

Scientific Contribution



EAACI 2004

12-16 June, Amsterdam



Dear Colleague

This booklet contains sixteen abstracts within the field of allergy vaccination and diagnostics. The abstracts demonstrate the collaboration between allergy specialists and ALK-Abelló and illustrate our long-standing tradition of research in cooperation with the international community.

Advancing evidence – new approaches in allergy vaccination

At our Symposium we will focus on the increasing body of evidence for allergy treatment. Future discussions should result in an international consensus rather than the confusion that appears when different guidelines do not suggest similar approaches to allergic diseases.

We will present the latest documentation on specific allergy treatment from the largest allergy vaccination study ever performed, as well as the most recent approaches to sublingual immunotherapy.

Modern guidelines are founded on evidence based medicine, and the evidence in support of allergy vaccination as well as sublingual immunotherapy is increasing significantly. It gives the opportunity to improve the international guidelines for specific allergy treatment for the benefit of the patient.

If you wish to comment on or further discuss the enclosed abstracts, please visit us at the ALK-Abelló stand number 5.

We look forward to meeting you during EAACI 2004.

Yours sincerely

ALK-Abelló A/S

The ALK-Abelló company sponsored symposium

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The ALK-Abelló company sponsored symposium

Advancing evidence – new approaches in allergy vaccination

Tuesday, 15 June from 13.45 to 15.30 (Forum)

Chairpersons

Anthony Frew and Hans-Jørgen Malling

Allergy treatment – consensus or confusion

Jean Bousquet, France

The UK Immunotherapy Study (UKIS)

Richard Powell, United Kingdom

New approaches in sublingual immunotherapy

Walter Canonica, Italy

Allergy vaccination – evidence based consensus

Hans-Jørgen Malling, Denmark

The future of guidelines

Jean Bousquet, France

Clinical guidelines are an increasingly familiar part of clinical practice. They have potential benefits and harm if the recommendations are wrong. However, rigorously developed guidelines minimise the potential harm. Clinical guidelines are only one option for improving the quality of care.

1. Is there a need for guidelines in allergic diseases and asthma?

There is still a clear need for guidelines since:

- The diseases represent a global health problem.
- Morbidity and mortality (from asthma) are substantial.
- Treatments are not well understood by physicians and patients, and patients are far from being well controlled.
- Guidelines offer better control of allergic diseases and asthma than free treatment choice.

2. Are there limitations for guidelines?

Several limitations exist for all guidelines including those for allergic diseases and asthma.

- Guidelines were initially based on opinion based medicine. Recently, they have been developed using evidence based medicine (EBM), but EBM is not free from criticism.

- Guidelines are often too difficult to be easily used by physicians and although they are excellent teaching tools, many need to be simplified. Since they appear to be complex to the physicians, they are not often used.
- Recommendations should take into account health economics, the availability and affordability of treatments and medical devices.
- Guidelines should also be adapted to the patient's needs.

3. Do we need to update guidelines?

Guidelines need to be constantly updated with new information. Paradigms in medicine are often shifting and guidelines should reflect these changes. However, there is always a gap between the latest information and its inclusion in guidelines. As an example, the treatment of asthma in GINA was initially based on severity assessed in untreated patients (1995). Then, in 2002, it was also based on the treatment currently received by the patient, and may well be based on asthma control in a future update.

4. Is there a future for guidelines?

Major changes will occur in the generation and implementation of guidelines.

- Guidelines will be made simpler and adapted to the physician's needs.
- Guidelines will be adapted to symptoms and to a patient based approach. Till now most guidelines were vertical (single disease), they will soon be transversal incorporating many diseases and consider co-morbidities.
- Since many patients with allergic diseases and asthma are ageing and may suffer from multiple chronic diseases, their integration into a single platform will be the next in order to devise "meta-guidelines" that combine the contents of individual practice guidelines that can easily be applied by general practitioners and other healthcare workers.

The UK Immunotherapy Study (UKIS)

Richard Powell, United Kingdom

Background

Specific allergen vaccination (SAV) is an effective treatment for seasonal allergic rhinitis (SAR), but has often been studied in patients with mild disease that would not normally be considered for SAV in the UK.

Methods

In order to assess the efficacy of SAV in subjects with more severe SAR, a one-year, double-blind, placebo-controlled efficacy and safety study was conducted in a large group of patients with SAR who were dissatisfied with the effects of standard drug therapy in relieving their symptoms. 410 subjects with SAR due to grass pollen were randomised (203 to 100,000 SQ-U, 104 to 10,000 SQ-U and 103 to placebo) of whom 347 (85%) completed treatment. The groups were matched for age, gender and duration and nature of symptoms.

Results

Across the whole grass pollen season, subjects in the 100,000 SQ-U group had 28% lower symptom scores and 32% lower medication scores than the placebo group (both $p < 0.001$). Subjects in the 10,000 SQ-U group reported significantly lower symptom scores (22% lower, $p < 0.01$) and a reduction in medication score (16%, $p = 0.16$) than those on placebo over the whole season. Quality of Life (QoL) during the pollen season measured by a rhinitis specific quality of life questionnaire (RQLQ) showed significantly better QoL in both active groups compared to placebo (100,000 SQ-U, $p < 0.0001$ and 10,000 SQ-U, $p = 0.031$). Adverse events occurred at similar rates in all 3 groups. Local reactions to SAV were common and found more often in the

100,000 SQ-U group. Early systemic reactions were mostly mild. 9 subjects (4.4%) had a clinically significant early systemic reaction, all in the 100,000 SQ-U group. No life-threatening systemic reactions occurred. Delayed systemic reactions were common and mostly mild. 4 subjects (2%) in the 100,000 SQ-U group had clinically significant delayed systemic reactions. There were no episodes of anaphylaxis.

Conclusion

SAV with Alutard SQ grass pollen was effective in reducing symptoms and medication usage in patients with grass pollen SAR who were dissatisfied with the effects of previous standard drug therapy. Treatment with 100,000 SQ-U was more effective in reducing symptom and need for medication during the pollen season, but also resulted in a higher frequency of allergic side reactions.

New approaches in sublingual immunotherapy

Walter Canonica, Italy

So far, the only reliable parameter for judging the efficacy of allergen immunotherapy in general is the clinical one. This involves the measurement (by clinical diaries or visual analog scales) of the symptom score and medication intake. The cumulative symptom + drug scores or each component *per se* can be used for statistical analysis. Based on the clinical parameter, SLIT has recently been validated in rhinitis by means of meta-analysis. Indeed, there are some evaluation criteria other than clinical scores that can be used. They are grouped under the term of paraclinical parameters and include: systemic immunological changes, specific and non-specific hyperreactivity at the target organs (nose, eye or bronchi), QoL assessment, *in vitro ex vivo* evaluations. Looking at the 25 double-blind, randomised trials of SLIT published so far, it can be observed that the systemic immunological changes (IgE and IgG subclasses) resemble, in general, those seen with injection immunotherapy, but those changes are not constant and sometimes negligible. Therefore, they only suggest that an immunological effect takes place. Also, the decrease of peripheral circulating ECP can reflect the anti-inflammatory activity of SLIT, but it has been assessed only in few studies. The recent finding that SLIT reduces the circulating IL-13 is more relevant since it suggests that also SLIT affects the Th2/Th1 balance. This is in agreement with the previous *in vitro* observation that SLIT reduces the proliferation of allergen specific T-cells from peripheral blood. From a clinical viewpoint, it is noticeable that SLIT was able to reduce the *in vivo* expression of adhesion molecules in humans and consequently the influx of inflammatory cells to the nose and conjunctiva upon specific allergen challenge. Another important observation is that SLIT can significantly reduce the non-specific bronchial hyperresponsiveness in asthmatic subjects. In this regard, it was shown that the change in bronchial responsiveness correlated well with the decrease of the ICAM-1 molecule expression. All these facts are indirect (paraclinical) evidence of the effect of SLIT on bronchial inflammation since bronchial responsiveness is sustained at least in part by inflammation itself. Quality of Life (QoL) is an important paraclinical parameter that correlates well with the clinical efficacy and provides a more general assessment of SLIT from the patient's viewpoint. So far, there is a single study in house dust mite allergic subjects confirming that an effect of SLIT on QoL can be detected by the SF-36 questionnaire. Another large long-lasting study (the QoLIT) is presently ongoing to confirm this observation. In conclusion, paraclinical parameters are in general useful criteria for investigating the mode and site of action of SLIT, but presently none of them can replace the clinical evaluation carried on during the natural exposure to the offending allergen.

Allergy vaccination – evidence based consensus

Hans-Jørgen Malling, Denmark

Allergen specific immunotherapy is a clinically effective and disease modifying treatment of allergic diseases. Requirements in modern medicine necessitate clear documentation of the cost effectiveness of disease interventions. The classification of statements of evidence and the consequent recommendations for clinical use is generally accepted. Allergic diseases are handled by several specialties and the recommendations for applying various interventions depend on the specific guidelines developed by different groups of specialists.

A problem is that the clinical importance of a specific allergy treatment depends on the eyes – looking or not looking for the basic pathophysiologic mechanisms of the clinical manifestations. If allergy is not considered of importance in the clinical symptoms, allergen specific interventions will probably not be considered. Evidence based consensus is only partly applicable to the daily use of treatments that are widely used based on empiric information and tradition rather than on the scientific documentation.

A fundamental requirement for any treatment of a disease is documentation of clinical efficacy. Clinical efficacy is a reduction in clinical symptoms and/or demand for pharmacotherapy that from a clinical point of view reduce the disease severity. An inverse relation exists between the frequency and severity of side effects and the demand for a high clinical efficacy. Based on multiple placebo-controlled, double-blind studies, the clinical efficacy during treatment has been documented for subcutaneous immunotherapy in patients with rhinoconjunctivitis, asthma and *Hymenoptera* venom anaphylaxis. With respect to sublingual immunotherapy, efficacy has been convincingly shown for rhinitis and asthma, although the magnitude of efficacy may be slightly less than for subcutaneous immunotherapy. An important issue in disease modifying treatment is the long-term efficacy, i.e. efficacy lasting after terminating treatment. Evidence exists for subcutaneous immunotherapy but studies evaluating sublingual immunotherapy are insufficient to unequivocally claim this effect. Another important aspect is the preventive capacity in terms of preventing progression in disease severity and the development of new sensitisations. Some studies have shown this capacity for subcutaneous immunotherapy, but not yet for sublingual immunotherapy.

In daily clinical practice, it is important clearly to differentiate between what we know, what we believe and what we hope. Patients deserve treatments based on scientific background and not on feelings. Insufficient documentation of evidence should not result in unavailing academic discussions, but be an inspiration to initiate clinical studies to unravel the uncertainty.

The safety of bee venom immunotherapy with purified extracts

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Allergic side effects are considered to be the main problem in specific immunotherapy, especially in honey bee venom allergic patients treated with both fast and slow dose regimens. For this reason, alternatives to the standard aqueous extracts have been developed, including purified extracts, also coupled with adjuvants such as aluminium hydroxide for the depot ones. The aim of the study was to compare the safety of immunotherapy with 2 different preparations of bee venom: the standard aqueous extracts and the purified ones.

44 patients with a case history of systemic reactions after bee stings were randomised to immunotherapy with 1 of the 2 bee venom extracts: 1) standard aqueous extracts (Pharmalgen ALK-Abelló S.p.A.) 2) bee venom extract prepared by purification techniques which eliminate vasoactive amines and reduce potentially toxic peptides (Aquagen SQ ALK-Abelló S.p.A.). Before treatment, IgE mediated sensitivity was determined by positive bee venom skin tests. The dose increase phase was administered following a rush protocol regimen over 5 days until the maintenance dose of 100 mcg, without any premedication, was reached. During the rush therapy, all patients were kept under strict observation in our out-patient clinic.

20 patients (13M, 7F; mean age 42.3) with history of systemic reactions (4 grade II, 5 grade III, 11 grade IV reactions as per Müller's classification) were given an Aquagen extract. 24 patients (20M, 4F; mean age 39.62) with history of systemic reactions (3 grade II, 9 grade III, 12 grade IV reactions as per Müller's classification) were given a Pharmalgen extract. All of the 20 patients tolerated the purified extract up to the maintenance dose of 100 mcg without any side effects and switched to the aluminum hydroxide adsorbed extract (Alutard SQ ALK-Abelló S.p.A.) during the maintenance phase. 8 out of the 24 patients (33%) who were treated with the standard aqueous extract experienced mild to moderate side effects (5 generalised itching, 2 urticaria, 1 dyspnoea) ($Z=2.443$; $p=0.015$) completed the dose increase phase with the aluminum hydroxide adsorbed extract (Alutard SQ) and continued ITS without any side effects.

In conclusion, purified extracts are safer than the standard aqueous ones in bee venom immunotherapy. Further trials with larger numbers of patients will have to be carried out to substantiate these findings.

Monoclonal antibody based assay for a major olive pollen allergen Ole e 9

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Background

Olea europaea pollen is one of the most important causes of allergic disorders affecting people in the Mediterranean areas. A novel major allergen, Ole e 9, is involved in the allergic responses of 65% of patients suffering from olive pollinosis. Ole e 9 has a molecular mass of 46.4 kDa, displays 1,3 β -endoglucanase activity and belongs to the group 2 pathogenesis related protein family. It is known that the prevalence of sensitisation to olive allergens depends on the environmental allergen load, and hence new analytical methods to measure the concentration of a second major allergen, in addition to Ole e 1, will improve the standardisation of olive allergenic products. We will here describe a solid phase immunoassay to measure Ole e 9 and discuss its potential applications.

Method

Ole e 9 was purified from olive pollen by sequential gel filtration and hydrophobic affinity chromatography. This preparation was used as immunogen to produce mouse mAb to Ole e 9. 40 hybrid cells producing Ole e 9 specific mAb were obtained after fusion of mouse myeloma cells with spleen cells from an immunised Balb/c mouse.

A solid phase sandwich ELISA for measuring Ole e 9 was developed using 1 purified mAb as the capture antibody and a polyclonal rabbit antibody to olive pollen as the tracer. Quantitation of Ole e 9 was achieved using, as primary reference, an affinity purified Ole e 9 preparation, the protein content of which was determined by amino acid analysis. The purity of the allergen was assessed by SDS-PAGE and N-terminal sequence analysis. The allergenic activity of the reference was determined by RAST inhibition.

Results

The dose response curves obtained in the assay with purified Ole e 9 and olive extracts were parallel, thus validating the use of the reference for quantitative purposes. The detection limit of the ELISA is about 10 ng/mL, and the practical working range lies between 20 and 200 ng/mL. Significant differences in the content of Ole e 9 in 7 different batches of olive extracts were found.

Conclusion

A mAb based ELISA for the major olive allergen Ole e 9 was set up. Due to its reproducibility and sensitivity, the assay is especially useful for standardisation of *Olea* extracts intended for clinical use, as well as for raw material screening and process validation. The availability of this assay has a high relevance, since significant differences in composition have been detected in olive pollen extracts from different origins.

Retrospective follow-up study of tolerance to a new commercial injected immunotherapy treatment allowing for a short build-up schedule

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Background

The conventional immunotherapy (IT) treatment schedule required 13 injections to reach the maintenance dose. A new treatment set of allergen extract recently commercialised allows for a more convenient administration, based on a shorter build-up phase which is completed between 3 and 6 weeks with 8 or 7 injections, respectively. We set out to assess the tolerance of this new product.

Method

A retrospective tolerance study has been carried out by 6 clinical groups. A 6-month follow-up was performed from September 2002 to February 2003. All patients who initiated IT in this period in the participant centres were included. Adverse reactions, systemic (SR) and local (LR), were registered in standardised forms and the percentage of reactions per patient and per administered dose calculated. The treatment schedules were as recommended by the manufacturer: 7 injections/7 visits called PLUS schedule and 8 injections/4 visits called CLUSTER schedule. In both cases, it was a weekly visit.

Results

353 patients were included (261 PLUS and 91 CLUSTER) and 2,886 doses administered (2,166 build-up and 720 maintenance). Of these doses, 800 corresponded to grass extract, 1,141 to grass & *Olea*, 273 to *Olea*, 73 to *Dermatophagoides mix* and 599 to *D. pteronyssinus*.

2.8% of the patients presented SR and 4.8% LR; a total of 1.2% of doses caused some sort of reaction (0.3% SR and 0.9% LR). No significant differences in the frequency of adverse reactions were observed, neither as a function of the build-up schedule, the allergens nor the first dose. Still, grass extract had the highest percentage of LR. 67% of SR and 68% of LR were not immediate but delayed. 64% of reactions resolved spontaneously and the rest responded positively to treatment. Epinephrine shot was necessary once as a consequence of immediate asthma. No anaphylactic shock, hospitalisation or life threatening situations occurred.

Conclusion

The new injected schedule proved to be well tolerated allowing for a shorter build-up phase while maintaining a tolerance similar to the conventional schedules.

The outcome of determination of specific IgE is dependent upon the population being investigated

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Background

Method comparisons are often used to demonstrate the characteristics of a new assay compared to an existing one. The aim of this study was to investigate what effect the choice of population and the choice of reference method may have on such comparisons, through measuring specific IgE (sIgE) in 2 populations in 2 different sIgE methods: the ADVIA Centaur (AC) and Pharmacia CAP (CAP) systems.

Method

Serum samples from a Scandinavian population were tested for specific IgE against Japanese cedar (t17) and wall pellitory (w21) in the AC and CAP sIgE assays. In addition, t17 was tested on a sensitised Japanese population, and w21 was tested on a sensitised Spanish population for method comparison. Sensitivity and specificity was calculated for both methods, using the other as reference.

Results

For the Scandinavian population which may likely be assumed not to be truly sensitised to neither t17 nor w21, the concordance between the 2 methods was 92% for t17 and 73% for w21. For t17, AC showed a sensitivity of 87% and a specificity of 100% vs. CAP. Conversely for t17, CAP showed a sensitivity of 100% and a specificity of 83% vs. AC. For w21, AC showed a sensitivity of 49% and a specificity of 100% vs. CAP. Conversely for w21, CAP showed a sensitivity of 100% and a specificity of 63% vs. AC. All of the discordant Scandinavian samples were due to a positive response in the CAP system. These samples were tested for other sIgE reactivities. All AC negative and CAP positive samples were found to have sIgE response against 1 or more other allergens, primarily inhalants being present in the local environment. All of the discordant samples in t17 were tested positive on grass, tree and weed pollen prevalent in the Nordic countries. All of the discordant samples in w21 were tested positive on grass and tree pollen prevalent in the Nordic countries and/or mugwort (w6) or other weed pollen. When tested on a more relevant population, the concordance between the 2 methods was 100% for t17 and 98% for w21. For t17, both AC and CAP showed a sensitivity and specificity of 100%. For w21, AC showed a sensitivity of 98% and specificity of 100% vs. CAP, whereas CAP showed a sensitivity of 100% and specificity of 96%.

Conclusion

The selection and inclusion of patient samples for direct method comparison should be planned carefully with respect to bias towards one method and relevant patient exposure to allergens compared.

Different shrimp specific IgE assays may give various test results

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Background

Tropomyosin has been defined as the only major allergen in shrimp and extensive cross reactivity exists to other tropomyosins from other *Crustacean* species, mites and insects. A method comparison between 2 specific IgE assays, Pharmacia CAP (CAP) and ADVIA Centaur (AC), for shrimp (f24) revealed 39% discrepancy between the 2 systems. Selected discrepant samples (AC negative and CAP positive) were further investigated.

Method

Purified tropomyosin Pan b 1 from shrimp and recombinant mite tropomyosin Der p 10 were biotinylated. Available concordant and discrepant samples were tested against the 2 reagents in the AC specific IgE assay and compared to response against shrimp allergens (f24) in AC or CAP. Serum inhibition CAP assay with extracts of shrimp, crab, mite, cockroach and olive (a nonsense allergen) was performed on 5 discordant samples.

Results

There was 98% and 100% concordance between Pan b 1 and Der p 10, and the AC f24 assay, respectively. There was 56% and 65% concordance between Pan b 1 and Der 10, and the CAP f24 assay, respectively. All concordant samples in the AC and CAP f24 assay were concordant to the tropomyosin assay, while discrepant samples were also negative against tropomyosin. At 50 µg/ml inhibition allergen, only 1 out of 5 discordant samples were inhibited completely with shrimp and 1 other sample was partly inhibited with shrimp and crab. 3 out of 5 samples were inhibited completely with mite and cockroach. Skin prick tests (SPT) were negative for 6 other discrepant samples. Serum samples from verified seafood allergic patients in Spain showed 7 out of 9 were AC f24 positive and 6 of these were Pan b 1 positive. All 9 patients were AC squid positive and 8 were CAP f24 positive. Serum samples from verified mite allergic and non-seafood allergic patients showed 11 out of 12 were negative against AC f24, Pan b 1, Der p 10, but 4 of these samples were CAP f24 positive.

Conclusion

These results suggest that the Pharmacia CAP f24 assay measures different sIgE than the ADVIA Centaur f24 assay. The ADVIA Centaur f24 assay is concordant with tropomyosin sensitisation, while sIgE measured with Pharmacia CAP in some cases are not related to any known allergen.

Immunological characterisation of recombinant Phl p 6 and comparison with natural Phl p 6

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Rationale

Phl p 6 is one of the major allergens of *Pheleum pratense* grass pollen. Recombinant expression of Phl p 6 allows a thorough characterisation of the protein. We explored the authenticity of the recombinant molecule (rPhl p 6) with its natural counterpart.

Methods

rPhl p 6 was expressed in *Pichia pastoris* and both the recombinant and the natural molecules were purified to homogeneity by identical methods including a Cu-chelate column and size exclusion chromatography. The immunological properties of the molecules were compared by their ability to inhibit the binding of nPhl p 6 to grass pollen allergic patients' IgE, by histamine release assay with grass allergic patients' blood, T-cell reactivity and by using monoclonal antibody based immunoassays.

Results

rPhl p 6 and nPhl p 6 inhibit the binding of biotinylated nPhl p 6 to serum IgE to a similar extent. Blood cells from grass allergic patients were incubated with either of the molecules and degranulation was measured as release of histamine in a similar allergen dose dependent manner. Phl p 6 specific T-cell reactivity was investigated using Phl p 6 specific T-cell lines and clones. The readouts used were proliferation and cytokine production. All cultures tested responded equally to both nPhl p 6 and rPhl p 6. The kinetic parameters of the binding of 3 monoclonal antibodies raised against Phl p 6 were identical for the 2 molecules.

Conclusion

Large amounts of recombinant Phl p 6 can be produced by heterologous expression in yeast. The recombinant and the natural molecule show similar activity in the immunological assays.

Influence of airborne pollen, pollution and meteorology on the symptoms of pollen allergic patients

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Background

The aim of the study was to determine the impact of airborne grass pollen, environmental pollutants and meteorological factors on the symptom and medication (S/M) score of grass pollen allergic patients with asthma, rhinitis or both.

Methods

108 patients from 4 Spanish cities (25 from Madrid, 25 from Plasencia, 24 from Salamanca and 34 from Sevilla) completed an S/M diary card between May and June of 2002. Daily values of grass pollen, pollutants and meteorological parameters were collected. Grouped variables were generated for each patient, S/M score and the relationship with environmental variables was analysed.

Results

Allergenic pressure was greater in Plasencia (219 grains/m³ daily average) than in Madrid (77 grains/m³), Sevilla (99 grains/m³) or Salamanca (101 grains/m³). S/M scores, adjusted for pollen and severity of disease, were similar in these 3 cities, although patients in Madrid showed higher values that were only significant for asthma symptoms. S/M scores intensely and significantly correlated with pollen counts. The relation between these 2 and the meteorological variables was not constant in all the cities, although a direct correlation with temperature and inverse with humidity prevailed. The relation between pollutants and S/M scores did not show a defined pattern except for a significant positive correlation with particles under 10 µm. Moreover, step regression introduces pollen count as the most relevant variable to explain S/M scores. As in the analysis of daily correlations, the rest of environmental variables behaved heterogeneously between the cities. Other analytical procedures of classification returned similar results.

Conclusion

Airborne grass pollen is the principal determinant of S/M scores. It has not been possible to demonstrate that meteorological or pollution differences among the cities induce differences in S/M scores.

Purification of recombinant Ves v 5 and Pol a 5 and their use on the Bayer ADVIA Centaur in the diagnosis of hymenoptera allergy

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Background

Recombinant (r) Ves v 5 and rPol a 5, antigen 5s from yellow jacket (*Vespula vulgaris*) and paper wasp (*Polistes annularis*), are immunochemically analogous to their natural counterparts and are major allergens of the venoms of these species. These molecules have structural differences that give them different antigenic properties. Therefore, they are here used to discriminate between sensitivities towards these vespid species. Their use could also be important in the diagnosis of allergies to other hymenoptera such as bees, which could be due to homologous proteins or to cross reacting carbohydrate determinants.

Methods

Recombinant antigen 5s are produced in *Pichia pastoris* and are purified by a novel procedure that employs size exclusion chromatography. The purified molecules are biotinylated and used to measure, in an ADVIA Centaur System (Bayer Diagnostics), the specific IgE present in the sera of patients allergic to hymenoptera. Sera from 53 patients were tested against both recombinant proteins.

Results

rVes v 5 and rPol a 5 are obtained as pure proteins from the culture fluid of *P. pastoris*. 62.5% of the patients tested had specific IgEs directed against these recombinant molecules. Among these patients, 85% had IgEs specific only either to Ves v 5 or Pol a 5, indicating that the measuring strategy followed allowed a good discrimination of the sensitivity exhibited by the allergic patients. Moreover, being proteins that are specific to the venom of vespids and considering also that they are not glycoproteins, antigen 5s seem to be specific reagents for the correct diagnosis of hymenoptera venom allergy.

Conclusion

The use of recombinant antigen 5s from 2 species of vespids in the ADVIA Centaur system clearly define the sensitivity of more than 60% of patients allergic to the venom of these hymenoptera.

Treatment history of patients with allergic airway disease before onset of specific immunotherapy

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Background

It is known that in Germany specific immunotherapy (SIT) is insufficiently used as a therapy option for patients with allergic airway disease. The purpose of this study was to determine how many patients receive SIT only after longer periods of pre-treatment and to investigate whether patients with delayed access to SIT would benefit less.

Method

Patients were sampled from the practices of 180 dermatologists, ENT physicians, pulmonologists and paediatricians who were all specialised in allergology. The current analysis is based on data from 6,791 patients who were undergoing their first hypo-sensitisation at the time of data collection. Patients filled in a questionnaire dealing with their treatment history and the efficacy of the allergy treatments they had received. The physicians recorded data on each patient's diagnosis and current treatment.

Results

32% of the patients had previously consulted a different doctor with their allergic airway disease. 74% of these patients indicated that their condition had not improved at all or only a little as a result of pre-treatment. Only 28% of them had been referred from their pre-treating physician to the allergological specialist while most patients (53%) had changed doctors on their own account. The most frequent reason for changing the doctor was insufficient improvement of allergy symptoms. The physicians taking part in the study classified the point-in-time of beginning the SIT treatment significantly more often as "delayed" in pre-treated patients. This partly explains why the proportion of patients having improved as a result of current SIT treatment was slightly lower among patients with pre-treatment.

Conclusion

The study emphasises the deficiencies in the provision of SIT in Germany. Most allergy patients are initially treated by general practitioners who do not provide SIT themselves. These physicians apparently regard hypo-sensitisation only rarely as a therapeutic option and hesitate to refer their patients to a specialised allergologist.

Multicentre study to validate a symptom and medication diary card in patients sensitised to grass pollen

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Background

We carried out a follow-up study in 6 Spanish cities to assess the reliability and sensitivity of a symptom and medication (S/M) diary card model.

Method

142 patients sensitised to grass pollen completed the diary card during 2 4-week periods, in spring and fall. 42 of these patients were also sensitised to a perennial allergen. Reliability was defined as the capability to detect the severity of the disease according to the result of the evaluation performed by the physicians at the beginning of the study, and to the subjective self evaluation of the patients on a visual analogical scale. Sensitivity was determined as the capability to reflect changes after different pollen exposure levels.

Results

Reliability; consistently with the physician's evaluation, patients diagnosed with mild or severe rhinitis scored significantly higher in nasal symptoms and medication, and had a lower percentage of days without medication than patients with absent or mild rhinitis. Similarly, asthmatic patients had higher scores in pulmonary symptoms and medication, and a lower percentage of days without symptoms, medication or both. All symptom scores and the percentages of days without symptoms, medication or both were significantly correlated with the patients' subjective evaluation. Sensitivity; during fall, all measured variables dropped. Differently from the previous stage, disparities appeared between the scores of patients sensitised also to perennial allergens and those who were not. The latter showed lower nasal, ocular and total symptom scores, and a higher percentage of days without symptoms and medication. We found a remarkably high daily correlation between pollen counts and S/M scores in patients with mild rhinitis in 4 of the 6 studied cities. Total medication scores correlated with pollen to a lower degree.

Conclusion

The proposed S/M diary card model is a sensitive and reliable instrument for the evaluation of the status of the allergic disease and the clinical evolution of allergic patients.

Anaphylaxis to pomegranate: detection of IgE reactivity to a 13 kDa protein

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Rationale

Pomegranate is the fruit of the *Punica granatum* tree. The fruit pulp is made of a cluster of red-violet seeds. Hypersensitivity to pomegranate has been rarely reported, although systemic reactions are frequently implicated. Nevertheless, the allergens involved have not been identified so far and only IgE reactivity to a 29 kDa protein has been reported.

Case report

A 31-year old female suffered an acute episode of red itchy palms and soles, abdominal pain, cough, generalised urticaria and shortness of breath after ingesting a pomegranate. She had a past history of oropharyngeal pruritus when eating peach peel and seasonal allergic rhinoconjunctivitis to *Platanus acerifolia* for the last 6 years. An allergic study was carried out. *In vitro* immunoassays were performed in order to determine the pattern of IgE reactivity and cross reactivity.

Results

Skin prick tests to commercial extracts of common inhalants and foods (ALK-Abelló, Madrid, Spain) were positive to *Platanus acerifolia* pollen, peach peel and nuts. Prick-by-prick with fresh pomegranate fruit pulp showed an immediate positive wheal (12x6 mm). Common blood parameters were within normal limits, being total serum IgE 228 kU/L. Patient serum showed high levels of specific IgE (Pharmacia CAP system) to *Platanus acerifolia* (48.4 kU/L), peach (13.4 kU/L) and walnut (0.85 kU/L). Determination of specific IgE by ELISA to purified Pru p 3 gave >27 kU/L. SDS-PAGE immunoblotting of pomegranate and peach extracts showed a strong IgE binding band of \approx 13 kDa and another weak one of \approx 27-29 kDa.

Conclusion

Pomegranate is an uncommon but potential allergen of severe systemic reactions. To our knowledge, this is the first report of a low-molecular-weight allergen of 13 kDa (possibly a nSLTP) involved in pomegranate allergy.

Randomised, controlled trial of specific immunotherapy on allergic inflammation in atopic dermatitis patients with sensitisation to house dust mites

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Sensitisation to house dust mite allergens, which is detectable with specific IgE tests, is very common in patients suffering from atopic dermatitis. Specific immunotherapy is an effective therapy in respiratory IgE mediated allergic diseases and has been described to be effective in atopic dermatitis in some open clinical trials. “Classical” specific immunotherapy has not been investigated in double-blind, placebo-controlled trials in atopic dermatitis so far. In a multi-centre, double-blind, randomised trial, we treated patients with atopic dermatitis, IgE mediated sensitisation against house dust mites and a SCORAD score higher than 40 points with subcutaneous specific immunotherapy. Patients were randomised into 3 dose groups with maintenance doses of 20 (“active placebo”), 2,000 and 20,000 SQ-U (“active treatment”) *Dermaphagoides pteronyssinus/farinae* extracts for a 1-year treatment. 79 patients with at least 1 SCORAD assessment (equivalent to treatment for 2 months up to 1 year) could be evaluated as full analysis set (intention-to-treat). The SCORAD score declined in all 3 study groups. The differences in the SCORAD score between baseline and the last 3 months of treatment were significantly greater in patients actively treated (2,000 and 20,000 SQ-U) compared to patients with active placebo (20 SQ-U). The application of topical corticosteroids in the 2 active treatment groups was significantly reduced compared to active placebo. The final assessment of the physician confirmed a better skin condition of patients with active treatment compared with active placebo at the end of therapy. Here we show for the first time in a double-blind, placebo-controlled trial that “classical” allergen specific immunotherapy with house dust mite extracts is effective in patients with atopic dermatitis sensitised to house dust mite allergens, and may be valuable in the treatment of this chronic skin disease.

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Curing Allergy

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