Modified extracts for allergen immunotherapy

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Allergen immunotherapy reverses the TH2-skewed immune response towards TH1 (1;2). For effective allergen immunotherapy, maintenance doses of approximately 10 µg major allergen have been proposed (3). However, cross-linking of mast cell bound IgE by allergen extracts may lead to adverse reactions. With unmodified allergen extracts, several injections with increasing allergen doses are thus required to achieve an effective maintenance dose.

To avoid high systemic allergen concentrations shortly after injection and to improve immunogenicity, sustained release forms of allergen extracts have been developed. Frequently, allergens are adsorbed to aluminium hydroxide (Al(OH)<sub>3</sub>). Allergoids have been developed with the aim to further reduce the number of injections necessary to reach the maintenance dose. Formaldehyde- or glutaraldehyde modified allergen extracts reveal reduced IgE reactivity and thus less potential to elicit adverse reactions (4;5), while their T-cell dependent immunogenic effects are preserved (6). This allows an accelerated dosing schedule and effective preseasonal short-term treatment. The clinical efficacy of allergoid immunotherapy has been demonstrated in various studies (7;8).

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## **Different allergenicity of different apples in birch-sensitive patients with oral allergy syndrome: in vivo study**

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### **Background**

The amounts of apple allergens vary considerably in different strains of apples, and are influenced by maturation, ripening and storage conditions. In vitro studies revealed that the allergenicity of apple is related to the level of expressed Mal d 1. Strains with high relative amount of Mal d 1 (Granny Smith, Golden Delicious, Jonagold, Braeburn, etc.) showed greater allergenicity than those with medium (Sternrenette, Alkmene), low (Boskoop), and very low amount (Jamba, Gloster, Altländer, etc.). Apples purchased from stores contained higher amounts of Mal d 1 than freshly picked ones. Furthermore, the amount of Mal d 1 showed the tendency for the increase during prolonged storage at 4°C.

Instability of commercial apple allergen extracts created a persistent problem of false negative results of skin prick testing (SPT). For that matter, SPT with fresh fruits is considered a useful diagnostic tool. Oral challenge with apple is complicated by several factors: susceptibility of apple allergens to all types of processing, different allergenicity between apple strains and maturation stages, occasional development of tachyphylaxis, and a lack of a consistent standardized model.

The aim of this study was to investigate the difference in allergenicity of fresh and ripe apples of five different strains, in birch-sensitive patients with OAS to apple. We also wanted to develop a simple and accurate model for oral challenge. For that purpose we designed a special scoring system.

### **Material and methods**

Thirty patients with clinically manifested allergy and confirmed sensitization to birch pollen and a history of OAS to apple were included into the study. Twelve patients had previously received a birch pollen immunotherapy. Control group consisted of 15 nonatopic subjects. Five different apple strains (Granny Smith, Jonagold, Idared, Gloster, and Golden Delicious) were used for skin prick tests and oral challenge tests. Fresh (fully developed, mature apples, without signs of ripening), and ripe (stored, with signs of ripening) apples of each strain were purchased from local grocery stores. All patients and control subjects were tested with one fresh and one ripe apple of each strain.

SPTs were performed with a drop of juice squeezed from an apple slice directly to the forearm. All tests were done in duplicate (a total of 10 SPTs at each forearm), and the mean wheal diameters were used for analyses. Patients were also tested with commercial SPT apple allergen.

Oral challenge tests were performed with a slice of each apple chewed for a minute. Symptoms were recorded during the next 15 minutes, and the severity of reaction was evaluated. A scale from 6 to 10 was used to grade reactions from very mild to very strong. Each apple strain to which no symptoms occurred was retested with a whole apple eaten cautiously, bite for bite. Symptoms were recorded and severity of reactions evaluated, only this time a gradation from very mild to very strong reactions was marked 1 to 5. Finally, a scale from 0 (indicating no reaction to a greater amount of sample) to 10 (indicating the strongest reaction to a smaller amount of sample) was obtained, and used for analyses.

### **Results**

All ripe apples, regardless of the strain, produced significantly stronger reactions in SPT than fresh ones (mean wheal diameters: Granny Smith – 7.35; 5.63\*, Jonagold – 7.93; 6.06\*, Idared – 7.70; 6.02\*, Gloster – 7.33; 5.59\*, Golden Delicious – 7.32; 5.45\*). As there was no significant difference between different strains, we conclude that degree of maturity is more important for skin reactivity than the apple strain itself.

SPTs with a commercial apple allergen were negative in all subjects.

Severity of reactions in oral challenge tests was also greater with ripe than with fresh apples, although significant difference was reached only for Granny Smith and Golden Delicious (mean score values:

Granny Smith – 7.03; 5.53\*, Jonagold – 6.83; 5.40, Idared – 6.53; 5.20, Gloster – 5.50; 4.17, Golden Delicious – 6.97; 5.37\*). As opposed to SPT, different apple strains showed different allergenicity. Deduction revealed that the allergenicity decreased in the following order: Granny Smith > Golden Delicious > Jonagold > Idared > Gloster. This result is in full agreement with earlier in vitro studies. Control subjects had negative results in all performed tests.

The issue of a birch pollen immunotherapy as the effective therapy for cross-reactive food allergies is still controversial. Symptom scores of our patients who have received a birch pollen immunotherapy were significantly lower in comparison with patients who have not. Contrary to oral challenges, there was no difference in SPTs between patients who have, and those who have not received a birch pollen immunotherapy.

To improve the accuracy of evaluation of reaction severity in oral challenges, we designed a scoring system, named PEACE score (PEroral Apple Challenge Evaluation score). Contrary to other scoring systems which mostly used scales from 0 to 3 or 0 to 4, our system uses a scale from 0 to 10, which makes it more sensitive. Other systems (including the VAS score) were based exclusively on a patient's judgment of the reaction severity, and thus highly subjective, whereas our system considers the intensity of symptoms, and their different combinations, which makes it more objective and final scores comparable between patients and between different time points in the same patient. Furthermore, our system considers the amount of sample used for challenge.

### **Conclusion**

Results of this study confirmed earlier in vitro findings that particular apple strains, and ripe apples regardless of the strain, have greater allergenicity. These findings may help to advise patients in apple selection. The results also confirmed that a birch pollen specific immunotherapy may decrease sensitivity to apple.

SPT with apples poorly predicts a severity of oral allergic reactions. To evaluate the effect of specific immunotherapy, oral challenge test should be performed. In order to avoid severe reactions, oral challenge should start with fresh apple followed with ripe one of the same strain. Although not standardized, both SPT and oral challenge test are suitable for practice, as they reflect a real allergenicity of apples available to patients.

Proposed scoring system could, with slight modifications, be used to assess the severity of reactions in challenges with other vegetable foodstuffs.

## Measurement of the wheal parameters at the skin prick testing with laser profilometer

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*\*\*University of Ljubljana, Faculty of Mechanical Engineering, Slovenia*

The allergological tests are basic methods for detecting allergens, who are responsible for different clinical manifestations. In vivo and in vitro tests are used. In vivo tests are based on IgE-mediated allergic reaction of the skin with the measurable wheal. The wheals at positive and negative control are compared with the wheal of tested allergens. The size of the wheal is determined with millimeter scale. The standardization of skin prick test is proposed with position paper of European Academy of Allergology and Clinical Immunology (EAACI)(1). The size of wheal is determined with arithmetic mean of the longest and shortest diameter.

Pijnenborg and al. used a scanning program to measure the wheal area. The contours of the wheal were encircled on transparency paper and used in scanning program (2). Because this method is time consuming and requires special equipment is used only for research.

The response of the small skin vessels in skin prick test was monitored with laser-Doppler flowmetry(3). The method is used only in research project.

The reproducibility and the precision of skin prick test we would like to improve with the use of the laser profilometer. Laser profilometer measurement would better determine parameters of the wheal: diameter, area, volume and height of the wheal. The result of measurement would be expressed as ratio between the volume of allergen caused and histamine caused wheal.

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## Value of specific IgE antibodies as a screening procedure in reactions to antibiotics

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### Introduction

Allergic reactions to antibiotics are one of the most common drug reactions. Among antibiotics, allergic reactions to betalactams are the most common cause of drug reactions mediated by specific immunological mechanisms (1).

Immediate reactions usually appear within a maximum interval of one hour after drug intake and are mediated by specific IgE-antibodies. They can be evaluated by different methods: clinical history, skin tests, *in vitro* quantification of IgE antibodies and drug provocation test.

Clinical picture typical for immediate reactions are urticaria, angioedema and anaphylaxis.

Two classical methods for skin testing are used: prick and intradermal test.

The most widely used methods for the quantification of specific IgE are RAST and FEIA. FEIA system is a commercial method available and used world-wide. The specificity of the test is reported to be very high, ranging from 95-100% in the study done by Blanca et al (2), to 87% in the study of Sanz et al (3).

In our clinical practise we use the commercial method Pharmacia CAP FEIA® for detecting sIgE antibodies. We have encountered on a patient with positive sIgE to betalactams and no clinical history of reaction to betalactam, but the patient also had a high level of total IgE. The patient was tested with drug provocation test and he didn't have any symptoms after maximum daily dose of the drug. In our study we wanted to find out whether sIgE to betalactams can be falsely positive at high levels of total IgE.

### Methods

Serums from 30 patients with high total IgE level and no history of reaction to betalactam and 10 serums from patients with low total IgE level and no history of reaction to betalactam were tested with Pharmacia CAP FEIA® for the presence of sIgE for penicilloyl G and V, amoxicilloyl and ampicilloyl.

### Results

In the group of patients with low total IgE ranging from 8 to 263 kU<sub>A</sub>/l and no history of reaction to betalactam we found no positive sIgE for any of the above mentioned betalactams. In the group of 10 patients with total IgE level above 500 kU<sub>A</sub>/l (up to 685 kU<sub>A</sub>/l) we found 4 patients with positive sIgE to at least one betalactam. In the group of 10 patients with total IgE between 1000 and 2000 kU<sub>A</sub>/l we found 7 patients with positive sIgE to at least one betalactam. In the group of 10 patients with total IgE above 2000 kU<sub>A</sub>/l we found 8 patients with positive sIgE to at least one betalactam. In that group all the patients with total IgE level above 3000 kU<sub>A</sub>/l had positive sIgE to at least one betalactams. In most of patients with positive sIgE to betalactams more than one sIgE (penicilloyl G, penicilloyl V, amoxicilloyl or ampicilloyl) was positive.

### Conclusion

High sIgE for betalactams that are probably falsely positive can be found in patients with total IgE above 500 kU<sub>A</sub>/l.

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## Cellular In Vitro Tests for Allergy Diagnosis

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The recent insight in the cellular immunological background of allergic diseases has urged an interest in using of cellular systems for in vitro diagnosis of allergic diseases. Those tests are defined as stimulation of cells from a patient by a potential allergen and observation of biological response. The response could be measured by secretion of soluble products or surface up regulation of activation markers, by the form of proliferation or by induction of intracellular expression and/or phosphorylation. Despite a vast publication on cellular in vitro tests for allergy diagnosis only a few tests have actually been introduced in the clinic and their use is still limited to relatively few centres. Those methods are leukocyte histamine release test, CAST-ELISA (production of sulfidoleukotrienes by leukocytes), antigen-induced proliferation of T-lymphocytes and the very recent CD63 basophil activation test.

Flow cytometry quantification of basophil activation by measurement of CD63 expression is at the moment the most promising cellular allergy test. The level of CD63 expression and identification of basophils can be exactly quantified and analysed by flow cytometry using specific antibodies conjugated to fluorescent dyes. In resting basophils CD63 is located within granule membranes and absent from cell surface, but during exocytosis, when the granule membranes fuse with the cell membrane, it is translocated to the cell surface (1). This surface up-regulation is closely correlated with basophil histamine release (2). CD63 activation test was suggested to be useful in diagnosis of food, pollen, latex, drugs and especially Hymenoptera venom allergy (2-4). The focal point of all those approaches was the measuring of maximal response to evaluate possible sensitization to specific allergen. However, basophil response to different allergens is concentration dependent; curves are usually bell-shaped and rather highly individual from donor to donor. From that point of view we decide not just to measure maximal response, but also to evaluate basophil sensitivity (shift of the increasing dose dependent activation) by recalculating CD63 response induced by two different optimal log allergen concentrations. Namely, basophil sensitivity appears to be an independent intrinsic property connected with the intracellular signalling elements regardless of the cell surface density of the specific IgE. Since effector cells are the key cells for manifestation of allergic disease, we questioned whether responsiveness of those cells have any correlation with clinical symptoms. To explore this hypothesis, we examined the basophil sensitivity in patients before receiving Hymenoptera venom immunotherapy for possible predicting of the allergic adverse reactions. The results showed that increased basophil sensitivity is associated with major side effects during VIT and that monitoring of CD63 concentration-dependent venom response could be a relevant tool for identification of patients at higher risk for side effects (5). Moreover, we also demonstrated a significant positive correlation between individual sensitivity ratio and clinical severity of allergic side reactions. This study was the first to show clear correlation between clinical symptoms and basophil CD63 expression

In conclusion, measurement of sensitization by skin tests or serum levels of specific IgE does not necessarily imply clinical allergy. On the other hand new data suggest that basophil and possibly mast cell allergen specific responsiveness might correlate with clinical allergy symptoms and/or disease. Implantation of those observations in allergy diagnosis is one of the major points for further development, validation and using of cellular in vitro tests.

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