Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY
EAACI

Poster Discussion Session PDS 1

New clinical data in AIT

217
Is premedication important to prevent reactions in cluster immunotherapy?

Olgaç, M; Demir, S; Ünal, D; Gelincik, A; Çolakoğlu, B; Büyüköztürk, S
Istanbul Faculty of Medicine, Internal Medicine Department, Division of Immunology and Allergic Diseases, Istanbul University, İstanbul, Turkey

Background: Although pretreatment is not routinely used before immunotherapy, it has been shown to reduce the reactions in patients using rush, cluster and conventional schedules. Our aim is to determine the possible preventive effects of various premedication usage on systemic and local reactions during cluster immunotherapy.
Method: One hundred and eighteen patients receiving a total of 137 cluster immunotherapy protocols to mites, pollens, bee and wasp venoms were recruited in the study. Patients were randomized into 5 different groups according to premedication status as shown here below: Group 1: daily levosetirizine, Group 2: levosetirizine only 2 h prior to injections, Group 3: daily montelukast, Group 4: combinations of montelukast and levosetirizine and, Group 5: no premedication Patients were followed during build up and maintenance phase of immunotherapy, systemic and local reactions were reported. Reactions are reported according to World Allergy Organization Subcutaneous Systemic Reaction Grading System.
Results: Most of the patients were female (%64.2), the most frequent allergen was house dust mites (50.4%). Of 69.3% of patients took premedication and 19.7% of patients had reactions during the build up phase. Reactions were more frequent in patients who have pollen allergy and have received pollen immunotherapy [$P = 0.034$; OR = 2.65 CI:95%, 1.05–6.63)]. The total frequency of the hypersensitivity reaction was significantly higher in patients not receiving premedication [14 (14.7%) vs 13 (31.0%), $P = 0.028$]. However no difference was detected between groups taking various drugs for premedication. The patients in the premedication group significantly experienced less local reactions ($P = 0.033$) although no difference in the frequency of systemic reactions was seen between the groups.
Conclusion: These preliminary results suggest that the reaction risk is increased in pollen immunotherapy and premedication does not seem to prevent the frequency or severity of systemic reactions although it decreases the frequency of local reactions. As the number of patients recruited in the study increases, the effect of premedication on reactions may be clearer.

218
Correlation of field data with environmental challenge chamber (ECC) data in grass pollen allergic patients

Plückhahn, K; Meyer, H; Praechter, C; Narkus, A; Häfner, D
Allergopharma GmbH & Co. KG, Reinbek, Germany

Background: This comparative trial was designed to prove the specificity of reported symptoms by comparing symptoms from healthy subjects with grass pollen allergic patients. The trial also evaluated the correlation between the rhinoconjunctivitis symptoms experienced by grass pollen allergic patients in the ECC upon exposure to grass pollen allergen and symptoms experienced by these patients during the grass pollen season in Canada.
Method: Adult grass pollen allergic patients and healthy subjects were exposed to grass pollen in the ECC before, shortly after the peak, and after the natural grass pollen season. The total symptom score (TSS) in the ECC was achieved by calculating the sum of 4 individual nasal and 3 ocular symptoms: nasal congestion, rhinorrhea, nasal itching, and sneezing, as well as ocular itching, tearing/watery eyes, and ocular redness. In the natural pollen season of 2014, subjects reported their rhinoconjunctivitis symptoms and use of rescue medication to assess the rhinoconjunctivitis symptom medication score (RC-SMS). For correlation analysis the areas under the curves of TSS and RC-SMS were calculated.
Results: The specificity of reported symptoms could be demonstrated since only grass pollen allergic patients reported significant symptoms after exposure. A statistically significant positive correlation between the ECC and the natural pollen season could be shown.
Conclusion: Although a good correlation between field and ECC data has been reported in the literature for patients with ragweed allergy, this trial shows a modest correlation for patients suffering from grass pollen allergy. Further investigations are warranted to determine whether the correlation may be dependent on the specific allergen causing the allergic disease or on other interacting confounding factors.

219
Correlation of the intracutaneous test (ICT) and environmental challenge chamber (ECC) data from a *Phleum pratense* pollen allergoid trial

Hansen, S; Tribanek, M; Praechter, C; Narkus, A; Häfner, D
Allergopharma GmbH & Co. KG, Reinbek, Germany

Background: We performed a randomized, double-blind, placebo and active-controlled trial to investigate the dose response relationship of a *Phleum pratense* allergoid preparation for subcutaneous specific immunotherapy.
Method: Adult patients ($n = 98$) with allergic rhinitis/rhinoconjunctivitis +/- bronchial asthma were randomized to 1 of 5 groups. Three groups received 9 preseasonal injections of the investigational medicinal product (IMP) in different doses: standard dose (10 000 TU/mL; $n = 18$), 3-fold lower ($n = 20$), and 3-fold higher ($n = 18$) than standard dose. The active comparator group ($n = 22$) was treated with a standard dose of a commercial 6-grasses allergoid. Twenty patients received placebo. The primary efficacy endpoint was the change in the size of the wheal (area in mm^2) 6 h after ICT injection pre and post treatment. In the ECC, rhinitis symptoms were assessed by measuring the total nasal symptom score (TNSS). Nasal allergic symptoms were evoked by a standardized exposure to pollen from *Dactylis glomerata*. Measurements were performed for 2 h every 20 min. TNSS integrated 4 symptoms: nasal congestion, rhinorrhea, nasal itching, and sneezing. Pearson's correlation coefficients were calculated between the baseline adjusted change of AUC in the TNSS and the change in the ICT wheal size area.
Results: For all doses of IMP or active comparator, statistically significant ICT wheal size reductions after treatment

© 2015 The Authors

($P < 0.001$ for each comparison of one active treatment group to placebo) were observed. Furthermore, a decrease in TNSS of 18.6% in the low-dose group and 38.4% in the standard-dose group was seen. Regarding the changes ICT wheal size and TNSS, a statistically significant correlation ($r = 0.34$) was shown.
Conclusion: There is a positive correlation between the ICT wheal size and the TNSS.

220
Relationship between pollen dispersal and the severity of allergy symptoms and immunotherpy: allergen immunotherapy regulates symptoms at the peak, but not in the early period, of pollen dispersal

Okamoto, Y[1]; Sakurai, T[1]; Iinuma, T[1]; Sakurai, D[1]; Yonekura, S[1]; Okubo, K[2]; Kaneko, S[3]; Konno, A[4]
[1]Chiba University Graduate School of Medicine, Otorhinolaryngology, Chiba, Japan; [2]Nippon Medical School, Otorhinolaryngology, Tokyo, Japan; [3]Torii Pharmaceutical Co.,Ltd., Tokyo, Japan; [4]South Tohoku General Hospital, Otolaryngology Unit, Koriyama, Japan

Background: The severity of symptoms of pollinosis is affected by the amount of scattered pollen. However, the details of this mechanism have not been clarified with respect to the pollen dispersal pattern, timing of the pollen season and impact of medications.
Method: The relationships between the daily amount of pollen dispersal and the severity of symptoms as well as effects of sublingual immunotherapy (SLIT) were examined in order to clarify the efficacy of treatment with SLIT. A randomized, placebo-controlled study was carried out from 2007 to 2012 among patients with Japanese cedar pollinosis. The patients were asked not to use any rescue medicines during the cedar pollen season to the extent possible. The daily amount of pollen dispersal was quantitated according to the Durham method, and the subjects recorded their nasal symptoms in allergy diaries.
Results: A total of 1313 patients were enrolled, with 119, 112, 53, 65, 482 and 482 subjects in 2007, 2008, 2009, 2010, 2011 and 2012, respectively. The cedar pollen season was divided into three periods: the early period, from the start of the pollen season to the first peak of pollen dispersal (>100 grains/ cm^2/day); the middle period, from the first peak to the last peak (>100 grains/ cm^2/day); and the late period, from the last peak to the end of dispersal. In the placebo group, a correlation between the amount of pollen dispersal and the severity of allergies was observed only in the early period, with no evidence of such a correlation in the middle or late periods, each year. Treatment with SLIT significantly improved the patients' symptoms in the middle, but not clearly early, period.
Conclusion: The daily amount of pollen dispersal affects the severity of symptoms in patients with pollinosis only in the early period of the pollen season, and SLIT therapy is notably not effective during this period.

221
Real life safety experience with 5-grass pollen sublingual tablet for the treatment of grass pollen-induced allergic rhinoconjunctivitis

Didier, A[1]; Bons, B[2]; Zeldin, RK[2]
[1]Larrey Hospital, Toulouse, France; [2]Stallergenes S.A., Antony, France

Background: Treatment with 5-grass pollen sublingual tablet has proven efficacious, safe, and well tolerated in a multinational clinical development program consisting of eight randomised, double-blind, placebo-controlled studies that enrolled a total of 2512 subjects. As a result, the tablet has received marketing authorisation for the treatment of patients with grass pollen-induced allergic rhinoconjunctivitis in over 30 countries. Here we present the post-marketing safety experience.
Method: Since the first Marketing Authorisation in 2008, safety data consisting of adverse event reports have been collected from spontaneous reporting and post-marketing authorisation safety studies (PASS) through the pharmacovigilance system.
Results: Through 2014, the post-marketing exposure to the 5-grass tablet is estimated at 170 000 patients (55 000 < 18 years old). In addition, 1728 patients participated in one of two PASS. The cumulative rate (i.e. number of cases / total number of patients exposed) for spontaneously reported cases of adverse reactions is 58/10 000 patients. Most were application site reactions such as mouth oedema or throat irritation. No deaths were reported. Cases of anaphylaxis and severe laryngopharyngeal reactions have been reported rarely. All resolved without sequelae. In the PASS, the nature, intensity and frequency of adverse reactions were consistent with those observed in the clinical development program.
Conclusion: The favourable safety and tolerability profile of the 5-grass pollen sublingual tablet observed in the clinical development program is confirmed in the post-marketing experience of more than 170 000 patients.

222
Consistent efficacy of a 300IR birch pollen sublingual immunotherapy solution assessed by proportion of symptom-controlled days

Worm, M[1]; Rak, S[2]; Arvidsson, M[2]; Radu, C[3]; De Blay, F[3]; Malling, H-J[4]
[1]Campus Charité Mitte, Universitätsmedizin Berlin, Allergie-Centrum-Charité, Berlin, Germany; [2]Asthma and Allergy Research Group, Department of Respiratory Medicine and Allergology, Sahlgrenska University Hospital, Göteborg, Sweden; [3]Chest Diseases Department, Strasbourg University Hospital, Strasbourg, France; [4]Allergy Clinic, University Hospital, Copenhagen, Denmark

Background: Birch pollen 300IR sublingual immunotherapy (SLIT) demonstrated sustained efficacy and safety in adults with alergic rhinocojunctivitis over 2 pollen seasons[1]. An alternative approach to combining symptom score and intake of rescue medication is the number of days with symptom control (i.e., days without intake of rescue medication and a symptom score below a pre-defined and clinically justified threshold).
Methods: In a randomised, double-blind, placebo-controlled, multi-national, multicentre, Phase IIIb study, adults (aged 18 to 65 years) with a birch pollen ARC were randomly assigned to a 300IR SLIT solution of birch-pollen ($n = 284$) or Placebo ($n = 290$), daily starting 4 months before and continuing through the pollen season for two pollen seasons. Of those, 496 completed the study. Proportion of Symptom-Controlled Days for each patient (PSCDs) was calculated as the proportion of days during the pollen period while the patient was on treatment with no rescue medication intake (RMS = 0) and controlled symptoms (RTSS = 0 or 2).
Results: In Period 2, patients in the 300 IR group had a greater proportion of totally controlled days (PSCD0-0, i.e. proportion of days during the pollen period while the patient was on treatment with RTSS = 0 and RMS = 0) than did the patients in the Placebo group (23.8% vs 13.9%). The relative mean difference between the 300IR and Placebo group was 70.9%. The proportion of well-controlled days (RTSS≤ 2 and RMS = 0) was also greater among the patients in the 300IR group (34.7%) than in the Placebo group (22.2%). Similar results for PSCD0-0 were seen during the first pollen period (14.0% in 300IR group vs 9.8% in Placebo group, with a relative mean difference of 43.3%).
Conclusion: The results obtained with the Proportion of Symptom-Controlled Days were consistent with those observed for the primary efficacy variable with significant differences during the pollen period in favour of the 300IR group.

© 2015 The Authors

[1]Worm M, Rak S, de Blay F, Malling HJ, Melac M, Cadic V, Zeldin RK. Sustained efficacy and safety of a 300IR daily dose of a sublingual solution of birch pollen allergen extract in adults with allergic rhinoconjunctivitis: results of a double-blind, placebo-controlled study. Clin Transl Allergy. 2014 Feb 11;4(1):7.

223
House dust mite sublingual immunotherapy for respiratory allergies in clinical practice (the ACTIVE study)

Migueres, M[1]; Delaisi, B[2]; Jeanpetit, Y[3]; Ebbo, D[4]; Massabie, Y-P[5]; De Blay, F[6]
[1]Clinique de L'Union, Service de Pneumologie et Allergologie, Saint-Jean, France; [2]Centre de Pneumologie de l'enfant, Boulogne Billancourt, France; [3]Stallergenes, Antony, France; [4]Private Practice, Paris, France; [5]Private Practice, Marseille, France; [6]Nouvel Hopital Civil, Service de Pneumologie, Strasbourg, France

Background: House dust mites (HDM) are the most prevalent inhaled allergen wolrdwide impacting the patient's quality of life. Sublingual immunotherapy (SLIT) is a treatment option for HDM allergy.
Method: ACTIVE is a large observational, prospective study conducted in France and designed to describe (i) the clinical profiles of patients initiating HDM SLIT and (ii) SLIT's impact on allergic symptoms during the first year of treatment.
Results: Eight hundred and fifteen HDM allergic patients (children and adolescents: 45%; adults: 55%) consulting an allergy specialist for HDM-induced allergy were included in the study. Almost all patients (97%) suffered from allergic rhinitis (AR) and 70% had persistent, moderate-severe AR. Comorbidities included conjunctivitis (40.5%) and asthma (54%). Additional allergies to grass pollens, animal dander and tree pollens were respectively reported in 14%, 11% and 8% of the 212 polyallergic patients. In line with the inclusion criteria, all 815 patients initiated HDM SLIT; 59 (28%) of the 212 polyallergic patients received concomitant immunotherapy (mainly with grass or tree pollen extracts). HDM SLIT included an initiation phase (with a daily dose of 10IR in 88% of treated patients) and a maintenance phase (with a daily dose of 300IR in 99.0% of treated patients). The mean ± SD dose per administration was 7.5 ± 2.1 actuations, and 97.5% of patients prescribed with a year-round regimen. The prescribed duration of HDM SLIT was 3 years for 65.5% of the patients and 5 years for 21%. Patients reported benefit from SLIT from the first months of treatment (months 1 to 4) onwards. Overall efficacy was evaluated as very good or good by 70% of the patients after 6 months and by 77% after one year. The physicians' evaluations of efficacy gave similar results. After one year of HDM SLIT, 99% of the patients reported a reduction in their asthma symptoms.
Conclusion: In real-life practice in France, HDM SLIT is primarily prescribed to patients suffering from persistent, moderate/severe AR and co-morbid asthma. The majority of patients reported benefit from the first months of treatment onwards and then throughout the first year.

224
A randomized DBPC dose-finding multicenter trial of SLIT allergoids tablets in house dust mites (HDM) allergic patients

Mösges, R[1]; Dieterich, P[1]; Hüser, C[1]; Singh, J[1]; Allekotte, S[1]; Shah-Hosseini, K[1]; Compalati, E[2]
[1]University Hospital of Cologne, Institute of Medical Statistics, Informatics and Epidemioloy, Cologne, Germany; [2]Lofarma S.p.A., Milano, Italy

Background: Mites tablets of monomeric allergoids have been developed for sublingual immunotherapy in patients suffering from allergic rhinoconjunctivitis (ARC). The purpose of this trial was to determine the efficacy and safety of four different doses of mites tablets compared to placebo.
Method: Out of 160 patients recruited, 131 adult patients with ARC induced by HDMs were randomized for this dbpc phase II study (EudraCT No 2013-000617-20) conducted in Germany. Treatment consisted of either 300 UA/d; 1000 UA/d; 2000 UA/d; 3000 UA/d or placebo over a course of 12 weeks. Efficacy was assessed by the improvement of reactions to a titrated conjunctival allergen challenge. Safety was assessed by frequency, type and severity of treatment-related adverse events (TRAE).
Results: After a 12-week course of immunotherapy, 88.5% and 76.0% of the patients treated with 2000 UA/d and 1000 UA/d, respectively, showed a tenfold improvement in the threshold of allergen concentration compared to 64.2% under placebo ($P < 0.05$ and $P = 0.358$). Neither treatment related SAEs nor cases of anaphylaxis were reported, so there was no need for the use of epinephrine. In total, of all patients under active treatment 4.95% experienced local TRAEs while 6.93% had systemic TRAEs.
Conclusion: The treatment with mites monomeric allergoids is a well-tolerated and safe treatment for patients suffering from HDM induced ARC. The highest proportion of patients with improvement in the CPT threshold of allergen concentration was found in patients treated with 2.000 UA/d corresponding to approximately 168.000 UA cumulative dose during the course of the trial.

225
Early onset and sustained year-round effect of the SQ HDM SLIT-tablet in HDM respiratory allergy

Klimek, L[1]; Horak, F[2]; Kuna, P[3]; Zieglmayer, P[4]; Seitzberg, D[5]; Broge, L[5]; Nolte, H[6]
[1]Center for Rhinology and Allergology, Wiesbaden, Germany; [2]Research Consult GmbH, Dptm. Vienna Challenge Chamber, Vienna, Austria; [3]Medical University of Lodz, Lodz, Poland; [4]Vienna Challenge Chamber, Institute for Allergy Research, Vienna, Austria; [5]ALK, Horsholm, Denmark; [6]Merck & Co., Inc, Whitehouse Station, United States

Background: House dust mite (HDM) allergy is a perennial allergy but HDM exposure may vary. The immunological changes leading to the clinical effect of successful allergy immunotherapy take time to develop. This abstract investigates onset and development of the effect of treatment with the SQ HDM sublingual immunotherapy (SLIT)-tablet.
Method: This abstract includes 3 evenly randomised, double-blind, placebo-controlled efficacy trials with once-daily treatment with the SQ HDM SLIT-tablet. Treatment was initiated throughout the year.

- P003: Chamber trial, $N = 124$, 6 and 12 SQ-HDM, efficacy in AR assessed by the total nasal symptoms score after 8, 16 and 24 weeks of treatment.
- MT-04: Field trial, $N = 834$, 6 and 12 SQ-HDM, efficacy in AA assessed by time to first asthma exacerbation under inhaled corticosteroid reduction over the last 6 months of 13–18 months of treatment.
- MT-06: Field trial, $N = 992$, 6 and 12 SQ-HDM, efficacy in AR assessed by the total combined rhinitis score (TCRS, sum of AR symptoms and AR medication scores) after approximately 4, 14, 24, 34 and 44–52 weeks of treatment.

Results: All 3 trials confirmed efficacy against placebo. For 12 SQ-HDM, onset of a clinically relevant effect was demonstrated after 8 weeks of treatment under controlled exposure (P003) and after 14 weeks of treatment in a field setting (MT-06). For 6 SQ-HDM, onset of a clinically relevant effect was demonstrated after 16 weeks of treatment under controlled exposure (P003) and after 14 weeks of treatment in a field setting (MT-06). For both P003 and MT-06, the magnitude of effect increased for both doses until week 24 (end of P003). Treatment continued for an additional 6 months in MT-06, with the

© 2015 The Authors

treatment effect reaching a plateau which was then maintained. The period in which efficacy was shown at regular intervals in MT-06 was May-January. Mean TCRS in the placebo group was around or higher than 7 at all time points. MT-04 showed reduced risk of having a moderate or severe asthma exacerbation over the last 6 months of 13–18 months of treatment. The 6-months efficacy evaluation period was in September-April.

Conclusion: The data indicate that HDM-allergic patients have year-round symptoms which can be alleviated by treatment with the SQ HDM SLIT-tablet. Onset of effect of 12 SQ-HDM was observed 8–14 weeks after initiation of treatment, followed by further increase in magnitude of effect with continued treatment, after which a plateau was reached.

226

The SQ HDM SLIT-tablet is well tolerated in adults with HDM respiratory allergic disease (HDM allergic asthma (AA) and/or allergic rhinitis (AR)): evidence from 2500 subjects in 4 clinical trials

Emminger, W[1]; Calderón, M[2]; Maloney, J[3]; Svanholm Fogh, B[4]; Fejerskov, P[4]; Hernandez, D[5]
[1]Allergy Outpatient Clinic, Vienna, Austria; [2]Section of Allergy and Clinical Immunology, Imperial College, London, United Kingdom; [3]Merck & Co., Inc., Kenilworth, NJ, United States; [4]ALK, Hoersholm, Denmark; [5]IIS Hospital La Fe, Valencia, Spain

Background: 4 randomised, double-blind, placebo-controlled multi-centre phase II and III clinical trials were conducted with the SQ house dust mite (HDM) SLIT-tablet. Here tolerability data from the adult population are presented.

Method: This pooled analysis comprises:

- 565 adults with mild/moderate HDM AA and mild HDM AR on average, randomised 1:1:1:1 to placebo, 1, 3 or 6 SQ-HDM for ˜1 year (MT-02)
- 124 adults with moderate/severe HDM AR +/- mild AA, randomised 1:1:1 to placebo, 6 or 12 SQ-HDM for 24 weeks (P003)
- 834 adults with HDM AA not well-controlled by inhaled corticosteroid (ICS) randomised 1:1:1 to placebo, 6 or 12 SQ-HDM for 13–18 months (232 subjects had uncontrolled asthma according to GINA) (MT-04)
- 992 adults with moderate/severe HDM AR despite frequent use of pharmacotherapy randomised 1:1:1 to placebo, 6 or 12 SQ-HDM for ˜1 year (MT-06)

For all trials, hospitalisation due to asthma exacerbation within 3 months prior to randomisation and FEV_1< 70% were exclusion criteria.

Results: The proportion of subjects with AEs was dose-dependent. With 12 SQ-HDM 72% of AEs were mild and 26% were moderate. Four percentage of subjects experienced severe AEs. The most common AEs were local allergic reactions (with 12 SQ-HDM: oral pruritus 20%, throat irritation 15%, mouth oedema 11%, lip oedema 6%, ear pruritus 5%, paraesthesia oral 5% and tongue pruritus 5%).

The majority of the AEs started on day 1 or 2 (from phase I trials a median onset within 5 min after tablet intake and a duration from a few minutes to hours is known). After the first week AE onset was uncommon, and the proportion of subjects with ongoing AEs decreased with time.

Serious AEs were reported by 3% with placebo and 1% with 12 SQ-HDM. Six treatment-related serious AEs occurred (migraine, dizziness, arthralgia, laryngeal oedema, idiopathic thrombocytopenic purpura, asthma). No AEs were reported as anaphylactic reactions.

There was a single case of adrenaline use on day 1 administered in the physician's office due to an event of mild local reaction, reported in the 12 SQ-HDM group. The subject continued treatment and completed the trial with mild oral pruritus as the only subsequent AE.

There was no indication of the risk of treatment-induced events being greater for the 232 subjects with uncontrolled asthma according to GINA.

Conclusion: The investigated doses of SQ HDM SLIT-tablet were well tolerated in adults, regardless of the severity or control of the HDM respiratory allergic disease.

227

Characteristics of adult patients with house dust mite respiratory allergy in allergist practice

Demoly, P[1]; Broué-Chabbert, A[2]; Wessel, F[3]; Chartier, A[4]
[1]Département de Pneumologie et Addictologie, Centre Hospitalier Universitaire de Montpellier, Montpellier, France; [2]Département de Pédiatrie, Pneumologie et Allergie, Centre Hospitalier Universitaire de Toulouse, Toulouse, France; [3]Département de Pneumologie, Centre Hospitalier Universitaire de Nantes, Nantes, France; [4]Département Médical, ALK, Courbevoie, France

Background: House dust mite (HDM) respiratory allergy is a common chronic disease with a negative impact on quality of life. The role of allergen immunotherapy (AIT) as a possible therapeutic alternative in patients not well controlled by pharmacologic treatment and HDM avoidance remains to be better clarified. This study aims at describing the characteristics of HDM allergic adults seen in clinical practice with or without AIT initiation.

Method: ANTARES is a French epidemiological, observational, transversal study, performed from October 2013 to March 2014 by allergists. Data were prospectively collected using physician and patient self-administered questionnaires. Any patient over 5 years of age with documented HDM respiratory allergy not treated with AIT within the last 12 months was eligible. Hereunder, are shown the results within the adult's subgroup.

Results: 195 physicians enrolled 1589 patients to this ANTARES survey. A subgroup of 938 adults (median age 31 years; 60% of women) is described: 709 initiated AIT (693 with sublingual AIT, 16 with subcutaneous AIT) [AIT group] vs 229 patients without AIT [w/o AIT Group]. In AIT vs w/o AIT groups, allergic rhinitis (AR) duration since diagnosis was 4 years vs 3 years; the mean total nasal symptom score was 8 vs 7 out of 12; 64% vs 38% had moderate to severe persistent AR according to ARIA; 62% vs 50% were polysensitised and 58% vs 48% experienced conjunctivitis; 82% vs 62% reported to be impacted by AR in everyday life [mini Juniper RQLQ]. Allergic asthma (AA) was observed in 39% [AIT] vs 24% [w/o AIT], mostly for both with a mild-to-moderate obstruction [$FEV_1 \geq 70\%$]; according to GINA guidelines, AA was controlled in 59% vs 62%, partly controlled in 32% vs 29% and uncontrolled in 9% vs 10%; the asthma control ACQ score was < 1 (37% vs 50%), between 1 and 1.5 (19% vs 21%) and ≥ 1.5 (43% vs 29%). Ninety four percentage [AIT] were on symptomatic medication vs 89% [w/o AIT] (mainly antihistamines +/- nasal corticosteroids) and about 60% [AIT] declared being unsatisfied by symptomatic medication vs 43% [w/o AIT].

Conclusion: Adults suffering from HDM respiratory allergy undergo AIT mainly due to the global impact of the condition on the daily quality of life. HDM respiratory allergy in adults is characterized by the high rate of polysensitisation, the poor control of AR by symptomatic medication and the frequency of the association with not well controlled allergic asthma.

228

Latex immunotherapy effectiveness

Aruanno, A; Nucera, E; Buonomo, A; Pecora, V; Rizzi, A; Pascolini, L; Ricci, AG; Di Rienzo, A; Centrone, M; Romiti, R; Mezzacappa, S; Schiavino, D
Policlinico A. Gemelli, Roma, Italy

Background: Since 1970 allergic reactions to natural rubber latex (NRL) have been reported and during the last decades they have become an emerging clinical problem

© 2015 The Authors

of public health. Proper diagnosis of latex allergy is important for appropriate preventive measures and treatment. The only etiological and decisive therapy is represented by the specific desensitization. This treatment has a very small incidence of adverse reactions, good patient compliance and especially by a high success rate. Our protocol of rush latex desensitization treatment is performed in 4 days, during which increasing doses of latex extract are administered under patient's tongue until the highest dose of 500 μg of latex. A maintenance therapy is followed at home. Every patient is equipped with an emergency kit and suggested to undergo future specialist visits in latex-safe environment until the latex tolerance is not been acquired. The aim of the study was to verify the clinical efficacy of NRL SLIT in patients that finished the treatment or were treated for at least three years.

Methods: We studied 76 NRL allergic patients, who finished or are still performing a sublingual desensitization treatment according to our protocol.

Primary endpoint was assessed by the changes in the response to challenge tests (cutaneous, sublingual, mucous-oral, conjunctival, nasal), performed before and after at least 3 years of therapy.

The secondary endpoint was to evaluate the possible immunological changes determined by the immunotherapy by means of skin prick tests with latex (Alk-abellò, Milan) and the assay of latex specific IgE.

Results: We detected a significant negativity ($P < 0.01$) of all challenge tests (cutaneous, mucous, nasal and conjunctival) in our patients. Concerning the immunological changes, we found a significant reduction of skin prick test wheal areas ($P = 0.01$), while we observed a reduction of latex specific IgE values but these data didn't seem to be statistically significant. Moreover 25 patients of those who were exposed again to latex (dental and gynecological visits or professional exposure) didn't present adverse reactions after almost three years of desensitization, while 10 patients manifested mild symptoms after latex contact.

Conclusion: Latex sublingual desensitization treatment seem to be safe and can be use as an effective treatment for the NRL allergic patients who have difficulties in applying adequate avoidance measures.

229

Deciphering the dose-response effect of peanut Epicutaneous ImmunoTherapy (EPIT) in peanut allergic subjects

Dupont, C[1,2]; Agbotounou, WK[3]; Thébault, C[3]; Ruban, C[3]; Martin, L[3]; Yang, WH[4]; Sussman, GL[5]; Brown-Whitehorn, TF[6]; Nadeau, KC[7]; Cheema, AS[8]; Leonard, SA[9]; Pongracic, JA[10]; Sauvage-Delebarre, C[11]; Assa'ad, AH[12]; De Blay, F[13]; Bird, JA[14]; Tilles, SA[15]; Boralevi, F[16]; Bourrier, T[17]; Shreffler, WG[18]; Hébert, J[19]; Green, TD[20]; Gerk van Wijk, R[21]; Knulst, AC[22]; Kanny, G[23]; Kowalski, ML[24]; Schneider, LC[25]; Benhamou, P-H[3]; Sampson, HA[26]

[1]Hôpital Necker, Enfants Malades, Pediatrics-Gastroenterology, Paris, France; [2]Université Paris-Descartes, Paris, France; [3]DBV Technologies, Bagneux, France; [4]University of Ottawa Medical School, Ottawa, Canada; [5]University of Toronto Faculty of Medicine, Toronto, Canada; [6]Children's Hospital of Philadelphia, Philadelphia, United States; [7]Stanford University School Medicine, Palo Alto, United States; [8]Alpha Medical Research, Mississauga, Canada; [9]Rady Children's Hospital, University of California, San Diego, United States; [10]Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, United States; [11]Hôpital St Vincent de Paul, Lille, France; [12]Cincinnati Children's Hospital Medical Center, Cincinnati, United States; [13]Nouvel Hôpital Civil, Strasbourg, France; [14]University of Texas South Western Medical Center, Dallas, United States; [15]Northwest Asthma and Allergy Center, Seattle, United States; [16]Hôpital Pellegrin-Enfants, Bordeaux, France; [17]Hôpitaux Pédiatriques de Nice CHU-Lenval, Nice, France; [18]Massachusetts General Hospital for Children, Boston, United States; [19]CRAAQ, Québec, Canada; [20]Children's Hospital of Pittsburgh of UPMC, Pittsburgh, United States; [21]Erasmus Medical Center, Rotterdam, Netherlands; [22]University Medical Center, Utrecht, Netherlands; [23]Hôpitaux de Brabois, Vandoeuvre-lès-Nancy, France; [24]Medical University of Lodz, Lodz, Poland; [25]Boston Children's Hospital, Boston, United States; [26]Icahn School of Medicine at Mont Sinai, New York, United States

Background: Peanut-specific EPIT proved safe and effective in a multicenter double-blind, placebo-controlled phase IIb trial (VIPES, AAAAI, 2015), using Viaskin® Peanut (VP) loaded with 50, 100 or 250 μg peanut protein (pp) or Viaskin® placebo. Subjects enrolled had an Eliciting Dose (ED) at their entry Double-Blind Placebo-Controlled Food Challenge (DBPCFC) ≤300 mg pp. The study was positive and the highest VP dose met its primary efficacy endpoint (proportion of responders at Month 12 with a pp ED during DBPCFC 10-fold greater than the pp ED at entry or reaching a post-treatment ED ≥1000 mg pp). To evaluate how robust was the desensitizing effect of VP against placebo in peanut-allergic subjects, a post-hoc analysis utilized a more stringent efficacy endpoint criterion.

Method: Data from the VIPES study (221 subjects, 6–55 years including 113 children, 6–11) were re-analyzed based on a more stringent definition of efficacy: subjects with an ED at entry challenge ≤30 mg are responders to treatment only if they reached post-treatment ED ≥300 mg; subjects with entry challenge ED>30 mg are responders if they reached post-treatment ED ≥1000 mg.

Results: Using this new efficacy criterion, a clear treatment dose-response effect was seen for the response rates in the whole population (placebo: 17.9%; VP50: 34.0% $P = 0.0787$; VP100: 39.3% $P = 0.0206$ and VP250: 48.2% $P = 0.0012$ vs placebo) and especially in children (respectively 12.9%, 39.3%, 42.3%, 50.0%, $P < 0.035$ for the 3 doses). A dose-response effect was also observed in the challenge pp Cumulative Reactive Dose (CRD). In children, the median [Min, Max] CRD changes from baseline were: placebo: 0.0 [-400, 1000] mg, VP50: 135.0 [-430, 3300] mg; VP100: 114.5 [-100, 4300] mg and VP250: 400 [-300, 4442] mg. Analyzing children CRD with the Least Square Mean technique (covariates: baseline CRD value and country), the differences [95% CI] vs placebo were: VP50: 120.5 [9.25, 361.65] mg; VP100: 141.1 [17.61, 411.43] mg and VP250: 390.4 [133.64, 947.24] mg. A dose effect was also seen for other secondary efficacy criteria at Month 12 including changes in IgE and IgG4 levels in the whole population and in children.

Conclusion: A post-hoc analysis of VIPES study with a more stringent criterion further supported the efficacy of VP, especially VP250 to desensitize peanut-allergic subjects, particularly children: decreased placebo response, barely any impact on VP responses, clearer dose-response effect.

© 2015 The Authors

Poster Discussion Session PDS 2

Diagnostic tools in nasal respiratory diseases

230
The role of infrared thermography in the assessment of nasal provocation test

Herknerova, M[1]; Padertova, B[2]; Cap, P[1]; Hozman, J[2]; Petru, V[1]
[1]Hospital Na Homolce, Allergology and Clinical Immunology, Prague, Czech Republic; [2]Czech Technical University, Faculty of Biomedical Engineering, Prague, Czech Republic

Background: To evaluate the relevance of facial infrared thermography (FIT) in the assessment of specific nasal challenge test and to investigate possible correlations among thermography and other diagnostic methods.
Method: Fifteen adult patients with the history of persistent allergic rhinitis over the last 2 years sensitized to house dust mites according to the skin prick tests.

Group A, entered the study together with 15 adult healthy patients.

Group B. The nasal challenge test was performed with Alyostal Nasal Provocation Test, Stallergenes.

D. pteronyssinus or D. farinae according to the instructions of the manufacturer. We assessed nasal symptoms score (NSS), active anterior rhinomanometry (AAR), MasterScope Rhino device, Jaeger, before and after each allergen extract concentration increase (0.1 IR/ml, 1 IR/ml, 10 IR/ml, 100 IR/ml) and FIT, thermovision camera FLIR E50, Flir Systems, every second minute during the interval (20 min) for each concentration of the allergen extract. The test is considered to be positive for the NSS $\geq$ 5 points. The FIT increase by $\geq$ 1°C and/or $\Delta t_{AUC} \geq 20.0$ °C x min (= area under the curve = sum of multiples of temperature changes and time intervals) is considered to be positive. The AAR positivity was defined as bilateral flow decrease ≥20% and/or bilateral resistence increase ≥30%.
Results: Group A: NSS was positive in 14/15 (93%) patients. The FIT positivity was observed mainly in the nasal area in 11/15 (73%) patients. The temperature increase reflected the gradual growth of the allergen extract concentration in 9/15(60%) patients. AAR was positive in 10/15 (67%) patients.

Group B: Some nasal clinical symptoms were observed in 3/15 (20%) of the patients, however, these symptoms did not reach the threshold of positivity of the test. FIT increase was observed in 4/15 (27%) patients. However, this finding was more heterogeneous than in group A and did not reflect the gradual growth of the allergen concentration. AAR was positive in 3/15 (20%) patients.
Conclusion: FIT may represent innovative tool in allergy diagnosis in general and can be useful for more exact assessment of the nasal response.

231
Anxiety and depression are directly related with allergy symptoms in patients with rhinitis, with differences depending on the type of allergy- Results of Animo Study

Valero, A[1]; Garani, V[2]; Perea, E[2]; García, J[2]; Giralt, E[3]
[1]Hospital Clínic, Department of Pulmonology and Respiratory Allergy, Barcelona, Spain; [2]Laboratorios Leti, Barcelona, Spain; [3]Grupo Saned, Barcelona, Spain

Background: To determine any significant differences in the proportion of patients with anxiety and/or depression among subjects recruited outside the allergy season, and those recruited during the allergy season.
Method: Multicenter, cross-sectional study with 2 phases. The 1st phase was performed outside the allergy season and the 2nd one was performed during the allergy season. Adult patients with allergic rhinitis, with or without bronchial asthma and/or conjunctivitis, were included. Patients receiving treatment for depression or anxiety were excluded. Sociodemographic and clinical variables were collected and the Hospital Anxiety and Depression Scale (HAD), the sleep scale of the Medical Outcomes Study (MOS) and the Esprint-15 quality of life questionnaire were completed in 2 study visits performed in both phases.
Results: A total of 670 patients were included in the study, 343 in the 1st and 327 in the 2nd phase; with a mean age of 34.60 ± 11.03 years. Main concomitant diseases were asthma (41.34%) and conjunctivitis (32.84%).

Mean rhinitis time evolution was 5.34 ± 6.43 years. Pollen allergy was more prevalent than non seasonal allergy (70.26% of patients in the 1st phase and 84.10% of patients in the 2nd phase).

Allergic symptoms, nasal obstruction, rhinorrhea, nasal pruritus and sneezing were present in about two thirds of patients for each symptom. 64.48% had persistent rhinitis. Regarding severity of rhinitis, 57.91% had moderate rhinitis (1–3 symptoms) and 22.69% had severe rhinitis.

In the HAD questionnaire, mean scores were obtained (anxiety and depression scales and globally) and within the normal values. In the MOS-sleep scale, mean scores values indicated the presence of sleep disturbances, sleep snoring and sleep shortness of breath.

In the 1st phase, the patients with non seasonal allergies had higher HAD global and anxiety scores than those patients with pollen allergies (6.39 vs 5.41 in the anxiety dimension), without differences in the depression dimension. Anxiety scores in patients with pollen allergies were higher in the 2nd phase than in the 1st phase.

In the 2nd phase, relevant differences were obtained in the depression dimension (17.65% of patients with non seasonal allergies had depression with scores ≥ 11, while only 4.76% of patients with pollen allergies had depression).
Conclusion: Anxiety and depression are directly related with allergy symptoms. Anxiety is more prevalent in the allergy season in patients with pollen allergies.

234
Relationship between irritant triggers and the severity of rhinitis in the children with allergic and nonallergic rhinitis

Baek, JH; Cho, E; Lee, SW; Kang, YS; Shin, YH; Jee, HM[1]; Jung, Y-H[1]; Han, MY[1]
CHA University School of Medicine, Seongnam, Korea

Background: Symptoms of patients with rhinitis are often aggravated by irritant factors. We investigated differences in clinical response to irritants and allergic triggers between children with allergic (AR) and nonallergic rhinitis (NAR).
Method: Children with chronic rhinitis (n = 208) were evaluated and classified into allergic or nonallergic rhinitis group on the basis of aeroallergen specific IgE results. Healthy controls (n = 24) were also

© 2015 The Authors

recruited for comparison. ARIA guideline was used to classify patients, and the patients' current symptom score and irritant score, the number of positive reactions to 21 non-allergic triggers, were measured.
Results: Mean age of the study subjects was 6.8 years (range: 1.8–16.0 years). Patients with allergic rhinitis constituted 68.8% of the study population, and the AR proportion increased as the patients aged ($P < .001$). The irritant score was higher among children with allergic (4.2, SD 3.0, $P = 0.002$) and nonallergic rhinitis (3.8, SD 2.9, $P = 0.017$) compared with the controls (2.2, SD 1.8, $P = 0.006$). There was no difference in irritant score between the AR and NAR patients ($P = 0.378$) or between mono- or poly-sensitization groups among the AR patients ($P = 0.590$). Moderate-severe rhinitis group had significantly higher irritant scores than the mild rhinitis group ($P = 0.046$), and patients with more severe symptoms had higher irritant scores ($P = 0.005$). Higher irritant score was related to history of diagnosis with atopic dermatitis (OR = 2.928, 95% CI 1.567–5.473, $P = 0.001$).
Conclusion: Irritant response was a useful tool in gauging severity of rhinitis, but not in differentiating between allergic and non-allergic rhinitis. Atopic dermatitis was a strong risk factor for nasal hyperreactivity to non-allergic triggers.

235
To estimate the major allergen in multi-sensitized children with rhinitis: is nasal provocation really needed?

Yuce, EG[1]; Ogulur, I[1]; Karakoc-Aydiner, E[1]; Baris, S[1]; Kiykim, A[1]; Sahin Yilmaz, A[2]; Oysu, C[2]; Ozen, A[1]; Barlan, I[1]
[1]Division of Pediatric Allergy-Immunology, Marmara University, Istanbul, Turkey; [2]Department of ENT, Umraniye Research and Training Hospital, Istanbul, Turkey

Background: Due to limited availability of standard allergens for provocation tests, current study aimed to set an algorithm to estimate the result of allergen specific nasal provocation test (NPT) by combination of SPT (skin prick test), diluted SPT, specific IgE's and basophile activation test (BAT).
Method: Fifty-two (13F/39M) aeroallergen multi-sensitized patients with Allergic Rhinitis (AR) were enrolled. All patients were evaluated with SPT, diluted SPT and spIgE's, bronchial provocation (BPT) with methocholine, NPT and BAT with house-dust-mite (HDM) and grass mix pollen.
Results: Among fifty-two patients (11 ± 3 years), 39 had AR+Allergic Asthma,13 only AR. Bronchial hyperreactivity was positive in 29 of them. SPTs (DP: 6.6 ± 2.3, DF: 6.2 ± 2.7 and grass-mix: 6.8 ± 2.5 mm) and spIgE (HDM-mix: 32 ± 24, grass-mix: 31 ± 4 IU/ml) were positive in all patients. SPTs were found to be positive in 5 patients for DP, 6 for DF and 6 for grass even at 1/10000 dilution. In addition, NPTs were provoked in 9/17 for HDM and 7/25 for grass. BATs were positive in 38/43 and 41/43 patients for HDM and grass mix, respectively. For grass mix or HDM, there was no correlation between the NPT scorings vs SPT, spIgE or BAT. Scores for grass NPT were found to be correlated with grass SPT at 1/10000 dilution (r: 0.668, $P < 0.001$). In the light of our preliminary data, in 5 patients BAT and NPT were simultaneously performed and NPT was found to be positive in 2 patients with HDM, 2 with grass mix and 1 both.
Conclusion: Although in-vivo or in-vitro sensitization tests remain as key elements in the diagnosis of allergic diseases, NPT still is the gold standard in determining the clinical relevance of sensitization and the choice of allergen for appropriate allergen immunotherapy.

236
Chronic cough in children

Manole, F
Faculty of Medicine, ENT, Oradea, Romania

Background: There are many different causes for a persistent or chronic cough in children: asthma, nasal and sinus disease, postnasal drip, post-viral Infections, bacterial infection of the lower airway, inhaled foreign body. The purpose of the study was to evaluate the alteration of the nasal mucosa in the selected children correlated with associate pathology.
Method: We include in our study 90 patients aged between 3–18 years who present at ENT examination for interdisciplinary approach of cough who persist in last 3 months and have repeated episode. Patients was divided into three equal number groups according to age. All of this was evaluated after ENT examination: anterior rhinoscopy, posterior rhinoscopy examination of oral cavity and otoscopy. We noticed the aspects of the turbinate, the aspects and dimension of the lymphoid tissue in nasopharynx and oropharynx, and the alteration of the tympanic membrane. Regarding subjective symptoms we notice: nasal obstruction, rhinorrhea, sneezing, nasal itching, headache, type of chronic cough, postnasal drip. After a complete examination the patients was send to pediatric and allergologist to evaluate the asthma and cough variant rhinitis.
Results: In the case of group one were was included patients between 3 and 7 years we found predominant nasal mucosa with normal coloration, mucopurulent secretion stagnated to the floor of the nasal fossa in 58% of cases and pale bluish discoloration of nasal mucosa, suggestive for allergic rhinitis in 22% of cases. In the group aged between 8 and 13 years we found hypertrophic nasal turbinate with secretion in middle meatus in 26% of cases suggestive for rhinosinussitis and in 30% of cases nasal mucosa was swollen, pale-bluish, suggestive for allergic rhinitis. In the group aged between 13 to 18 years we found turbinate hypertrophy and septal deviation with postnasal drip in 18% of cases and in 32% of cases mucosa was covered with clear mucoid secretion, swollen turbinate suggestive for allergic rhinitis.
Conclusion: Chronic cough in children is multifactorial and these factors can be individual or associated. In the case of preschool age child, chronic cough appear to be correlated with nasal obstruction with adenoid hyperplasia. In the next two group chronic cough appear to be correlated with nasal obstruction secondary to allergic rhinitis most of the cases and few cases with postnasal drip.

237
Oral administration of *Lactobacillus paracasei* NCC2461 for the modulation of grass pollen allergic rhinitis: a randomized, placebo-controlled study during the pollen season

Nembrini, C; Singh, A; Vissers, Y; Mercenier, A; Nutten, S; Blanchard, C
Nestlé Research Center, Lausanne, Switzerland

Background: The efficacy of *Lactobacillus paracasei* NCC2461 in modulating allergic rhinitis was previously evaluated in 2 exploratory, randomized, cross-over design studies. Oral administration with NCC2461 reduced specific subjective allergic symptoms and immune parameters following nasal challenges with controlled grass pollen allergen concentrations (1, 2). Our aim was to confirm the anti-allergic effect of NCC2461 in grass pollen allergic subjects exposed to natural doses of the allergen during the pollen season.
Method: A double-blind, randomized, placebo-controlled, parallel study was conducted with 131 grass pollen allergic subjects from May to July 2012 in concomitance with the pollen season in Berlin (ClinicalTrials.gov: NCT01653652). NCC2461 or placebo was administered daily for an 8-week period to adult subjects with clinical history of seasonal allergic rhinitis, positive skin prick test and IgE to grass pollen. For the 8-week trial period, symptoms and quality of life question-

© 2015 The Authors

naires were filled out electronically daily or weekly, respectively, and plasma was collected for IgE analysis at screening and at the end of the intervention. All subjects were included within a 5-day interval, ensuring exposure to similar air pollen counts for each individual during the trial period.

Results: Symptoms increased with pollen loads, confirming a natural exposure to the allergen and presence of seasonal allergic rhinitis. However, no significant differences were observed in allergic rhinitis symptoms scores, quality of life, or specific IgE levels between subjects receiving NCC2461 as compared to placebo administration. In reporting minor adverse events, subjects in the probiotic arm had a lower incidence of nasopharyngitis than subjects receiving the placebo.

Conclusion: In contrast to previous findings, oral administration of NCC2461 did not show a beneficial effect on allergic rhinitis when compared to placebo in a parallel study conducted during the pollen season. The influence of study design, allergen exposure and intervention window on the efficacy of NCC2461 in modulating respiratory allergy should be further evaluated.

1. Perrin, Y., *et al*. (2014) *Clin. Transl. Allergy*. 4, 1.

2. Wassenberg, J., *et al*. (2011) *Clin. Exp. Allergy*. 41, 565–573.

238
Imaging analysis of conjunctival provocation by placebo treatment

Astvatsatourov, A; Gloistein, C; Mösges, R
Institute of Medical Statistics, Informatics and Epidemiology, University Hospital of Cologne, Cologne, Germany

Background: The conjunctival provocation test (CPT) is a fast and easy diagnostic procedure, based on challenge of the eye's mucosa by instillation of allergen solutions of increasing concentrations onto the patient′s conjunctiva. The CPT is used for evaluation of allergic rhinoconjunctivitis.

Objective: To investigate placebo effect and local mucosa desensitization caused by the repeated conjunctival challenges on placebo treated patients, i.e. to analyse the reproducibility of the CPT diagnosis in immunotherapeutic clinical trials.

Methods: The CPT procedure was applied four times during one clinical study of sublingual immunotherapy with 1, 4 and 8 week intervals on 120 placebo-treated patients. Allergic reaction to CPT was assessed by numerical severity score and documented by study investigators. As a part of the CPT documentation, high-resolution digital photos were collected and analysed by an external observer and by digital image analysis software. Allergic reddening was documented twice before the sublingual immunotherapy at visit–0 and visit–1, once during the therapy at visit–3 and once after the therapy at visit–4. The reddening was analysed as an objective and independent CPT parameter.

Results: Conjunctival reddening was successfully assessed using the imaging based objective analysis, which is completely independent from investigators and patients subjectivity. Valid data of 70 patients was collected for the analysis, when 50 of 120 placebo treated patients had missing data. Between visit 0 and visit 4 a not significant reduction of allergic conjunctival reddening by 1.3% (paired t–Test $P = 0.116$, paired U–test $P = 0.244$) was observed, whereas between visit 1 and visit 4 reduction of the reddening by 3% (paired t–Test $P = 0.003$, paired U–test $P = 0.004$) was significant.

Conclusion: Two possible reasons may cause the decrease of allergic hyperaemia: 1) conjunctival desensitization from repeating local application of the allergens and 2) placebo effect. Both desensitization and placebo effects cannot be distinguished due to setup of therapy studies. To attribute observed effects to conjunctival desensitization, further investigations by pilot studies without any treatment including placebo administration are needed.

239
The impact of allergic rhinitis on the sense of smell in pediatric population (The OLFAPEDRIAL study)

Langdon, C[1]; Guilemany, JM[1]; Alobid, I[1]; Bartra, J[2]; Dávila, I[3]; del Cuvillo, A[4]; Ferrer, M[5]; Jáuregui, I[6]; Molina, X[7]; Montoro, J[8]; Sastre, J[9]; Valero, A[2]; Mullol, J[10]
[1]Hospital Clinic - IDIBAPS, Unitat de Rinologia i Clínica de l'Olfacte, Servei d'Otorinolaringologia, Barcelona, Spain; [2]Unitat d'Al.lèrgia, Servei de Pneumologia i Al.lèrgia Respiratòria, Hospital Clinic - IDIBAPS, Barcelona, Spain; [3]Servicio de Inmunoalergia, Hospital Clínico, Salamanca, Spain; [4]Clínica Dr. Lobatón Cádiz, Otorrinolaringología, Cádiz, Spain; [5]Clínica Universitaria de Navarra, Alergia, Pamplona, Spain; [6]Hospital de Basurto, Alergología, Bilbao, Spain; [7]Infociencia, RPS, Barcelona, Spain; [8]Hospital La Plana, Alergia, Villarreal, Spain; [9]Fundación Jiménez Díaz, Alergia, Madrid, Spain; [10]Hospital Clinic - IDIBAPS, Clinical & Experimental Respiratory Immunoallergy, Barcelona, Spain

Background: Allergic rhinitis (AR) is the commonest chronic disease in children. Olfactory dysfunction in allergic paediatric population has been scarcely studied. The aim of this study was to evaluate the prevalence and severity of smell disorders in children with AR using the ARIA *(Allergic Rhinitis and its Impact on Asthma)* classification.

Methods: A multicentre, epidemiological, cross-sectional, population-based study was performed in children (6–12 yo) with a diagnosis of AR. Patients were classified according to ARIA guidelines for both symptom duration (intermittent [IAR] and persistent [PER]) and disease by m-ARIA 4-item (sleep, daily activities/sport/leisure, school performance, and troublesome symptoms) severity classification: mild (no affected items), moderate (1–3 affected items) and severe (all 4 items affected). Loss of smell was scored using a Likert scale (0–3).

Results: Almost 44% (551 out of 1260) of children had smell dysfunction. The loss of smell was more prevalent in children with PER than with IAR (52.1% vs 38%, $P < 0.0001$), and at any degree of disease severity: mild (17.9% vs 3.4%; $P < 0.001$), moderate (45.8% vs 38.4%; $P < 0.01$), and severe (72.2% vs 55.3%; $P < 0.0001$). The loss of smell was also more severe in children with PER than with IAR (0.75 ± 0.84 vs 0.51 ± 0.73, $P < 0.0001$), and at any degree of disease severity: mild (0.21 ± 0.47 vs 0.05 ± 0.26; $P < 0.001$), moderate (0.66 ± 0.84 vs 0.50 ± 0.71; $P < 0.01$), and severe (1.05 ± 0.82 vs 0.76 ± 0.8; $P < 0.0001$).

Conclusion: Almost half of children with AR presented a moderate loss of smell with a higher impairment related to the disease duration (persistent AR) and severity (severe AR). In consequence, the loss of smell may be considered as a symptom-guide to define AR severity in children.

This study was supported in part by an educational grant from UCB Pharma.

240
Allergic rhinitis phenotypes based on mono-allergy or poly-allergy

Gelardi, M[1]; Leo, E[1]; Iannuzzi, L[1]; Quaranta, N[1]; Ciprandi, G[2]; Incorvaia, C[3]; Buttafava, S[4]; Frati, F[4]
[1]Section of Otolaryngology, Department of Neuroscience and Sensory Organs, University of Bari, Bari, Italy; [2]IRCCS-AOU San Martino, Genoa, Italy; [3]Allergy/Pulmonary Rehabilitation, ICP Hospital, Milan, Italy; [4]Stallergenes Italia, Medical and Scientific Department, Milan, Italy

Background: Allergic rhinitis (AR) is characterized by typical symptoms that are the clinical expression of the underlying inflammation. Because of the very frequent occurrence of poly-allergy, phenotyping AR represents an up-to-date issue. The aim of this study was to evaluate whether the number of allergies is able to define different phenotypes in patients with AR.

Method: Eighty-three patients (43 males, 40 females, mean age 34.7 years) suffering from AR were included in the study. The type of allergic sensitization, the result of

© 2015 The Authors

the visual analogue score (VAS) for nasal symptoms perception, including sneezing, rhinorrhea and obstruction, and the results of nasal cytology, performed by standard technique, were evaluated. The data from poly-allergic and mono-allergic patients were compared by statistical analysis using non-parametric tests.

Results: Poly-allergic patients perceived, as assessed by VAS, significantly more severe nasal obstruction than mono-allergic ones ($P = 0.0006$), and they had a significantly more frequent sneezing ($P < 0.0001$). Moreover, poly-allergic patients had a significantly more intense infiltrate of inflammatory cells in the nasal mucosa, that concerned both eosinophils ($P = 0.0005$) and mast cells ($P = 0.0001$), than mono-allergic patients.

Conclusion: The findings from this study demonstrate that the presence of poly-allergy could define a distinct AR phenotype in comparison with mono-allergic patients. In fact, the occurrence in poly-allergic patients of highly significantly more intense inflammation and more severe symptoms than mono-allergic ones makes this event clinically relevant.

241
Therapeutic effects of intranasal cyclosporine for eosinophilic rhinosinusitis with nasal polyps in a mouse model

Jeon, S-Y[1,2]; Joo, Y-H[1]; Kim, S-W[1,2]; Cho, HJ[1]
[1]Gyeongsang National University Hospital, Otorhinolaryngology, Jinju, Korea; [2]Institute of Health Sciences, Jinju, Korea

Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is a principally Th2-mediated inflammatory disease. Systemic corticosteroids currently represent the most effective treatment for CRSwNP, but their long-term use is constrained due to their detrimental side effects. Long-term use of topical steroids is safe, but their efficacy is often limited. Topical cyclosporine has proven to be safe and effective for Th2-mediated diseases such as allergic conjunctivitis. It was hypothesized that topical cyclosporine would be an effective novel drug for the treatment of CRSwNP; its therapeutic efficacy was assessed using a previously established mouse model.

Methods: After induction of eosinophilic CRSwNP in 4-week-old BALB/c mice according to previous protocols, the therapeutic effects of intranasal cyclosporine were evaluated and compared with those of triamcinolone acetonide (TAC). Histopathologic changes were evaluated using hematoxylin and eosin for polyp formation and Sirius red staining for eosinophilic infiltration. The production of cytokines in sinonasal tissues, including tumor necrosis factor, interleukin (IL)-2, interferon-γ, IL-4, IL-5, IL-13 and IL-17A, were measured using a cytometric bead array.

Results: The number of polyp-like lesions was reduced significantly only by systemic TAC, but the degree of eosinophilic infiltration was decreased significantly by topical cyclosporine, the potency of which was similar to that of topical or systemic TAC. Except for interferon-γ, the majority of measured cytokines were reduced significantly by topical cyclosporine, although its effects on IL-2 and IL-13 were less potent than those of systemic TAC.

Conclusion: Topical cyclosporine might be an effective drug for the management of CRSwNP.

242
Effect of lipopolysaccharide on glucocorticoid receptor function in control nasal mucosa fibroblasts and in fibroblasts from patients with chronic rhinosinusitis with nasal polyps and asthma

Fernández-Bertolín, L[1,2]; Mullol, J[1,2,3]; Fuentes-Prado, M[1,2]; Roca-Ferrer, J[1,2]; Alobid, I[1,2,3]; Picado, C[1,2,4]; Pujols, L[1,2]
[1]IDIBAPS, Clinical and Experimental Respir. Immunoallergy, Barcelona, Spain; [2]Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias CIBERes, Madrid, Spain; [3]Rhinology and Smell Unit, ENT Department, Hospital Clínic, Barcelona, Spain; [4]Hospital Clínic, Pneumology and Allergy Department, University of Barcelona, Barcelona, Spain

Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is a chronic inflammatory disease of the upper airways frequently associated with asthma. Bacterial infection is a feature of CRSwNP that can aggravate the disease and the response to glucocorticoid treatment.

Objective: We examined whether the bacterial product lipopolysaccharide (LPS) reduces glucocorticoid receptor (GR) function in control nasal mucosa (NM) fibroblasts and in nasal polyp (NP) fibroblasts from patients with CRSwNP and asthma.

Methods: NP ($n = 12$) and NM fibroblasts ($n = 10$) were *in vitro* pre-incubated with LPS (24 h) prior to the addition of dexamethasone. Cytokine/chemokine secretion was measured by ELISA and Cytometric Bead Array. GRα, GRβ, mitogen-activated protein-kinase phosphatase-1 (MKP-1) and glucocorticoid-induced leucine zipper (GILZ) expression was measured by RT-PCR and immunoblotting, GRα nuclear translocation by immunocytochemistry, and GRβ localisation by immunoblotting. The role of MKP-1 and GILZ on dexamethasone-mediated cytokine inhibition was analyzed by small interfering RNA silencing.

Results: Pre-incubation of nasal fibroblasts with LPS enhanced the secretion of IL-6, CXCL8, RANTES, and GM-CSF induced by FBS. FBS-induced CXCL8 secretion was higher in NP than in NM fibroblasts. LPS effects on IL-6 and CXCL8 were mediated via activation of p38α/β MAPK and IKK/NF-kB pathways. Additionally, LPS pre-incubation:

(1) Reduced dexamethasone's capacity to inhibit FBS-induced IL-6, CXCL8 and RANTES,
(2) Reduced dexamethasone-induced GRα nuclear translocation (only in NM fibroblasts),
(3) Did not alter GRα/GRβ expression
(4) Decreased GILZ expression, and
(5) Did not affect dexamethasone's capacity to induce MKP-1 and GILZ expression.

MKP-1 knockdown reduced dexamethasone's capacity to suppress FBS-induced CXCL8 release.

Conclusion: The bacterial product LPS negatively affects GR function in control NM and NP fibroblasts by interfering with the capacity of the activated receptor to inhibit the production of pro-inflammatory mediators. This study contributes to the understanding of how bacterial infection of the upper airways may limit the efficacy of glucocorticoid treatment.

243
Mesenchymal stem cells administered intraperitoneally have a potent effects on the experimental allergic airway disease

IŞIK, S[1]; Adan-Gökbulut, A[2]; Karaman, M[3]; Kiray, M[4]; Bağrıyanik, HA[4]; Çağlayan-Sözmen, Ş[1]; Kozanoğlu, I[5]; Karaman, Ö[1]; Baran, Y[2]; Uzuner, N[1]
[1]Pediatric Allergy and Immunology, Dokuz Eylul University Faculty of Medicine, Izmir, Turkey; [2]Izmir Institute of Technology, Izmir, Turkey; [3]Dokuz Eylul University Faculty of Medicine, Department of Microbiology, Izmir, Turkey; [4]Dokuz Eylul University Faculty of Medicine, Department of Histology, Izmir, Turkey; [5]Başkent University, Department of Physiology, Ankara, Turkey

Background: Previous studies showed that bone marrow-derived mesenchymal stem cells (BMSCc) could ameliorate a variety of immune-mediated and inflammatory disease by its immunmodulatory and antiinflammatory effects. In this study, we developed a mouse model of ovalbumin (OVA) induced allergic inflammation in the upper airways and evaluated the effects of the intraperitoneal administration of BMSCs on allergic inflammation.

Method: Twenty-five BALB/c mice were divided into five groups; Group I (control group), Group II (sensitized and challenged with OVA and treated with saline), Group III (sensitized and challenged with

© 2015 The Authors

OVA and treated with 1x106 BMSCs), Group IV (sensitized and challenged with OVA and treated with 2x106 BMSCs) and Group V (sensitized and challenged with phosphate buffered saline and treated with 1x106 BMSCs). Histopathological features (numbers of goblet, eosinophil and mast cells, epithelium thickness and subepithelial smooth muscle thickness) of the airways and BMSCs migration to nasal and lung tissue were evaluated by light and confocal microscopes. The levels of cytokines in the nasal lavage fluid and lung tissue supernatants were measured by enzyme-linked immunosorbent assay.

Results: Confocal microscopy analysis showed that no noteworthy amount of BMSCs were present in the nasal and lung tissues of Group V while significant amount of BMSCs was detected in Group III and IV. In OVA induced AR groups (group II, III and IV), histopathological findings of chronic asthma, such as elevated subepithelial smooth muscle thickness, epithelium thickness and number of goblet and mast cells were determined. Also, we found that, in both BMSCs treated groups, the numbers of nasal goblet and eosinophil cells, histopathological findings of chronic asthma and levels of IL-4, IL-5, IL-13 and NO were significantly lower than the placebo group.

Conclusion: Our findings indicated that in murine model of AR, histopathological findings of chronic asthma were present, as well. BMSCs migrated to the nasal and lung tissue following intraperitoneal delivery and ameliorated to the airway remodeling and inflammation both the upper and lower airway through the inhibition of T helper 2 immune response in the murine model of AR.

244
miR-143 inhibits interleukin-13-induced inflammatory cytokine and mucus production in primary nasal epithelial cells from allergic rhinitis patients

Teng, Y; Li, Y; Gao, Y; Li, J
Hangzhou First People's Hospital, Otorhinolaryngology, Hangzhou, China

Background: Allergic rhinitis (AR) is a common chronic inflammatory condition of the nasal mucosal tissue. The interleukin-13 (IL-13) signaling pathway is of great importance in the pathogenesis of AR. However, how the signaling molecules in this pathway are regulated, particularly through microRNAs (miRNAs), remains unclear.

Method: In the present study, human primary NECs were isolated and established from the inferior turbinate tissues of 11 patients with AR. miRNA-143 (miR-143) was upregulated by miR-143 lentiviral vector in IL-13-stimulated nasal epithelial cells (NECs) from AR patients. The expression levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), eotaxin and mucin 5AC (MUC5AC) were measured by qRT-PCR and ELISA. IL-13 receptor α1 chain (IL13Rα1), as a target gene of miR-143, was validated by luciferase reporter assay.

Results: Our results showed that forced expression of miR-143 significantly decreased the mRNA and protein expression levels of GM-CSF, eotaxin and MUC5AC in IL-13-stimulated NECs. Moreover, we confirmed that miR-143 directly targeted and significantly suppressed IL13Rα1 gene expression.

Conclusion: This study thus suggests that miR-143 regulation of IL-13-induced inflammatory cytokine and mucus production in NECs from AR patients probably partly depends on inhibition of IL13Rα1. Therefore, the IL13Rα1 signaling pathway may be a potential target for the prevention and treatment of AR by miR-143.

© 2015 The Authors

Poster Discussion Session PDS 3

Phenotypes and biomarkers in asthma

245
Psychological status of adolescents with atopic asthma

Klyucharova, A; Artemyeva, A
Kazan State Medical University, Kazan, Russian Federation

Background: According to psychologists, bronchial asthma (BA) is a psychosomatic disease, the cause of which is overprotective parents. Lack of opportunity to express their opinions manifested in these patients breathlessness. Usually doctors find other causes of asthma: genetic (tendency to atopy and bronchial hyperreactivity), contact with allergens, infections, smoking, and others. However, psychoemotional stress is a trigger of asthma.
Objective: To determine the personality traits of adolescents with atopic asthma (ABA) and explore the features of the relationship among patients and their parents parents.
Materials and methods: 21 male patients aged 20 ± 2 years with diagnosis of atopic asthma were examined by the standards of diagnostics of allergic diseases. Twelve patients had intermittent asthma, over patients had mild persistent asthma.71 9% of patients had sensitization to house dust mites, over patients had sensitization domestic and pollen allergens. Patients had psychological examination with MMPI questionnaire and test "parent-child interaction". The control group was represented by adolescents of the same age and sex.
Results: According to the results of the test MMPI 66.7% of patients with ABA had increased self-control (scored more than 80T to 1 scale), 47.6% were uncertainty and anxious (more than 80T to 7 scale), 42.8% had analytical thinking (more than 80T 8 scale), creativity. Significant differences in the results of test "parent-child interaction" control group and patients with intermittent ABA didn't have. At the same time, median parental control (Me = 30.5 (27;32)) of patients with mild persistent ABA was higher than in healthy volunteers (Me = 25 (21; 28)) $P = 0.043$. Parents were less autoritarian for patients with mild persistent ABA (Me = 29 (24; 37) than for healthy volunteers (Me = 33 (27; 41) $P = 0.045$. Patients with ABA had bad relationships with parents (Me = 26.5 (21; 33) more often than healthy respondents (Me = 32 (28; 43) $P = 0.042$.
Conclusions: 1. Patients with ABA characterized an anxious persons, have an analytical mind, is suppressing natural emotions and have high self-control.

2. Excessive parental control performs a psychological pressure on a adolescent and forms more asthma.

246
Coping attitudes of parents with stressful events may affect asthma control in school-age children

Yavuz, ST[1]; Sari, O[2]; Aydogan, U[2]; Yesillik, S[3]; Saldir, M[4]; Kartal, O[3]; Gok, F[4]
[1]Department of Pediatric Allergy, GATA School of Medicine, Ankara, Turkey; [2]Department of Family Physicians, GATA School of Medicine, Ankara, Turkey; [3]Department of Adult Allergy and Immunology, GATA School of Medicine, Ankara, Turkey; [4]Department of Pediatrics, GATA School of Medicine, Ankara, Turkey

Background: Asthma causes a significant social burden for patients and family members. The ability of parents to cope with the disease may affect the treatment response of children with asthma. The aim of this study is to evaluate the association between coping styles and asthma control in a population-based sample of children with asthma.
Method: Children who attended to a tertiary outpatient pediatric allergy and asthma department and diagnosed with asthma were enrolled in the study. A questionnaire including demographic features and parameters to determine socioeconomic status were applied to the parents of the children. Asthma control status was assessed according to GINA guidelines. Assessment of Coping Attenuations (COPE) Inventory was used to evaluate the coping patterns of parents relevant to the management of childrens' asthma.
Results: A total of 162 children (107 male, 66.0%) with a median age of 9.0 (7.2–11.6) years were included. Asthma control status was determined as controlled in 102 children (63%), partially controlled in 30 children (18.5%) and uncontrolled in 30 children (18.5%). Parents of children with uncontrolled asthma were less likely to use the three major subscales (problem-focused coping, emotion-focused coping and nonfunctional coping) when compared to parents of children with better asthma control ($P = 0.005$, $P = 0.009$, $P = 0.002$, respectively). Moreover, we found out that parents of children with better asthma control were more likely to use several coping patterns such as focus on and venting of emotions ($P = 0.009$), seeking social support-emotional ($P = 0.004$), turning to religion ($P = 0.007$) and restraint coping ($P < 0.001$) when compared to parents of children with uncontrolled asthma.
Conclusion: Results of our study revealed that asthma control status of children may be correlated with coping patterns of parents. Appropriate attitudes of families to deal with the stressful events may contribute to better asthma control in children.

247
Differences in cognitive profile in patients with asthma and chronic obstructive pulmonary disease (COPD)

Ali, A; Mohammed, A; Mourad, S
Sohag University, Sohag, Egypt

Background: Asthma and Chronic obstructive pulmonary disease (COPD), especially in severe forms, is commonly associated with multiple cognitive problems. Aim of study was to evaluate the impact of COPD and asthma on cognitive status using MoCA questionnaire and P 300 test.
Method: We enrolled 40 patients with COPD, 40 patients with bronchial asthma and 20 healthy subjects (control group). We analyzed the differences in MoCA score and P300 latency between these three groups and also the correlation between these scores and inflammatory markers.
Results: Mild cognitive impairement (MCI) was found in 85% of patients with COPD compared with 60% of asthmatic subjects($P < 0.01$). Patients with COPD had a significant ($P < 0.0001$) decreased MoCA score (16.4 ± 6.30) compared to asthma (20.4 ± 4.64) and controls (28.05 ± 4.72). When both COPD group and asthma were compared, it was seen that latency of P300 was shorter in the asthma group ($P < 0.001$). In COPD, the MoCA score was significantly correlated with WBC ($r = 0.45$) Hypertension ($r = -0.38$), Triglycerides ($r = -0.35$) and

© 2015 The Authors

Cholesterol ($r = -0.66$). However, P300 latency correlated significantly with age ($r = 0.423$, $P < 0.007$), sex ($r = -0.314$, $P < 0.049$), duration of disease ($r = 0.622$, $P < 0.0001$) PaO_2 ($r = -0.490$, $P < 0.001$), SaO_2 ($r = -0.496$, $P < 0.003$) and FEV_1/FVC ($r = -0.353$, $P < 0.026$). In asthma, only MoCA is correlated with BMI ($r = 0.236$, $P < 0.05$).

Conclusion: According to this study, COPD significantly decreases the cognitive status compared to bronchial asthma. Longer latency of P300 appears to be an expected sequel of COPD.

248

A three year follow-up of asthma, respiratory symptoms and self-reported allergy, among pilots and cabin attendants

Fu, X[1]; Wieslander, G[1]; Lindgren, T[1]; Janson, C[2]; Norback, D[1]
[1]Department of Medical Sciences / Occupational and Environmental Medicine, Uppsala University, Uppsala, Sweden; [2]Department of Medical Sciences / Respiratory Medicine and Allergology, Uppsala University, Uppsala, Sweden

Background: There are few studies of the incidence of asthma and allergy in pilots and cabin crew. The aim was to study the associations between occupation and, personal factors (age, sex, pollen or furry pet allergy (atopy), smoking habits and onset of (incidence) of asthma and allergies among pilots and cabin attendants in one Scandinavian airline company.

Methods: A standardized questionnaire (Örebro form MM040 NA) was answered the spring of 1997 by 577 pilots and 936 cabin attendants and a 3-year follow-up was performed spring of 2000 with the same questionnaire answered by 436 pilots and 698 cabin attendants. Proportion of new cases (three year incidence) were calculated and related to personal factors with logistic regression.

Results: The 3-year incidence of doctor's diagnosed asthma was 1.2% among pilots and 0.6% among cabin attendants. The incidence of some respiratory symptoms (wheeze, attacks of breathlessness) was 4.6% among pilots and 12.5% among cabin attendants. The incidence of chronic bronchitis (daily phlegm cough) was 1.1% among pilots and 3.5% among cabin attendants. The incidence of pollen and furry pet allergy was 5.4% and 1.6% among pilots and 5.7 respectively 2.4% among cabin attendants. The incidence of food allergy/ intolerance was 1.8% among pilots and 3.2% among cabin attendants. The incidence of respiratory symptoms was lower among pilots as compared to cabin attendants ($P < 0.05$). The incidence of doctor's diagnosed asthma and food allergy/intolerance was higher in atopic subjects ($P < 0.01$). Younger staff and pursers had a higher incidence of pollen/fury pet allergy ($P < 0.01$). No association was found between incidence of asthma and allergies and gender or tobacco smoking.

249

Loss of smell as symptom-guide to discriminate chronic rhinosinusitis from rhinitis alone in asthma patients

Castillo, JA[1]; Picado, C[2]; Plaza, V[3]; Rodrigo, G[4]; Juliá, B[5]; Mullol, J[6]; IRIS-Asma Investigators
[1]Pneumology, Hospital Universitario Quirón Dexeus, Barcelona, Spain; [2]Pneumology, Hospital Clínic, Barcelona, Spain; [3]Pneumology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; [4]Pneumology, Hospital de las Fuerzas Armadas, Montevideo, Uruguay; [5]Departamento Médico MSD-España, Madrid, Spain; [6]Hospital Clínic, IDIBAPS&Rhinology Unit, ENT, Barcelona, Spain

Background: Rhinitis and chronic rhinosinusitis (CRS) are frequently associated with asthma. International guidelines (ARIA, EPOS) recommend, in addition to nasal endoscopy, CT scan, and skin prick test (SPT), the use of sinonasal symptoms to define rhinitis and CRS.

Objective: We aimed to assess the disease prevalence and the reliability of sinonasal symptoms to discriminate between rhinitis, allergic or non-allergic, and CRS, with or without nasal polyps, in asthma patients.

Method: Asthmatic patients ($N = 492$; mean age 45 ± 15 yo; female 70.5%) were recruited according to GINA: 17.3% intermittent and 82.7% persistent [24.6% mild, 31.4% moderate, 26.7% severe] in a prospective study carried out in 2010–2011 by pneumonologists and ENT specialists in 23 centers. Allergic (AR) and non-allergic (NAR) rhinitis and CRS with (CRSwNP) and without (CRSsNP) nasal polyps were evaluated according to ARIA and EPOS guidelines based on nasal symptoms, SPT, nasal endoscopy, and sinus CT scan.

Results: Half of the asthma patients (49.6%) had rhinitis (AR: 37.0%; NAR: 12.6%), 36.2% CRS (CRSsNP: 16.7%; CRSwNP: 19.5%), while 14.2% had no sinonasal disease. Loss of smell significantly discriminated between CRS and rhinitis (76.4% vs 41.0%, $P < 0.001$). In addition, loss of smell was more prevalent in CRSwNP compared to CRSsNP (83.3% vs 68.3%, $P < 0.05$) but similar in AR compared to NAR (43.5% vs 41.7%). Differences on other common symptoms didn't clearly discriminate between CRS and rhinitis: sneezing (87.1% vs 86.6%), ocular itching (73.6% vs 68.6%), rhinorrhea (83.1% vs 78.2%), and nasal obstruction (88.8% vs 82.8%).

Conclusions: 1st) Most asthmatic patients (86%) have nasal symptoms, 50% of them having rhinitis alone and 36% CRS; and 2nd) the loss of smell may be considered the symptom-guide to discriminate CRS (mainly with nasal polyps) from rhinitis alone in asthma patients.

250

Asthma is less age-dependently associated with allergic sensitization compared to rhinitis

Warm, K[1]; Hedman, L[2]; Lindberg, A[1]; Lötvall, J[3]; Lundbäck, B[3]; Rönmark, E[2]
[1]Department of Public Health and Clinical Medicine, Division of Medicine, the OLIN Unit, Umeå University, Luleå, Sweden; [2]Department of Public Health and Clinical Medicine, Occupational and Environmental Medicine, the OLIN Unit, Umeå University, Luleå, Sweden; [3]Krefting Research Center, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden

Background: Few studies report on the association between allergic sensitization and asthma and allergic rhinitis in relation to age among adults. The study focuses on the impact of specific sensitization to airborne allergens on asthma and allergic rhinitis in different ages among adults.

Method: A randomly selected population sample of adults in ages 22–86 years was invited to participate in a structured interview and blood sampling for specific IgE to ten common airborne allergens. Of invited, 68% ($n = 692$) participated in blood sampling. IgE ≥ 0.35 U/ml to the specific allergen was defined as a positive test.

Results: The prevalence of allergic rhinitis decreased with increasing age whereas the prevalence of asthma decreased not significantly. Allergic sensitization decreased with increasing age, both in the study population and among subjects with asthma and allergic rhinitis, respectively. In a multivariate model adjusted for confounders, sensitization to furry animals was significantly positively associated with asthma, OR 4.80 (95% CI 2.68–8.60). Both sensitization to furry animals, OR 3.90 (95% CI 2.31–6.58), and to pollen, 4.25 (2.55–7.06) were significantly associated with allergic rhinitis. In general, the association between allergic sensitization and allergic rhinitis was consistently strongest in ages 20–40 years and decreased with increasing age. This pattern was not found for asthma. However, a strong association between asthma and specific sensitization to furry animals was found in all age groups, especially in age ≥ 61 years, OR 6.78 (95% CI 2.09–21.99).

Conclusion: Allergic rhinitis was associated with both sensitization to pollen and furry animals and consistently stronger among younger compared to older adults. Sensiti-

© 2015 The Authors

zation to furry animals was significantly associated with asthma across all age groups.

251
Wheeze phenotypes and its relation to asthma persistence until adolescence

Pite, H[1,2]; Pereira, AM[1,3]; Gaspar, Â[1]; Morais-Almeida, M[1]
[1]CUF Descobertas and CUF Infante Santo Hospitals, Allergy Center, Lisbon, Portugal; [2]NOVA Medical School / Faculdade de Ciências Médicas, Universidade Nova de Lisboa, CEDOC, Chronic Diseases Research Center, Lisbon, Portugal; [3]CUF-Porto Hospital and Instituto, Allergy Unit, Porto, Portugal

Background: Several childhood wheeze phenotypes have been proposed. Their usefulness in predicting wheeze long-term outcome is of major clinical relevance. The purpose of our study was to identify and characterize childhood wheeze phenotypes in relation to asthma persistence until adolescence, in a multidimensional statistical model, independent of predefined hypotheses.
Method: Prospective cohort study including 308 children aged <7 years with recurrent wheeze, systematically evaluated at 3, 8 and 13 years of follow-up. Evaluations included medical observation, questionnaires, skin prick tests and, at school age and adolescence, lung function tests. Asthma prevalence was estimated at each time point. Risk factors associated with asthma persistence in adolescence were analyzed by multivariable logistic regression (LR). K-means clusters analysis was used to identify early childhood wheeze phenotypes, including variables selected according to LR analysis. These phenotypes were compared for asthma prevalence, use of preventive treatment, spirometry and bronchodilation test results at school-age and adolescence.
Results: Asthma prevalence was 66.1% (n = 287), 58.3% (n = 249) and 53.5% (n = 170), at 3, 8 and 13 years of follow-up, respectively. Having atopy (odds ratio (OR) [95% confidence interval]: 11.4 [3.8–34.4]) and rhinitis (OR 10.6 [3.7–29.8]) at preschool age were independent risk factors for asthma persistence in adolescence. We identified three childhood wheeze phenotypes, which were different regarding age of onset of wheeze, parental asthma, day care attendance before 12 months and personal history of atopy, rhinitis, asthma and food allergy at preschool age ($P < 0.01$). These phenotypes were predictive for asthma symptoms persistence and use of preventive treatment in school-aged children and adolescents ($P < 0.001$).
Conclusion: The hypothesis-free early childhood wheeze phenotypes differed significantly in asthma persistence at school age and adolescence. Our results support the distinction between persistent atopic and transient non-atopic early wheeze and the recognition of a third wheeze phenotype that, while early and non-atopic, is mostly persistent and related to rhinitis, eczema, maternal asthma and no day care attendance during infancy.

252
Blood lipids associates with childhood asthma, altered lung function and aeroallergen sensitization

Vinding, RK[1]; Stokholm, J[1,2]; Chawes, B[2]; Bisgaard, H[2]
[1]Copenhagen Prospective Studies on Asthma in Childhood, Faculty of Health and Medical Sciences, University of Copenhagen & Danish Pediatric Asthma Center, University of Copenhagen, Nasteved Hospital, Pediatrics, Nastved, Denmark; [2]Copenhagen Prospective Studies on Asthma in Childhood, Faculty of Health and Medical Sciences, University of Copenhagen & Danish Pediatric Asthma Center, University of Copenhagen, Gentofte, Denmark

Background: Studies on children's lipid profile and asthma are few and the results ambiguous.
Objective: To examine if the lipid profile is associated with concurrent asthma, altered lung function and allergic sensitization in school-aged children.
Methods: Blood lipid levels including high- and low-density lipoprotein cholesterol (HDL-C and LDL-C) and triglycerides were measured at ages 5–8 years in 301 children from the Copenhagen Prospective Studies on Asthma in Childhood$_{2000}$ (COPSAC$_{2000}$) at-risk birth cohort. Asthma and allergic rhinitis were diagnosed exclusively by the COPSAC pediatricians based on predefined algorithms at age 7 years along with numerous assessments of lung function and allergic sensitization. The associations between the lipid profile and clinical outcomes were examined with conventional statistics and by principal component analysis (PCA) adjusting for gender, passive smoking and BMI.
Results: High levels of LDL-C were associated with an increased risk of concurrent asthma: (adjusted odds ratio (aOR 1.93 [1.06–3.55], $P = 0.03$) and poorer lung function (PCA, $P = 0.02$). High levels of HDL-C levels were associated with improved lung function (PCA, $P = 0.02$ for both), decreased bronchial hyper responsiveness(Est; 0.53[0.00–1.6], $P = 0.05$) and a decreased risk of aeroallergen sensitization (aOR 0.27 [0.1–0.70], $P = 0.006$). High levels of triglycerides were associated with an increased risk of aeroallergen sensitization (aOR 2.01 [1.14–3.56], $P = 0.02$).
Conclusion: The observed blood lipid profile is associated with concurrent asthma, lung function, lung responsiveness and aeroallergen sensitization in 7-year-old children proposing a low-grade inflammatory systemic disorder.

253
Association of exposure to volatile organic compounds (VOCs) and phthalates with respiratory function in children with asthma

Kim, Y-M[1]; Kim, J[1,2]; Han, Y[1]; Lee, J[1]; Lee, J[1]; Hong, S[1]; Ahn, K[1,2]
[1]Samsung Medical Center, Environmental Health Center for Atopic Diseases, Seoul, Korea; [2]Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Pediatrics, Seoul, Korea

Background: Volatile organic compounds (VOCs) and phthalates have been associated with asthma in children, but few studies have evaluated their biomarkers in association with the aggravation of respiratory function. We explored the associations between urinary levels of phthalates and VOCs metabolites and respiratory function in children with asthma.
Method: For the evaluation of respiratory function, repeated measurements of forced expiratory volume in 1 sec (FEV_1), percentage of FEV_1 of predicted value (FEV_1(%)), forced vital capacity (FVC), FEV_1/FVC ratio (%), peak expiratory flow rate (PEFR), and fractional exhaled nitric oxide (FeNO) were conducted for 42 children with asthma from October 2013 to June 2014. We measured their urinary levels of three phthalate metabolites (including mono(2-ethyl-5-hydroxyhexyl)phthalate (MEHHP), mono(2-ethyl-5-oxohexyl)phthalate (MEOHP) and mono-n-butyl phthalate (MnBP)) and six VOC metabolites (including t,t-muconic acid (t,t-MU) for benzene, hippuric acid (HA) for toluene, three methyl hippuric acid (MHA) for xylene, and mandelic acid (MA) for styrene) using the first morning urine. We then analyzed the effect of VOCs and phthalates on the respiratory function based on linear mixed effect (LME) model with adjustment for age and sex.
Results: All three phthalate biomarkers showed negative effects on respiratory function including FEV_1(%) although all effects were not statistically significant. In particular, an increase in urinary level of MEOHP was negatively associated with lung function; FEV_1 decreased by 0.03 L/s (95% confidence interval (CI): -0.01~0.06) and PEFR by 4.90 L/min (95% CI: 1.01~8.78) as MEOHP increased by 10 μg/g creatinine. VOCs, particularly xylene biomarkers (MHAs), were strongly associated with increase in FeNO in asthmatic children. With an increase in 10 μg/g creatinine of o-MHA, m-MHA, and MA,

© 2015 The Authors

FeNO increased by 2.79 (95% CI: 1.58~4.00), 1.70 (95% CI: 1.14~2.26), and 1.81 (95% CI: 0.91~2.70) ppb, respectively.
Conclusion: VOCs and phthalates act as risk factors that aggravate respiratory function in asthmatic children. Careful efforts to reduce the exposure to VOCs and phthalates are required.

254
Prevalence of asthma severity signs in the West Sweden Asthma Study

Mincheva, R; Lundbäck, B; Lötvall, J
Krefting Research Centre, Gothenburg, Sweden

Background: Deeper comprehension of asthma severity is essential for addressing the needs of the patients that are uncontrolled on standard therapy. Nonetheless, the prevalence of asthma severity at a random population level has not previously been well described.
Aim: To tackle the issue of asthma severity from an epidemiological viewpoint, to determine

(A) its relative prevalence,
(B) whether any phenotype characteristics are evident, and
(C) to describe risk factors.

Method: A large random population study (18 092 questionnaire responses from 30 000 sent) was carried out in West Sweden, and a group of 744 people with active asthma was extensively phenotyped through clinical workup and detailed questionnaires. Different parameters of severity were outlined as follows:

(1) Low lung function as FEV1% predicted below 70%,
(2) Multi-symptom asthma defined daytime symptoms,
(3) Night-time awakenings most nights of the week,
(4) Use of rescue medications daily or almost daily and
(5) Any respiratory emergency visits or use of oral steroids regularly or on exacerbations in the last year. We outlined subgroups of asthmatics who presented with different extend of overlap of these characteristics and looked into possible risk factors.

Results: Over one third of the active asthmatics (n = 269.36.2%) showed any sign of significant disease severity. On further dissection, groups of people with just one isolated complaint were described - only with daytime symptoms (n = 45), low lung function (n = 37), using rescue medications (n = 31), having night-time awakenings (n = 31) and emergency visits/use of oral steroids (n = 27). Furthermore, those who showed at least two overlapping complaints represented 13.2% of the active asthma (n = 98). Risk factor analysis for the groups of asthmatics who had any or at least two symptoms showed that smoking and obesity are significant risk factors for both - for those having clustered symptoms smoking showed OR (CI) of 2.8 (1.5–5) and obesity 2.3 (1.5–3.6) and for those with any severity symptom the results were 2.4 (1.5–3.8) for smoking and 1.9 (1.4–2.7) for obesity, all significant at $P < 0.001$.
Conclusion: Prevalence of severe asthma is often estimated to be around 5–10% of a total asthma population, but our data show that on a population level significant asthma severity is much more common. Further dissection into asthma severity phenotypes in a random population setting can facilitate understanding of the dimensions of this burdening problem.

255
Associations between dendritic cells, farm-related exposures and childhood asthma

Martikainen, M-V[1]; Kääriö, H[1]; Karvonen, A[2]; Schröder, PC[3]; Renz, H[4]; Kaulek, V[5]; Dalphin, J-C[5]; von Mutius, E[3]; Schaub, B[3]; Pekkanen, J[6]; Hirvonen, M-R[1,2]; Roponen, M[1]
[1]Department of Environmental Science, University of Eastern Finland, Kuopio, Finland; [2]Department of Health Protection, National Institute for Health and Welfare, Kuopio, Finland; [3]Department of Allergy and Pulmonary, Dr. von Hauner Children's Hospital LMU Munich, Munich, Germany; [4]Institute of Laboratory Medicine, Pathobiochemistry and Molecular Diagnostics, Philipps University, Marburg, Germany; [5]Department of Respiratory Disease, University Hospital of Besançon, Besançon, France; [6]Department of Public Health, University of Helsinki, Helsinki, Finland

Background: Farm exposures early in life have been associated with the decreased risk of allergic diseases. Dendritic cells (DCs), the main antigen presenting cells of immune system, might modulate asthma-protective effect of farm exposures as they play an important role in the induction of immunity and tolerance. Our aim was to investigate whether farming and asthma are associated with cell numbers and immunogenic and tolerogenic properties of circulating DC subsets in a subpopulation of children from the PASTURE birth cohort study.
Method: In this case-control study, 82 farm and 86 non-farm French and Finnish children with and without asthma were studied at age of 6 years. DC numbers and expression of functional markers were assessed using flow cytometry. DC subsets were identified as BDCA1 + CD11c+mDC1s, BDCA3+^{high}mDC2s and BDCA2 + pDCs. Expression of functional markers CD86, immunoglobulin-like transcript 3 (ILT3) and ILT4 in mDC1s and pDCs were analyzed. Questionnaires were used to assess prenatal and lifetime pattern of farm exposures and to define asthma.
Results: The number of mDC2 cells was lower in farm children and in children with prenatal and lifetime exposure to farm milk and stables. Farm children also had a decreased number of ILT3 + mDC1s and, as a trend, less ILT3 + pDCs. DCs did not associate with asthma in whole study population. When stratified with farming, the ratio of mDC1s to pDCs (OR 2.05, CI 95% 1.06–3.97), the number of ILT4 + mDC1s (OR 1.77, CI 95% 1.01–3.12) and the expression level of CD86 in pDCs (aOR 2.17, CI 95% 1.25–3.79) were positively associated with asthma in non-farm children, whereas the number of CD86 + pDCs (OR 0.49, CI 95% 0.26–0.95) showed protection against asthma. Similar associations were not observed in farm children.
Conclusion: We found associations between DCs, farm exposures and asthma, but we could not demonstrate that farm exposure mediates asthma-protection by inducing immunogenic or tolerogenic responses in DCs. The complex interactions between farm exposure, asthma and DC immunology requires more investigation.

256
Systematic review of childhood asthma prediction models

Pinart, M[1,2,3]; Smit, HA[4]; Lodrup-Carlsen, K[5]; Keil, T[6,7]; Antó, JM[1,2,3]
[1]Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain; [2]Hospital del Mar Research Institute (IMIM), Barcelona, Spain; [3]CIBER Epidemiologia y Salud Publica (CIBERESP), Barcelona, Spain; [4]Julius Center for Health Sciences and Primary Care, Public Health, Health Technology Assessment, Medical Humanities, Utrecht, Netherlands; [5]Department of Pediatrics, Oslo University Hospital and University of Oslo, Oslo, Norway; [6]Institute for Social Medicine, Epidemiology and Health Economics, Charité - Universitätsmedizin Berlin, Berlin, Germany; [7]Institute of Clinical Epidemiology and Biometry, University of Würzburg, Würzburg, Germany

Background: There is a need for evidence-based guidelines to identify which preschool wheezing children will develop asthma at school age and may need treatment. We aimed to provide the available evidence on characteristics of existing risk prediction models to identify preschool children with early asthma-like symptoms who will develop clinical asthma at school age.
Method: We conducted a systematic review of original studies that developed new prediction models or modified existing prediction models. We searched PubMed and Embase up to June 2014 and selected English language prospective studies in

© 2015 The Authors

preschool children under age 4 years that assessed asthma as an outcome between ages 6 and 12 years. Early asthma-like symptoms was used as an eligibility criterion or as predictor in the model.

Results: We identified 14 prediction models and distinguished different eligibility criteria for the development population: health care visits for asthma-like symptoms (5 models), a high risk of asthma (5 models), parent-reported asthma-like symptoms (3 models) and 2 models without eligibility criteria, where early asthma-like symptoms was the predictor. There were 5 models with non-invasive, easy-to-obtain predictors. The most commonly used predictors were family history, comorbidities or precursors of asthma and severity of early symptoms. Nine models included additional clinical tests mostly positive specific IgE sensitization. The positive Likelihood Ratio varied from 1.06–7.43.

Conclusion: Although the positive predictive ability of the prediction models is poor to acceptable at best, the potential to improve clinical prediction by any of the models remains unclear until quantitative comparison is possible through external validation in a large study population with adequate population mix. A quantitative external validation is urgently needed to optimize evidence-based clinical decision making.

257
Vitamin D insufficiency in asthmatic patients

Popescu, A[1]; Greblescu, R[2]
[1]Pop de Basesti Medical Centre, Bucharest, Romania; [2]Medas Clinic, Bucharest, Romania

Background: Vitamin D has important functions in the immune system. In recent years, several studies have reported an association between vitamin D levels, atopy, asthma and respiratory tract infections.

Objective: We aimed to investigate whether vitamin D insufficiency in asthmatic adults associates with atopy, poor asthma control, and more frequent respiratory tract infections, and whether these patients can benefit from vitamin D supplementation.

Methods: The study included 62 adult patients diagnosed with asthma. At the first visit, patients underwent pulmonary function testing, skin prick testing with panel of aeroallergens, and 25-Hydroxyvitamin D levels were measured. Asthma Control Test (ACT) questionnaires were administered and records were obtained regarding number of visits to a medical centre for respiratory tract infections for the previous year. Subsequently, patients with vitamin D deficiency received 2000 I.U. vitamin D3 daily for 6 months (from October to March). At the end of that period, all patients were reassessed.

Results: Seventeen patients (27%) were found to have vitamin D levels lower than 30 ng/ml, considered to be insufficient. These patients had poorer asthma control as indicated by ACT scores, and had a significantly higher rate of allergic sensitization, compared to the vitamin D sufficient asthmatics. Patients with vitamin D insufficiency had an average number of medical visits for respiratory infections the previous year of 3.94, while after vitamin D supplementation the average was 2.17. ACT scores increased significantly at the end of the 6 month period, while spirometric values did not change significantly.

Conclusions: We found that vitamin D insufficiency in patients with asthma correlates with allergic sensitization. Vitamin D supplementation in these patients resulted in better asthma control, possibly due to fewer episodes or respiratory tract infections.

© 2015 The Authors

Poster Discussion Session PDS 4

Environmental heterogeneity and asthma and allergies

258

Ragweed (*Ambrosia artemisiifolia*): How does *Ambrosia* pollen react to different air pollution? A systems biology approach

Frank, U[1,2]; Zhao, F[1]; El-Kelish, A[1]; Heller, W[1]; Braun, P[3]; Durner, J[1]; Winkler, JB[1]; Engel, M[1]; Pfeifer, M[1]; von Törne, C[1]; Hauck, SM[1]; Öder, S[2,4]; Traidl-Hoffmann, C[2,5]; Ernst, D[1,2]
[1]Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; [2]CK-CARE, Christine Kühne - Center for Allergy Research & Education, Davos, Switzerland; [3]University of Applied Science Munich, Munich, Germany; [4]ZAUM - Center for Allergy & Environment Munich, Technical University and Helmholtz Zentrum München, Munich, Germany; [5]Institute of Environmental Medicine, UNIKA-T, Technical University München, Munich, Germany

Background: *Ambrosia artemisiifolia* (ragweed) is an invasive neophyte from Northern America, which is now spreading within Europe. The pollen has a very high allergenic potential and studies from recent years show an impact of climate change and pollution on the growth and flowering period of different plants. The aim of this project is to investigate whether climate-relevant, tropospheric ozone, elevated CO_2 and increased NO_2 also have an impact on the allergenic potential of ragweed pollen.
Material and methods: *Ambrosia* plants were grown in climate chambers or in the greenhouse under controlled conditions and were fumigated with 40 ppb ozone (control) and 80 ppb ozone (increased) or 380 ppm CO_2 (control) and 700 ppm CO_2 or 40 ppb NO_2 (control) and 80 ppb NO_2, respectively. Pollen of these plants were then compared and analyzed in a systems biology approach.
Results: Spectral analysis showed that the increase of the ozone to twice the ambient leads to a change in the pollen cell wall. Transcription analysis using 454 sequencing reflected this again. Similarly, an increase of so-called stress genes and an increase of isoformspecific *Ambrosia* allergen transcripts under elevated ozone was observed. The rise in CO_2 and NO_2 concentrations and drought stress resulted in elevated allergen transcripts as well as an increased content of allergenic proteins. Furthermore, a nitrosylation of Amb a 1 under elevated NO_2 was demonstrated, which could also have an impact on the allergenicity. Pollen extracts of CO_2 and drought stress-treated plants showed higher IgE levels in a mouse model.
Conclusion: Our data show that climate change and environmental gases have an influence on the transcript and protein amount of pollen allergens, thus supporting the idea of a direct effect of air pollution on the allergenicity.

259

The effect of CO2-enriched atmospheres to producing of allergenic pollen by ragweed

Oh, J-W[1]; Baek, W-K[2]; Kim, K-R[3]
[1]Pediatrics, Hanyang University Guri Hospital, Guri Kyunggi-Do, Korea; [2]Nature Science, Daejin University, Pocheon, Korea; [3]Applied Meteorology Lab, National Meteorological Research Institue, JeJu, Korea

Background: The prevalence of allergic diseases has increased result from exposure to environmental pollutants. Aeroallergen exposure is associated with allergic rhinitis and asthma. Pollens have long been known to be a significant cause of allergic disease. Pollen in heavily polluted zones can express a larger amount of proteins described as being allergenic. In the presence of high CO2 concentrations and temperatures, plants increase their pollen output.
Method: Ragweed establishment:

(1) Chamber study: Ten plants of ragweed were established in open-top chamber at different concentration of CO2 (380–400, 500–520, 600–620, 1000–1100 ppm).
(2) Field study: Beginning in March 2012 and 2013, a rural (Pocheon, Kyunggido, annual mean CO2: 230 ppm) and urban (Kangnam, Seoul, annual mean CO2: 440 ppm) locations were established.

Seeds of common ragweed (Ambrosia artemisiifolia) and giant ragweed (A. trifida) were obtained from Daejin University from a common seed lot of ragweed. At final harvest, entire plants were collected. To determine qualitative changes in pollen, harvested pollen grains were suspended in 95% ethanol. The crude soluble pollen protein preparations were stored at −20°C. Protein content of the extracts was quantified. Concentration of Allergens (common ragweed (Amb a 1) and giant ragweed (Amb t 5) was quantified through use of double sandwich ELISA.
Results:
(1) chamber study: Concentration of Amb a 1 was increased with increased CO2 Conc. (380–400, 500–520, 600–620, 1000–1100 ppm: 18.4 ± 5.0, 30.8 ± 13.1, 42.5 ± 11.2, 50.1 ± 21.2 ng/mL), Concentration of Amb t 5 was increased with increased CO2 Conc. (380–400, 500–520, 600–620, 1000–1100 ppm: 22.1 ± 6.8, 36.3 ± 11.6, 48.3 ± 19.5, 64.6 ± 21.3 ng/mL),
(2) Field study: There were not significantly different between Pocheon (CO2 230 ppm: 16.0 ± 2.0 ng/mL) and Seoul (CO2 440 ppm: 20.3 ± 8.6 ng/mL) in Conc. of Amb a 1, also Pocheon (CO2 230 ppm: 24.5 ± 6.9 ng/mL) and Seoul (CO2 440 ppm: 28.3 ± 6.2 ng/mL) in Conc. of Amb t 5, though Conc. of Amb a 1 and Amb t 5 were increased at Seoul than those at Pocheon.

Conclusion: Increased CO2 significantly influence allergenicity and pollen concentration of common ragweed through the chamber and field study. The elementary example given here demonstrates strong probable links between rising CO2 levels and increased allergic diseases. We suggest that urbanization might provide a alternative to current experimental methods evaluating plant responses to climate change.

260

Identification of IgE reactive proteins from pollens of *Lagerstroemia speciosa* and S*pathodea campanulata* - two avenue trees of West Bengal

Basak, T[1]; Bhattacharya, K[2]; Pandey, N[3]
[1]Department of Botany, Visva Bharati, Santiniketan, India; [2]Visva Bharati, Santiniketan, India; [3]Mediland Diagnostics Institute, Kolkata, India

Background: *Lagerstroemia speciosa*(LS) and *Spathodea campanulata*(SC) are 2 dominant avenue trees in Santiniketan and Kolkata, West Bengal (WB), India during summer and pre-monsoon time. The aim of the study was to determine the concentration and seasonal periodicity of the following pollen grains and attempted to identify the proteins released by the pollen

© 2015 The Authors

grains that may be responsible for immediate hypersensitivity reactions in seasonal respiratory allergic patients.

Method: An aerobiological survey was conducted with a personal Burkard volumetric sampler for two consecutive years both in Kolkata and Santiniketan, West Bengal. The allergenic potential of the pollen extracts was evaluated in patients with respiratory allergy by skin prick test (*in vivo*), IgE-enzyme-linked immunosorbent assay and IgE specific immunoblotting (*in vitro*) methods. Soluble pollen protein in PBS (pH 7.4) was fractionated in 0–30%, 30–50% and 50–90% using $(NH_4)_2SO_4$ precipitation. The total pollen protein resolved in 12% SDS-PAGE and IgE immunoblotting was done using sera from 6 patients.

Results: Both *Lagerstroemia speciosa* and *Spathodea campanulata* pollen grains were prevalent in the air from March to June in both Kolkata and Santiniketan while LS pollen was prevalent from June to November in Santiniketan only. LS and SC pollen antigen caused marked skin sensitivity among 22.5% and 26.4% of atopic populations respectively. Total pollen protein of LS and SC were resolved into 18 and 15 distinct bands over a wide molecular weight range respectively in 12% SDS-PAGE. A total of 7(21.5- 95 KDa) and 4 (21–95.2KDa) protein fractions were identified as a major IgE reactive pollen allergen in LS and SC respectively.

Conclusion: *Lagerstroemia speciosa* and *Spathodea campanulata* pollen grains are dominant in the atmosphere of Santiniketan and Kolkata during summer and pre-monsoon. LS pollen showed different peaks in two different sites while SC pollen showed a single peak in both of the two sites. SPT (*in vivo*), IgE-enzyme-linked immunosorbent assay and IgE specific immunoblotting (*in vitro*) analyses showed seven allergens (21.5 - 95 KDa) of LS pollen and four allergens (21 -95.2 KDa) of SC pollen, are major IgE reactive proteins. This information is very important to allergologist as these are the avenue trees and local inhabitants are frequently exposed to these pollens. This is the first report of allergenicity of *Lagerstroemia speciosa* (LS) and S*pathodea campanulata* (SC) pollens from WB.

261
The major olive pollen allergen, Ole e 1, interacts with raft-like microdomains

Lopez-Rodríguez, JC; Cruz, A; Hidalgo, A; San Segundo-Acosta, P; Martín-Pedraza, L; Villalba, M; Rodríguez, R; Pérez-Gil, J; Batanero, E; Barderas, R
Biochemistry & Molecular Biology I, Universidad Complutense de Madrid, Madrid, Spain

The airway epithelium barrier is a key player in the immune response against aeroallergens by contributing to the development of allergic inflammation. Several studies have suggested an important role of lipid raft microdomains in the regulation of signal pathways associated with innate and adaptive immunity. Lipid rafts are highly dynamic cholesterol and sphingolipid-enriched membrane microdomains, likely present in all eukaryotic cells. These membrane microstructures provide a spatial compartmentalization of receptors and signaling molecules that facilitates their interaction and functional activation. However, few studies have been performed to describe how the allergens interact with these lipid domains.

In the present study, we analyzed the interaction of Ole e 1, the major olive pollen allergen, with monolayers as model membranes using the Langmuir-Wilhelmy balance. This technique allows analyzing protein-lipid interactions observed as changes in the surface behavior of lipid monolayers of different compositions. In addition, epifluorescence microscopy images of supported lipid monolayers (Langmuir-Blodgett films) were obtained to visualize the allergen effect during the surface pressure-area compression and its distribution in the interfacial films.

We have found that Ole e 1 interacts at low surface pressures with monolayers of different compositions. Ole e 1 behavior is clearly different in monolayers from phosphatidylcholine/sphingomyelin/cholesterol, presenting lateral segregation of lipid domains mimicking lipid rafts, than in lipid monolayers containing the pulmonary surfactant dipalmitoylphosphatidylcholine, non-presenting lateral segregation or lateral phase transition. On the other hand, epifluorescence analysis revealed the preferential localization of a fluorescent-labeled Ole e 1 on raft-like microdomains.

In conclusion, our results show that Ole e 1 preferentially interacts with liquid-ordered raft-like microdomains. Further studies will be carried out to gain insight into the potential implication of such interaction in allergic reaction.

262
Asian sand dust increases MUC8 and MUC5B expressions via TLR4-dependent ERK2 and p38 MAPK in human airway epithelial cells

Kim, Y-D[1,2]; Choi, YS[1]; Na, HG[1]; Bae, CH[1]; Song, S-Y[1]
[1]College of Medicine, Yeungnam University, Otorhinolaryngology-Head and Neck Surgery, Daegu, Korea; [2]Regional Center for Respiratory Diseases, Yeungnam University Medical Center, Daegu, Korea

Background: Asian sand dust (ASD) is a natural phenomenon and originates from the deserts of China, and is known to contain various chemical and biomolecular components that enhance airway inflammation. The overproduction of airway mucins is an important pathologic finding in inflammatory airway diseases. However, the mechanism of ASD on mucin production of airway epithelial cells has not been elucidated.

Method: In the NCI-H292 cells and the primary cultures of human nasal epithelial cells, the effect and signaling pathway of ASD on MUC8 and MUC5B expressions were investigated using reverse transcriptase-polymerase chain reaction (RT-PCR), real-time PCR, enzyme immunoassay, and immunoblot analysis with several specific inhibitors and small interfering RNA (siRNA).

Results: ASD increased MUC8 and MUC5B expressions, and activated the phosphorylations of extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (MAPK). U0126 (ERK1/2 MAPK inhibitor) and SB203580 (p38 MAPK inhibitor) inhibited ASD-induced MUC8 and MUC5B expressions. In addition, knockdowns of ERK2 and p38 MAPK by siRNA blocked ASD-induced MUC8 and MUC5B mRNA expressions. Toll-like receptor 4 (TLR4) mRNA expression was increased after treatment with ASD. Knockdown of TLR4 by siRNA blocked ASD-induced MUC8 and MUC5B mRNA expressions. Furthermore, the phosphorylations of ERK1/2 and p38 MAPK were blocked by knockdown of TLR4.

Conclusion: These results show that ASD induces MUC8 and MUC5B expressions via TLR4-dependent ERK2 and p38 MAPK signaling pathway in human airway epithelial cells.

263
The effect of environmentally friendly wallpaper and flooring material on indoor air quality and atopic dermatitis: a pilot study

Byun, SY; Jeong, MY; Park, KC; Na, JI; Huh, CH
Seoul National University College of Medicine, Seoul National University Bundang Hospital, Dermatology, Seongnam-si, Korea

Background: Formaldehyde (FA) and volatile organic compounds (VOCs) are considered one of the main culprits behind atopic aggravation and the main sources of them are wallpaper, painting, adhesives and flooring. The purpose of this study is to assess the effects of environmentally friendly wall paper and flooring material on indoor air quality and atopic dermatitis severity.

Method: Thirty atopic dermatitis patients were enrolled in this study. To improve the air quality, wallpaper and flooring of sub-

© 2015 The Authors

jects′ houses were exchanged for plant or silica. The indoor air concentration of FA, total VOCs (TVOCs) was measured before remodeling and 2, 6 and 10 weeks after. Pruritus and severity of atopic eczema was evaluated using a questionnaire and EASI (Eczema Area and Severity Index) score before and 4, 8 and 12 weeks after remodeling. Subjects were instructed to keep their therapy on-going for atopic dermatitis.

Results: Twenty four subjects′ houses were remodeled and all of them finished the study. The FA in the ambient air significantly decreased within 2 weeks after remodeling. TVOCs level showed a decrease at week 2 but a re-increase at week 6 and 10. The reduction of pruritus and EASI score was statistically significant in patients whose baseline EASI score was over 3.

Conclusion: A replacement of environmentally friendly wallpaper and flooring material reduced FA in ambient air and improved pruritus and the severity of atopic eczema. The improvement of pruritus and eczema was statistically significant in patients whose baseline EASI score was over 3.

264
Pollution, pollen and OpenHealth data

Thibaudon, M[1]; Monnier, S[1]; Michelot, N[2]; Auvigne, V[3]
[1]RNSA, Brussieu, France; [2]MEDDE, Paris, France; [3]EKIPAJ, Angers, France

Background: Since the late nineteenth century pollinosis (pollen allergy) seems to progress regularly in line with the overall air pollution. Where this physico-chemical air pollution appears or increases, especially in cities, the prevalence of pollinosis strengthens.

The World Health Organization (WHO) rates allergic diseases in fourth rank worldwide among disorders and since the 2000s, allergy due to pollen affects 10 to 15% of the world's population.

Method: At the request of MEDDE (Ministry of Ecology, Sustainable Development and Energy), the RNSA (French Aerobiology Network) has developed a collaborative research project linking pollen data, air pollution data and health impact data for several French cities with different climates (Lyon, Paris, Toulouse, Strasbourg, Nice) from 2010 to 2013.

OpenHealth data were used as an indicator of the health impact. These data, which are transmitted daily by over 4600 pharmacies members of the Celtipharm network in France, relate to the sale of medicinal products (and first and foremost antihistamines) used in the symptomatic treatment of pollinosis (hayfever, hives…).

Results: Linking environmental data (pollen and pollutants) and the OpenHealth indicator of health impact generally confirms the health impact caused by pollen (birch, grass, oak, and ragweed). Indeed, the results show great similarities between OpenHealth data and pollen counts for birch (main peaks of drug consumption in March-April), grasses (secondary OpenHealth peaks in May-June) and ambrosia (last drug consumption peaks in August-September). Ozone pollution episodes in different cities of the study show peaks that overlap to some extent with pollen peaks and OpenHealth peaks.

265
Grass pollen allergy season 2014, a multidisciplinary case study in Vienna: temporal variation in pollination of grass species, impact on pollen allergy sufferers and species-dependent allergenicity

Kmenta, M[1,2]; Zetter, R[2]; Bastl, K[1]; Kramer, M[3]; Mwange, J[3]; Hewings, S[3]; Skinner, M[3]; Berger, U[1]
[1]Department of Otorhinolaryngology, Medical University of Vienna, Research Group Aerobiology and Pollen Information, Vienna, Austria; [2]Research Group Paleobotany and Palynology, Department of Paleontology, University of Vienna, Vienna, Austria; [3]Allergy Therapeutics Plc, Worthing, United Kingdom

Background: Grass pollen allergy is the most prevalent pollen allergy in Austria affecting about 50% of pollen allergy sufferers. Compared with pollen seasons of other aeroallergens (e.g. birch), the grass pollen season is not homogenous, since it is composed of the flowering periods of many different grass taxa. Hence, more than one peak can occur during the pollen season. Furthermore, the allergenicity of grass pollen varies from species to species and even sensitization to those might differ individually.

Method: Several grass species, that are most commonly distributed in the area of Vienna, were included in this study and monitored by use of phenology, pollen monitoring and symptom data. Phenological observations were performed twice a week in two different locations and evaluated with local pollen measurements and data entries from the Patient′s Hayfever Diary (PHD; www.pollendiary.com), including exclusively symptom data from people suffering from allergic symptoms in the area of Vienna. In addition, biochemical assessments of trends in allergen reactivity have been completed.

Results: Preliminary results indicate that the flowering of Kentucky bluegrass (*Poa pratensis*) and Orchard grass (*Dactylis glomerata*) induces the first peak in pollen and symptom load within the early grass pollen season. Moreover, the common distribution of false-oat grass (*Arrhenatherum elatius*), fescue grasses (*Festuca*) and English ryegrass (*Lolium perenne*) in the areas of observation assumes a greater importance of these taxa contributing to the main grass pollen season compared to grasses with poor distribution such as Timothy (*Phleum pratense*)- and Bermuda grass (*Cynodon dactylon*). Furthermore, the relevance of allergen reactivity within collected grass pollen is presented.

Conclusion: This study represents an unique approach combining phenology, pollen measurements and symptom data in order to gain insights into

(1) the contribution of different grass species to the grass pollen season,
(2) the impact on grass pollen allergy sufferers and their burden and
(3) allergenicity vs pollen count vs symptom load comparisons.

266
Real time monitoring of seasonal allergic rhinitis using drug sales data

Auvigne, V[1]; Annesi-Maesano, I[2,3]; Thibaudon, M[4]; Capone, C[5]; Guérin, P[6]
[1]OpenHealth, Vannes, France; [2]EPAR, UMR-S 1136, IPLESP, INSERM, Paris, France; [3]EPAR, UMR-S 1136, IPLESP, UPMC, Sorbonne University, Paris, France; [4]RNSA, Brussieu, France; [5]Carré Castan Consultants, Paris, France; [6]Celtipharm, Vannes, France

Background: Assessment of the epidemiological evidence of short-term links between pollen exposure and seasonal allergic rhinitis requires the availability of health indicators. Anti-allergic drug consumption within general population is a data source which can be used for this purpose. The purpose of the OpenHealth.fr project is to design and publish indicators to monitor the health of the French population. These indicators are based on the analysis of medication sales data from pharmacies. Currently five indicators are published daily, including one covering the incidence of allergies.

Method: The data that is used is data from the sale of medication used to treat seasonal allergic rhinitis. These are mainly antihistamines. Since 01/01/2009, the data has been sent every day by over 4600 pharmacies that are representative of pharmacies in metropolitan France (21% of all pharmacies). This data is used to build an Advanced Health Indicator ("Indicateur Avancé Sanitaire", or IAS). The IAS is standardised at the local level using the values observed in January each year. Spatio-temporal dynamics of allergic reactions are visualized using iso-demographic maps and time series.

Results: At a national level, Temporal dynamics are characterised by a seasonal

© 2015 The Authors

occurrence of allergic reactions with two main peaks: in March-April and in May-June. The dates for these peaks vary by about one month from one year to the next. At the local level many phenomena can be observed: an increase in symptoms at the end of January around the Mediterranean region, a peak of activity in late August and early September in the Rhône valley… At their peaks, the IAS values are 50 to 100% greater than in periods where there is no pollen activity.

Conclusion: The peaks in allergic reactions described by the IAS are concomitant with the main peaks in pollen activity: Cypress in late winter around the Mediterranean, broadleaved trees and then grasses at the national level in spring, ragweed in the Rhône valley in late summer. These results are to be compared with those of general practitioner networks. They could be used to better understand the relative importance of the different allergens in terms of public health, as well as the interactions between pollination and pollution.

267
Early airborne allergen exposure is not associated with sensitization in young children

Schoos, A-MM[1]; Chawes, BLK[1]; Jelding-Dannemand, E[1]; Elfman, LB[2]; Bisgaard, H[1]
[1]Copenhagen Prospective Studies on Asthma in Childhood, Faculty of Health and Medical Sciences, University of Copenhagen & Danish Pediatric Asthma Center, Gentofte Hospital, Gentofte, Denmark; [2]Department of Medical Sciences, Occupational and Environmental Medicine, Uppsala University, Uppsala, Sweden

Background: Allergen exposure is intuitively believed to be closely associated with the development of allergic sensitization in childhood. However, there is little evidence to substantiate such relationship. The objective of this study was to investigate the association between pre- and early postnatal airborne allergen exposure and sensitization to aeroallergens during childhood.

Method: Allergic sensitization to cat, dog, and house dust mites was diagnosed longitudinally from skin prick tests and specific IgE results at ages ½, 1½, 4, and 6 years in 392 children from the Copenhagen Prospective Study on Asthma in $Childhood_{2000}$ birth cohort born to mothers with asthma. Allergen exposure was defined as presence of dog or cat in the home during the 3rd trimester of pregnancy and the child's first year of life. Furthermore, objective measurements of dog, cat, and house dust mite allergen levels in bed dust samples were collected at age 1 year. Associations between exposure and allergic sensitization were analyzed by logistic regression.

Results: Allergic sensitization ever to dog was seen in 6.8% of the children ($N = 27$), cat in 7.1% ($N = 28$), *D. pteronyssinus* in 9.8% ($N = 39$), and *D. farinae* in 9.1% ($N = 36$). We found no association between pet exposure in early pre- or postnatal life and sensitization during childhood. Similarly, there was no association between levels of dog, cat, or house dust mite allergens in bed dust and sensitization during childhood. The results were adjusted for mother's sensitization and stratified for the child's eczema status.

Conclusion: Early life pre- and postnatal aeroallergen exposure does not seem to affect development of sensitization during childhood questioning the relevance of allergen avoidance.

268
Higher exposure to outdoor air pollution during infancy increase the risk of development of allergic rhinitis

Lee, JY[1,2]; Kim, J[1,2]; Kim, EH[2,3]; Han, Y[2]; Choi, J[1,2]; Kim, KH[1,2]; Hong, J-Y[2]; Oh, I-B[4]; Cheong, HK[3]; Ahn, K[1,2]
[1]Department of Pediatrics, Samsung Medical Center, Seoul, Korea; [2]Samsung Medical Center, Environmental Health Center for Atopic Diseases, Seoul, Korea; [3]Department of Social and Preventive Medicine, Sungkyunkwan University School of Medicine, Seoul, Korea; [4]University of Ulsan College of Medicine, Environmental Health Center, Ulsan, Korea

Background: The relationship between air pollution and allergic diseases in children is not yet fully understood, with epidemiological data being a matter of debate. The aim of this study was to investigate the association between exposure to outdoor air pollution during the first year of life and the development of allergic diseases in children.

Method: A nationwide cross-sectional survey was conducted in the first grade students from randomly selected 45 elementary schools. The participants of this survey were selected using a stratified two-stage cluster sampling design. Prevalence of physician-diagnosed atopic dermatitis (AD), allergic rhinitis (AR), and asthma was obtained through the Korean version of ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire. Daily ambient concentrations of sulfur dioxide (SO_2), nitrogen dioxides (NO_2), ozone (O_3), carbon monoxide (CO), and particulate matter with an aerodynamic diameter of 10 μm or less (PM_{10}) during the first year of life were monitored from 235 monitoring sites throughout the nation. In this study, children who lived within 2 km from the nearest monitoring sites were selected and analyzed. Multiple logistic regression analyses were conducted to examine the association between the levels of air pollution and the prevalence of allergic diseases, adjusting for the gender of the children, passive smoking, allergic diseases of the parents, education levels of parents, and income of family.

Results: Among the 4003 children, we finally selected 1828 subjects as study population through the exposure estimation process. For each increase of 0.1 ppm in daily mean and maximum CO, The adjusted odds ratio (aOR) for the presence of AR were 1.10 (95% CI: 1.03–1.19) and 1.05 (95% CI: 1.00–1.11), respectively. In addition, the aOR per increase of 1 ppb in daily maximum SO_2 was 1.05 (95% Cl: 1.00–1.07) for the presence of AR. However, exposure to SO_2, PM_{10}, NO_2, CO, and O_3 during the first year of life was not related to the development of AD and asthma.

Conclusion: Higher exposure to outdoor air pollution during the first year of life such as SO_2 or CO increase the risk of development of AR.

269
Particulate air pollution is associated with increased inflammatory and allergic symptoms in adolescents

Lambrechts, N[1]; Govarts, E[1]; Den Hond, E[1]; Franken, C[1]; Nelen, V[2]; Loots, I[3]; Baeyens, W[4]; Sioen, I[5]; Nawrot, T[6]; Bruckers, L[6]; Schoeters, G[1,3,7]; Koppen, G[8]
[1]VITO NV, Mol, Belgium; [2]Provincial Institute for Hygiene, Antwerp, Belgium; [3]University of Antwerp, Antwerp, Belgium; [4]Free University of Brussels, Brussels, Belgium; [5]University of Ghent, Ghent, Belgium; [6]University of Hasselt, Hasselt, Belgium; [7]University of Southern Denmark, Odense, Denmark; [8]Flemish Institute of Technological Research (VITO), Environmental Risk and Health, Mol, Belgium

Background: The northern part of Belgium (Flanders) has among the highest annual concentrations of air pollutants (PM10 and PM2.5) in Europe. The prevailing EU limit of 50 μg/m³ has been exceeded on 35 days in 2013. This can mainly be attributed to Western civilization hallmarks such as dense traffic and urbanization. At the same time, the prevalence of allergic sensitization in children and adolescents keeps on rising.

Method: Six hundred adolescents were recruited as part of the Flemish environmental health surveillance program (FLEHS3). In this study, it was hypothesized that exposure to particulate matter was associated with respiratory and allergic symptoms and related biological markers. The individual exposure to particulate matter (PM2.5 and PM10; ppb) at the home address was estimated based on an interpolation model and the monitored immission levels. pH of exhaled breath and exhaled nitric oxide (NO) was measured in 408 participants to monitor the inflammatory status of the airways. In urine samples of these 408 adolescents the concentration of 8-hydroxy-deoxyguanosine (8-OHdG) was

© 2015 The Authors

determined as a measure of systemic oxidative stress. Symptoms related to asthma, hay fever, eczema and respiratory infections were self-reported using a standardized questionnaire. Multiple linear or logistic regression models were used to calculate the change of effect for an increase of the exposure from P25 to P75.

Results: Multiple regression analysis showed 1.5% acidification of breath (95% CI = 0.1–3.0%) with an increase of PM10 exposure. The urinary concentration of 8-OHdG increased by a factor 1.065 (95% CI = 1.007–1.126) when PM2.5 changed. In addition, augmenting exposures to particulate matter (PM10 as well as PM2.5) were associated with more frequent reporting of allergies to household and personal care products (PM10: OR = 1.665, 95% CI: 1.182–2.344; PM2.5: OR = 1.834, 95% CI: 1.240–2.714), as well as allergies to pets (PM10: OR = 1.952, 95% CI = 1.284–2.967; PM2.5: OR = 1.875, 95% CI = 1.202–2.923). No significant associations were established between these air pollutants and exhaled NO. Prevalence of hay fever, eczema, asthma nor infections were correlated with PM exposure. All models were adjusted for gender, age, smoking status, familial allergy (only for respiratory and allergic symptoms) and a priori defined covariates.

Conclusion: The associated inflammatory and allergic symptoms confirm that the immune system is a target organ for particulate air pollutants.

271
Statistical analysis of the relationships between aeroallergens (pollen activities) and asthma admissions to hospitals in Abu Dhabi Emirate

Al Hashemi, RM[1]; Al Maini, DM[2]
[1]Masdar Institute of Science and Technology, Abu Dhabi, United Arab Emirates; [2]Mafraq Hospital, Rheumatology, Abu Dhabi, United Arab Emirates

Environmental factors have significant effects on the prevalence of asthma that depend on their state. Primarily, the asthma is a problem that cannot be resolved easily without research to determine it causative agents. In Abu Dhabi the number of asthmatic patients is increasing significantly especially among young ages (<15 years old). This paper aims to evaluate how urban air quality and vegetation cover conditions are affecting people with asthma in Abu Dhabi city. In this study, a scientific analytical research have been carried out to study the environmental factors that are triggering this chronic disease based on hospital admission data recorded between 2011 and 2013 in 12 public hospitals located in Abu Dhabi Emirate. Bi-weekly vegetation maps have been generated from NASA's high resolution satellite (LANDSAT) to assess the effects of vegetation (mainly pollen activities) on the number of asthmatics' admissions. In this regard, additional effort has been made to have better understanding on how pollen activities can be detected by Earth observation satellites. The importance of the monitoring has been discussed together with the other ways in which the monitoring becomes an effective technique of evaluation. It applies indexing strategies including the remote sensing from satellites. The Normalized Difference Vegetation Index (NDVI) has been used to classify the vegetation into different greenness indices. Seasonal variation of NDVI was found to have an effect on the variability of asthma cases. Descriptive analysis showing the correlation between vegetation variability and the admissions from various governmental hospitals and clinics was performed. Lastly, the vegetation variability was associated with rising cases of asthma admissions. It was concluded that vegetation condition has a direct effect on asthma-affected population with $R^2 \approx 0.5$ and p-value (≤ 0.05).

272
The effect of air pollutants on chronic cough

Jo, E-J[1]; Lee, W-S[2]; Lee, S-E[3]; Lee, S-H[1]; Mok, J-H[1]; Kim, M-H[1]; Lee, K[1]; Kim, K-U[1]; Lee, M-K[1]; Park, H-K[1]
[1]Department of Internal Medicine, Pusan National University School of Medicine, Busan, Korea; [2]Climate Research Department, APEC Climate Center, Busan, Korea; [3]Department of Internal Medicine, Pusan National University Yangsan Hospital, Yangsan, Korea

Background: Chronic cough, defined as a cough of >8 weeks, is a common symptom for which patients seek the hospital. Three common underlying causes of chronic cough were asthma, reflux disease, upper airway cough syndrome (UACS), however, some patients are not found to have a definable underlying cause. We investigated the association between the exposure of air pollutants and chronic cough.

Method: We reviewed retrospectively the clinical data of subjects who underwent skin prick tests to aeroallergens, induced sputum analysis, and methacholine bronchial provocation test between January 2011 and December 2013 to evaluate their chronic cough. We analyzed the effect of the ambient particulate matter (PM10), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3), and carbon monoxide (CO) on chronic cough.

Results: A total of 444 subjects were analyzed (mean age: 49.5 ± 14.3, female 68.9%). Airway hyperresponsiveness (AHR) was demonstrated in 6.3%; sputum eosinophilia, in 13.1%; and sensitization to at least one aeroallergen in skin tests, in 25%. Their diagnoses were asthma or eosinophilic bronchitis (19.4%), UACS (66.0%), reflux disease (5.0%), chronic obstructive pulmonary disease (1.1%), and idiopathic chronic cough (31.3%). In the patients suffering from chronic cough, non-asthma patients were exposed the higher level of air pollutants, PM10 (48.8 vs 46.5 μg/m^3, $P = 0.014$), NO_2 (21.0 vs 19.7 ppm, $P = 0.005$), and CO (4.1 vs 3.9 ppm, $P = 0.013$), comparing to asthma patients. The pollutants exposure also was higher in idiopathic chronic cough patients; NO_2 (21.3 vs 20.5 ppm, $P = 0.024$), and CO (4.2 vs 4.0 ppm, $P = 0.017$). AHR and sputum eosinophilia were not associated with air pollutants. However, in idiopathic cough patients, neutrophilic airway inflammation was related with PM10 ($\rho = 0.209$, $P = 0.033$), NO_2 ($\rho = 0.269$, $P = 0.006$), and CO ($\rho = 0.308$, $P = 0.001$).

Conclusion: Air pollutants had an effect on idiopathic chronic cough, and were related with neutrophilic airway inflammation.

© 2015 The Authors

Poster Discussion Session PDS 5

Clinical relevance of IgE to allergenic molecules

273

Molecular, proteomic and immunological parameters of allergens provide inclusion criteria for new candidates within established grass and tree homologous groups

Heath, MD; Collis, J; Hutchings, JW; Swan, N; Skinner, MA
Allergy Therapeutics Plc, Worthing, United Kingdom

Background: Our knowledge of allergen structure and function continues to rise and new scientific data on the homology and cross-reactivity of allergen sources should be considered to extend the work of Lorenz *et al*, 2009 and the concept of homologous groups. In addition to this, sophisticated techniques such as mass spectrometry (MS) is increasingly utilised to better characterise the complex mix and nature of allergen extracts.
Method: Homology models were built of Fag s 1 (Beech) and Cyn d 1 (Bermuda grass) and compared with template crystal structures of Bet v 1 and Phl p 1 from the 'exemplar' species of Birch and Timothy grass, respectively. ELISA experiments were performed to assess cross-reactivity of Beech (tree) and Bermuda (grass) extracts to rabbit sera raised to either "3 Tree" (Birch, Alder and Hazel) extract or "Grass" (12-grass mix extract), respectively. The comparability of biochemical stability of different allergen sources was assessed through statistical methods for a range of tree and grass species.
Results: Allergen cross-reactivity and/or structural homology have been described providing justification for inclusion of Beech within the Birch homologous tree group. Data from Bermuda grass (Cyn d 1) provides further justification for the inclusion of this species into the homologous group of the sweet grasses. However, further characterisation of relevant allergens from Bermuda grass and, in particular, comparison of cross-reactive patterns between subjects specifically in areas with high abundance of both *Pooideae* and *Chloridoideae* is sought.
Conclusion: MS allows the possibility to identify individual proteins or allergens from complex mixes by mass and/or sequence, and this has been extensively applied to the allergen field. New data on the homology, cross-reactivity and biological parameters of allergen sources has been considered to extend the work of Lorenz et al, 2009 in the context of Tree and Grass species. The concept of homologous groups is certainly dynamic allowing the flexibility and potential for streamlining quality parameters, such as stability profiles, due to extrapolation of exemplar data to a wider range of allergens. The work presented herein pays tribute to the concept of homologous groups providing an assessment of the current literature and supporting data for the inclusion of Beech and Bermuda grass species.

274

Evolution of serum levels of IgG vs IgE antibodies to Bet v 1 during childhood: a study at individual level

Hofmaier, S[1]; Hatzler, L[1]; Rohrbach, A[1]; Panetta, V[2]; Hakimeh, D[1]; Bauer, C-P[3]; Hoffmann, U[3]; Forster, J[4]; Zepp, F[5]; Schuster, A[6]; Stock, P[1]; Wahn, U[1]; Keil, T[7,8]; Lau, S[1]; Matricardi, PM[1]
[1]Department of Paediatric Pneumology & Immunology, Charité Universitätsmedizin Berlin, Berlin, Germany; [2]L'altrastatistica srl, Consultancy & Training, Biostatistics Office, Rome, Italy; [3]Department of Pediatrics, Technical University of Munich, Munich, Germany; [4]Department of Pediatrics, St Hedwig, St Josefs Hospital, Freiburg, Germany; [5]Department of Pediatrics and Adolescent Medicine, Johannes Gutenberg University Medical Centre, Mainz, Germany; [6]Department of Pediatric Cardiology and Pneumology, Heinrich-Heine-University, Düsseldorf, Germany; [7]Institute of Social Medicine, Epidemiology and Health Economics, Charité Universitätsmedizin Berlin, Berlin, Germany; [8]Institute of Clinical Epidemiology and Biometry, University of Würzburg, Würzburg, Germany

Background: We have recently reported that IgE responses to Bet v 1 in children are accompanied or preceded by IgG responses to the same molecule. The aim of the present study is to investigate the quantitative trends of both IgG and IgE responses and their reciprocal relation at individual level.
Method: The German Multicentre-Allergy-Study examined a birth cohort born in 1990. Blood samples were collected at age 1, 2, 3, 5, 6, 7, 10, 13 years. Participants were included in the present analysis if they had 1) at least 1 serum sample at each of the 4 age periods or time points: 1y-3y, 5y-7y, 10y, and 13y and 2) IgE responses to birch pollen. IgG and IgE responses have been tested with a microarray technique (ISAC).
Results: Twenty-eight birch-atopic children were examined. When tested with the microarray technique and examined on a logarithmic scale, the trajectories of the serum of IgE antibodies to Bet v 1 were in the majority of children relatively similar to those of their respective IgE responses. However, in some children, the levels of IgE and IgG antibodies to Bet v 1 diverged. In addition, the ratio between IgG and IgE antibody level was quite heterogeneous in the population examined and in some children changed with age.
Conclusion: The results suggest that the levels of IgG and IgE responses to Bet v 1 in childhood are only partially associated and may diverge in the individual child over time. This hypothesis must be further tested with a quantitative singleplex methodology.

275

Comparison of the different sIgE methods diagnostic capacity in patients sensitized to olive pollen components

Martínez-Aranguren, R[1]; Goikoetxea, MJ[1]; Villalba, M[2]; de la Hoz, B[3]; Martínez-Botas, J[4]; Feo Brito, F[5]; Alvarado, MI[6]; Sanz, ML[1]
[1]Clinica Universidad de Navarra, Allergology and Clinical Immunology, Pamplona, Spain; [2]Universidad Complutense de Madrid, Biochemistry and Mollecular Biology, Madrid, Spain; [3]Hospital Ramón y Cajal, Allergology, Madrid, Spain; [4]Hospital Ramón y Cajal, Biochemistry-Research, IRYCIS, Madrid, Spain; [5]Hospital Ciudad Real, Allergology, Ciudad Real, Spain; [6]Hospital Virgen del Puerto, Allergology, Plasencia, Spain

Background: Olive pollen is one of the principal causes of pollinosis in the Mediterranean area. Sensitization to their allergens can vary dependently to the pollen counts gradient. Our aim was to compare the sensitization to olive pollen allergens by different diagnostic methods.
Method: Twenty seven olive allergic patients (48% men, 16–54 years old) from two high pollen counts areas of Spain (Caceres and Ciudad Real) were studied showing positive SPT to olive pollen extract and manifested rhinitis and/or asthma in olive pollinization season. We studied the olive pollen sensitization profile of the patients by immunoblotting. sIgE was determined against Ole e 1, Ole e 7, Ole e 9 in all patients by ISAC 112 and

© 2015 The Authors

ELISA and in 17 patients by a homemade protein microarray (RIRAAF). sIgE against Ole e 2 (profilin), Ole e 3 (polcalcin) and Ole e 11 (pectin methylesterase) was measured by ELISA in all patients. Their allergens homologues were analyzed by the two multiplexed sIgE platform, ISAC 112 (Profilin: Phl p 12/Bet v 2/Hev b 8/Mer a 1/Pru p 4; Polcalcin: Phlp 7/Bet v 4; Pectin methylesterase: Sal k 1) performed in the whole sample and RIRAAF (Profilin: Che a 2/Pho d 2; Pectin methylesterase: Sal k 1) in 17 patients.

Results: The percentage of sensitization detected for each allergen by different techniques in patients is represented in table. The detection of sIgE by ELISA and ISAC had similar sensitivity, whereas homemade multiplex platform showed slightly lower sensitivity.

	Ole e 1, %	Ole e 7, %	Ole e 9, %	Ole e 2 / Porfilin, %	Ole e 3 / Pol-calcin, %	Ole e 11 / Sal k 1, %
Immuno-blotting	52	–	18	37	–	22
ELISA	67	11	18	41	15	35
ISAC	78	11	15	55	15	41
RIRAAF	59	0	12	29	–	17

[Percentage of sensitization to olive allergens]

Six patients did not show any reactive band in immunoblotting. However, only one of them had negative results in all the techniques employed. Six patients also had negative Ole e 1 sIgE values in all methods, but 5 of them were positive to Ole e 9, profilin or pectin methylesterase.

The most frequent sensitization observed was the mayor allergen Ole e 1.

The sensitization to olive minor allergens obtained in our patients has been greater than the obtained in previous studies in the same geographical areas.

Conclusion: The capacity of detection of olive allergens sIgE by ISAC is similar to sIgE detection in ELISA for the main allergens Ole e 1, Ole e 7, Ole e 9, Ole e 11. Olive minor allergen can be useful to diagnose olive pollen allergy.

276
Evaluation of Pru p 3 and Pru p 7 specific IgE in Japanese peach allergic patients

Mori, Y[1,2]; Inuo, C[1,2]; Kondo, Y[1,2]; Fukuie, T[2]; Okazaki, F[2]; Nagao, M[2]; Yagami, A[2]; Matsunaga, K[2]; Narita, H[2]; Fujisawa, T[2]; Ito, K[2]
[1]Fujita Health University, Pediatrics, Aichi, Japan; [2]Fruits Allergy Component Study Group, Aichi, Japan

Rationale: It has been reported that severe symptoms in peach allergic patients are related not only to Lipid Transfer Protein (LTP, Pru p3) but also to Gibberellin-Regulated Protein (GRP, Pru p7). In this study, we evaluated the clinical utility of LTP and GRP specific IgE measurements in Japanese peach allergic patients.

Methods: We evaluated 22 peach-allergic patients (9 male and 13 female, aged 3–39 years [median age: 12.5]) registered in Fruits Allergy Component Study Group (http://fruit-allergy.jp/) and 4 non-allergic subjects. Clinical types of these 22 patients were oral allergy syndrome (10), FDEIA (5), anaphylaxis (7). Specific IgE levels in serum were determined for peach, apple, PR-10, LTP, profilin, white birch, Japanese alder, Japanese cedar, Bet v1, Bet v2 and CCD using IMMULITE® 2000 3gAllergy™ Specific IgE assay. The specific IgE measurement for GRP was performed using ELISA method and the optical density was determined at 450 nm.

Results: The positive ratios of LTP-specific IgE by 3gAllergy™ were 0/10 for oral allergy syndrome, 1/5 for FDEIA and 2/7 for anaphylaxis. The median values of GRP-specific IgE measurement by ELISA were 0.066 Abs. (0.011–0.081) for non-allergic subject, 0.063 Abs. (0.026–0.104) for oral allergy syndrome, 0.123 Abs. (0.037–0.197) for FDEIA, and 0.288 Abs. (0–0.529) for anaphylaxis.

Conclusion: In addition to LTP, GRP-specific IgE determination may also be utilized as an important marker for peach-allergic patients with severe symptoms.

277
Improved diagnosis of sesame allergy by IgE measurements for Ses i 1 evaluated through food challenge

Maruyama, N[1]; Nakagawa, T[2]; Ito, K[2]; Cabanos, C[1]; Borres, MP[3]; Movérare, R[3]; Tanaka, A[4]; Sato, S[5]; Ebisawa, M[5]
[1]Graduate School of Agriculture, Kyoto University, Uji, Japan; [2]Department of Allergy, Aichi Children's Health and Medical Center, Obu, Japan; [3]Thermo Fisher Scientific, Uppsala, Sweden; [4]Thermo Fisher Scientific, Tokyo, Japan; [5]Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, Sagamihara, Japan

Background: The number of reported instances of allergic reactions to sesame (*Sesamum indicum*) seeds has increased significantly. However, presence of specific IgE (sIgE) and positive skin prick tests of sesame crude extract could not sufficiently predict true sesame allergic patients diagnosed by oral food challenge (OFC), because of their low clinical specificity.

Objective: In this study, we aim to identify allergen components useful in diagnosing true sesame allergy.

Methods: Ninety-five sesame-sensitized children were enrolled in the study and divided into symptomatic and asymptomatic groups. Diagnostic values of a specific IgE antibody measurement to native storage protein components, 11S globulin (nSes i 11S), 7S globulin (nSes i 7S), 2S albumin (nSes i 2S) and recombinant 2S albumin isoforms (rSes i 1 and rSes i 2) were evaluated by fluorescent ELISA and/or the streptavidin ImmunoCAP method.

Results: Based on area under curve (AUC) values of receiver operating characteristic (ROC) analysis by fluorescent ELISA, rSes i 1 had the highest diagnostic values among those 5 components. rSes i 1 streptavidin ImmunoCAP had the larger AUC (0.872) compared with 0.683 from the commercially available sesame ImmunoCAP test. Clinical sensitivity and specificity were 82.1% and 85.7%, respectively, at optimal cutoff value (3.98 kUA/L).

Conclusions: Sensitization to Ses i 1 was strongly related to positive challenge outcome and allergic reactions to sesame. Measurements of sIgE level to Ses i 1 will be certainly useful to reduce numbers necessary for OFC.

278
Clinical relevance of measuring IgE to cat allergen components

Patelis, A[1]; Gunnbjornsdottir, M[1]; Alving, K[2]; Borres, MP[2,3]; Högman, M[1]; Janson, C[1]; Malinovschi, A[4]
[1]Medical Sciences: Respiratory, Allergy and Sleep Research, Uppsala University, Uppsala, Sweden; [2]Women's and Children's Health, Uppsala University, Uppsala, Sweden; [3]Thermo Fisher Scientific, Immunodiagnostics, Uppsala, Sweden; [4]Medical Sciences: Clinical Physiology, Uppsala University, Uppsala, Sweden

Background: The absence of IgE sensitisation to allergen components in the presence of sensitisation to the corresponding extract has been reported, but its clinical importance has not been studied. The aim of this study was to evaluate the clinical significance of IgE sensitisation to cat allergen extracts vs allergen components in relation to the ongoing and future of respiratory disease.

Methods: A total of 451 adults participated in the European Community Respiratory Health Survey (ECRHS) II and 302 of those in ECRHS III, 12 years later. IgE sensitisation to allergen extract and components(ImmunoCAP ISAC), exhaled nitric oxide (F_ENO) and bronchial responsiveness to methacholine were measured in ECRHS II. Rhinitis and asthma symptoms were questionnaire-assessed in both ECRHS II and III.

Results: Subjects sensitised to both cat extract and any component ($n = 61$) had higher F_ENO_{50} ($P = 0.008$) and more bronchial responsiveness ($P = 0.002$) than

© 2015 The Authors

subjects sensitised only to the extract ($n = 56$). Furthermore, subjects sensitised to both cat extract and any component were more likely to develop asthma ($P = 0.005$) and rhinitis ($P = 0.007$) than subjects sensitised only to cat extract. Looking at individual components, Fel d 1 and Fel d 2 were associated with bronchial responsiveness ($P = 0.001$ and $P = 0.02$ respectively) and F_ENO_{50} ($P = 0.002$ and $P < 0.001$ respectively) but not Fel d 4 ($P = 0.36$ for bronchial responsiveness and $P = 0.74$ for F_ENO_{50}).

Conclusion: IgE sensitisation to cat allergen components appear to have a higher clinical value than extract-based measurement as it related better to airway inflammation and responsiveness and had a higher prognostic value for the development of asthma and rhinitis over a 12-yearperiod. Furthermore, sensitisation to certain cat allergen components might have a higher clinical value, but this should be further studied studied in asthma populations.

279
Sensitization to Fel d 1 in childhood predicts symptoms of cat allergy in adolescence - a BAMSE/MeDALL study

Asarnoj, A[1,2]; Wadén, K[1]; Hamsten, C[1,3]; Andersson, N[4]; Kull, I[4]; Lupinek, C[5]; Curin, M[5]; Anto, J[6]; Bousquet, J[7]; Valenta, R[5]; Wickman, M[4,8,9]; van Hage, M[1]
[1]Karolinska Institutet, Department of Medicine Solna, Clinical Immunology and Allergy Unit, Stockholm, Sweden; [2]Karolinska University Hospital Solna, Astrid Lindgren Children's Hospital, Stockholm, Sweden; [3]Karolinska Institutet, Center for Inflammatory Diseases, Stockholm, Sweden; [4]Karolinska Institutet, Institute of Environmental Medicine, Stockholm, Sweden; [5]Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria; [6]Universitat Pompeu Fabra (UPF), Centre for Research in Environmental Epidemiology (CREAL), IMIM (Hospital del Mar Research Institute), Departament de Ciències Experimentals i de la Salut, CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain; [7]Hôpital Arnaud de Villeneuve, University Hospital of Montpellier, Montpellier, INSERM 1018, Villejuif, France; [8]Södersjukhuset, Sachs' Children's Hospital, Stockholm, Sweden; [9]Karolinska Institutet, Centre for Allergy Research, Stockholm, Sweden

Background: Sensitization to cat allergen molecules may contribute differently to development of cat allergy. The aim of the study was to investigate the association between sensitization patterns to cat allergen molecules during childhood and prediction of trajectories of symptoms to cat up to 16 years of age.

Method: Sera and questionnaire data from 779 randomly collected children from the BAMSE birth cohort at 4, 8 and 16 years were used. IgE to the cat allergen molecules Fel d 1, Fel d 2 and Fel d 4 were analyzed with an allergen chip based on ISAC technology (MeDALL chip). Allergy was defined as reported rhinitis, conjunctivitis or asthma at exposure to cat.

Results: Fel d 1 was the dominating cat allergen at all ages (8.7% at 4 years, 13.9% at 8 years and 20.3% at 16 years) and induced the highest median IgE levels, but with no significant increase over time (2.9–3.3 ISU-E at 4–16 years). Fel d 1 was the only cat allergen that induced significantly higher median IgE levels in children with symptoms to cat than in cat tolerant children at all ages (P-values < 0.001). Sensitization to Fel d 1 at 4–8 years of age was independently associated with symptoms to cat at 16 years (OR 13.7, 95% CI 8.3–22.7, adjusted for co-sensitization to other cat allergens). Sensitization to multiple cat allergens increased the likelihood of reporting cat symptoms longitudinally. However, positive predictive values for cat symptoms did not differ at any age between sensitization to Fel d 1 or cat extract.

Conclusion: IgE testing with just one cat allergen molecule, Fel d 1, is as good as testing IgE to cat allergen extract (ImmunoCAP) for prediction of cat allergy up to 16 years.

280
Physico-chemical and lipid-binding characterization of the house dust mite allergen Der p 13 produced in *Pichia pastoris*

Satitsuksanoa, P[1]; Nony, E[2]; Pulsawat, P[1]; Piboonpocanun, S[3]; Le Mignon, M[2]; Jain, K[2]; Kennedy, M[4]; Jacquet, A[1]
[1]Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [2]Stallergenes, Antony, France; [3]Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand; [4]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Scotland, United Kingdom

Background: Amongst the allergens identified from the house dust mite (HDM) *D. pteronyssinus*, Der p 13 remains poorly characterized. According to its IgE reactivity, Der p 13 may be considered as a minor HDM allergen but it could nevertheless be important in the initiation of the HDM allergic response. This possibility is supported by Der p 13's sequence similarities with fatty acid binding proteins including the *B. tropicalis* lipid-binding allergen Blo t 13, indicating that Der p 13 could transport fatty acids/lipids able to activate innate immunity.

Objective: In the present study, Der p 13 was expressed in *P. pastoris* as a secreted protein. Physico-chemical characterization of rDer p 13 was performed to validate its structural integrity and its lipid-binding propensity was investigated for the first time.

Methods: The cDNA encoding full-length Der p 13 was cloned into the *P. pastoris* pPICZa A vector, downstream of the sequence encoding yeast mating factor leader peptide. Selected recombinant KM-71 *P.pastoris* clones were cultured in shake flask and rDer p 13 expression was induced by addition of methanol. After purification by cation exchange chromatography and ultrafiltration, the structure of rDer p 13 was characterized by circular dichroism (CD) and its molecular mass and sequence by mass spectrometry (MS). Polyclonal antibodies to rDer p 13 were produced to detect the natural allergen in HDM extracts. Finally, fluorescence-based lipid binding assays were performed to determine any hydrophobic ligand-binding activities exhibited by rDer p 13.

Results: rDer p 13 was expressed and secreted from KM71 *P. pastoris* strain as a 15 kDa soluble protein. MS analysis identified rDer p 13 with 90% sequence coverage. The physico-chemical characterization of purified rDer p 13 showed that the allergen preparation was homogenous, and displayed predominately β-sheet secondary structure content. Polyclonal antibodies to rDer p 13 were able to detect natural Der p 13 in HDM extracts. rDer p 13 was able to bind the hydrophobic surface probe 8-Anilinonaphthalene-1-sulfonic acid (ANS), and the natural fluorescent fatty acid *cis*-parinaric acid.

Conclusion: Our results not only suggest that rDer p 13 produced in *P. pastoris* is correctly folded but also support the hypothesis that Der p 13 is a member of the cytosolic lipid binding protein family. Such a recombinant product could be useful for future innate immune activation assays and HDM allergy diagnosis.

281
Prevalence of specific IgE to natural shrimp extracts and the allergens rPen a 1, nPen m 2, rPen m 3, rPen m 4 and rPen m 6 in shrimp allergic subjects

Ehrenberg, A; Porsch, H; Pontoppidan, B; Elfström, L; Larsson, H; Mattsson, L; Lidholm, J
Thermo Fisher Scientific, Uppsala, Sweden

Background: Shrimp is a common cause of severe food allergic reactions worldwide. Tropomyosin was initially considered to be the only relevant allergen in shrimp but several other components have since been identified. The aim of this study was to examine IgE antibody responses to natural extracts of different shrimp species (*Penaeus monodon, Pandalus borealis, Metapenaeus joyneri and Metapenaeus barbata*)

© 2015 The Authors

and shrimp components among shrimp allergic patients.

Methods: Sera from 79 European, North American and Japanese subjects with a doctor's diagnosis of shrimp allergy were analyzed. Recombinant shrimp allergens Pen m 3 (myosin light chain 2), Pen m 4 (sarcoplasmic Ca-binding protein) and Pen m 6 (troponin C) from the giant tiger prawn (*P. monodon*) and Pen a 1 (tropomyosin) from the northern brown shrimp (*Penaeus aztecus*) were expressed in *E. coli* while Pen m 2 (arginine kinase) was purified from *P. monodon* extract. Quantitative determinations of specific IgE to extract from the four shrimp species and individual components were performed by ImmunoCAP. Responses of 0.35 kU_A/L or above were considered positive.

Results: All 79 subjects tested positive to shrimp extracts from *M. joyneri* and *M. barbata* while 80% tested positive for the two other species. The frequencies of sensitisation to rPen a 1, nPen m 2, rPen m 3, rPen m 4 and rPen m 6 were 33%, 29%, 18%, 9% and 20%, respectively. Large regional differences in frequency of sensitization were observed for some components. In the Japanese subjects, nPen m 2 was the most frequently recognised component (12/22 subjects, 55%), in contrast to only 5/30 (17%) among the South European subjects. Instead, the most prevalent component sensitizations among those subjects were to rPen a 1 (12/30, 40%) and rPen m 6 (10/30, 33%) while only 5 (23%) and 2 (9%) of the 22 Japanese subjects were sensitized to the same components. Among the North European subjects, sensitization to Pen m 2, Pen m 3 and Pen m 4 was present in 13–17% while IgE to Pen a 1 was present in 6/23 (26%). In a majority of subjects in this study, the combined component responses were comparable to or greater than that to *P. monodon* extract.

Conclusions: The IgE reactivity to shrimp allergen components is diverse and shows substantial regional differences. In a subset of subjects, the IgE response to *P. monodon* extract could not be accounted for by any of the components studied.

282
Relevance of response to recombinant allergens of Aspergillus fumigatus in patients with chronic or acute respiratory diseases

Barocci, F[1]; De Bernardi, G[2]; Lodato, A[1]
[1]Hospital Circle of Rho - Company Hospital G. Salvini Garbagnate Milanese, Immunotransfusion Center, Rho, Italy; [2]Hospital Santa Corona - Garbagnate Milanese, Respiratory Medicine, Garbagnate Milanese, Italy

Background: This study was conducted in adults hospitalized in the Department of Respiratory Medicine suffering from chronic obstructive pulmonary disease - COPD, from pneumonia, from severe chronic asthma, from allergic bronchopulmonary aspergillosis - ABPA, or from severe chronic asthma. Our objective was to improve the use of diagnostic serological for diagnosis of aspergillus allergy.

Method: We identified 30 patients that were positive by dosing for IgE antibodies to the native allergen of the A. fumigates with the test system ImmunoCap of Phadia S.r.l. of Thermo Fisher Scientific. All the different components available, Asp f 1, Asp f 2, Asp f 3, Asp f 4, Asp f 6, were evaluated in the patients positive to the allergen native of A. fumigatus without distinction of pathology. Some patients had allergic predisposition being positive to other allergens such as mold and mites.

Results: The results obtained show that only 5 patients were positive, from 0.70 to 0.79 KUA/l and only one with 31.1 KUA/l, to the native allergen of A. fumigates, but they were negative to all the recombinant. Among 25 patients positive to the native allergen of A. fumigates and Asp f 4 and/or Asp f 6, which were the first components on the market and used in routine, only 10 patients were positive. Asp f 1 was positive alone or with other components in 19 patients, it was negative in 6 patients. Asp f 2 and Asp f 3 often were booth positive coupled.

Conclusion: From these initial evaluations we can highlight the greater frequency of positivity of patients at component Asp f 1. The differents of the results may depend on the patient's pathology and/or their consequent different immunological response to the various molecular components. Without distinction to pathology the patients positive to the allergen native of A. fumigatus should be evaluated with all the recombinant available with the aim to exclude false positivity.

283
Allergenic members of the defensin-like protein family share structural features but show distinct immunological properties

Pablos, IM[1]; Eichhorn, SP[1]; Machado, YJ[1]; Briza, P[1]; Ebner, C[2]; Arora, N[3]; Vieths, S[4]; Gadermaier, G[1]; Ferreira, F[1]
[1]Molecular Biology, University of Salzburg, Salzburg, Austria; [2]Allergy Clinic Reumannplatz, Vienna, Austria; [3]CSIR-Institute of Genomic and Integrative Biology, Allergy and Immunology Section, Delhi, India; [4]Paul-Ehrlich-Institut, Division of Allergology, Langen, Germany

Background: Mugwort (Artemisia vulgaris), ragweed (Ambrosia artemisiifolia) and feverfew (Parthenium hysterophorus) belong to the Compositae family and pollen of these weeds are important allergy triggers. Art v 1, Amb a 4 and Par h 1 are allergenic defensin-like proteins from mugwort, ragweed and feverfew. While some studies are available for Art v 1 and Amb a 4, information on the major allergen of feverfew is limited. Thus, we aimed to clone and produce recombinant Par h 1 and study the structural and immunological features shared by allergens of this protein family.

Method: Total RNA was extracted from feverfew pollen and the full-length cDNA sequence was obtained using a degenerated primer followed by 5'-RACE protocol. The recombinant allergens Art v 1.0101, Amb a 4.0101 and *Par h 1* were expressed in *E. coli*. Structural characterization was performed by gel electrophoresis, mass spectrometry, dynamic light scattering and two different spectroscopy methods. For immunological studies the purified proteins were subjected to endolysosomal degradation, *in vitro* antigen uptake using murine BMDCs and IgE ELISA experiments with sera from Austrian patients allergic to mugwort pollen.

Results: The full-length cDNA sequence and the coding region including the signal peptide of *Par h 1* were identified. The mature protein of 128 amino acids consists of a defensin domain fused to a proline-rich region showing 53 and 69% sequence identity with Art v 1 and Amb a 4, respectively. Recombinant allergens were purified from *E. coli* and protein identities and disulfide bond patterns were confirmed by mass analysis. Circular dichroism and Fourier transform infrared spectroscopy showed that purified allergens share similar secondary structure elements suggesting similar folding. However, proteins showed different proteolytic stability to endolysosomal degradation. Additionally, they presented marked differences in the uptake kinetics by BMDC, thus suggesting differences in the immunological properties. Analyzed patients' sera displayed IgE binding to Art v 1, Amb a 4 and *Par h 1* and partial cross-reactivity was observed.

Conclusion: For the first time the full-length cDNA sequence of the major feverfew allergen, Par h 1 was identified. The recombinant allergens were characterized and will enable to study sensitization and IgE cross-reactivity in large patients' cohorts as well as *in vivo* studies using a mouse model.

Funded by ERA-Net New INDIGO project I 1152.

© 2015 The Authors

284
IgE reactivity of 27 kDa glycoprotein from the pupa of silkworm, *Bombyx mori*

Jeong, KY; Son, M; Lee, JY; Park, KH; Lee, J-H; Park, J-W
Yonsei University College of Medicine, Internal Medicine, Institute of Allergy, Seoul, Korea

Background: Boiled silkworm pupa is a traditional food in Asia, and silkworm pupa food allergy patients are common in these regions. Still now only one allergen from silkworm, arginine kinase, has been identified. The purpose of this study was to identify novel food allergens in silkworm pupa by analyzing a protein extract after heat treatment.
Methods: Heat treated extracts were examined by proteomic analysis. A 27-kDa glycoprotein was identified, expressed in *Escherichia coli*, and purified. IgE reactivity of the recombinant protein was investigated by ELISA.
Results: High molecular weight proteins (above 100 kDa) elicited increased IgE binding after heat treatment compared to before heat treatment. The molecular identities of these proteins, however, could not be determined. IgE reactivity toward a 27-kDa glycoprotein was also increased after heating the protein extract. Recombinant protein was recognized by IgE antibodies from allergic subjects (33.3%). Glycation or aggregation of protein produced by heating may create new IgE binding epitopes.
Conclusions: Sensitization to a 27-kDa glycoprotein from silkworm may contribute to elevation of IgE to silkworm. However, the role of the 27-kDa glycoprotein in silkworm allergy remains to be determined.

285
Retrospective review of the results of specific IgE to penicilloyl V, penicilloyl G and amoxicilloyl in patients with suspected penicillin allergy: the immunology laboratory perspective

Dziadzio, M[1]; Harvey, J[1]; Mai, A[1]; Sperlich, J[1]; Rotiroti, G[2]; Kariyawasam, H[2]; Chee, R[1]; Seneviratne, SL[1]; Lukawska, J[2]
[1]Royal Free Hospital NHS Foundation Trust, London, United Kingdom; [2]Royal National Throat Nose Ear Hospital, University College London, London, United Kingdom

Background: The prevalence of penicillin allergy in the general population is unknown but probably overestimated. Immediate reactions are usually IgE-mediated while non-immediate reactions are often induced by sensitized T cells. Skin tests and drug challenge are the gold standards for diagnosing penicillin allergy.

Positive specific IgE to penicilloyl V, penicilloyl G and amoxicilloyl is consistent with IgE sensitization to the major (but not minor) atigenic determinants of penicillin and/or amoxicillin, with potential cross-reactivity. Evidence shows that side-chain specific reactions have become more common and sIgE to minor determinants cannot be detected by the ImmunoCAP method. A recent study reported 8% prevalence of positive sIgE to beta-lactams among patients with self-reported history of penicillin allergy [1].
Method: In our immunology laboratory, we carried out a retrospective analysis of the results of sIgE to penicilloyls and amoxicilloyl obtained in all samples received over the period of 5 years. Specific IgE was measured by ImmunoCAP. Results were reported as negative if sIgE was < 0.35 kU/L.
Results: One-hundred-and ninety samples were received from children and adult patients. As requested by the clinicians, 179 samples were tested for penicilloyl V, 116 for penicilloyl G (115 for both), 76 samples for amoxicilloyl and 60 samples for all three antigens. Patients' age ranged from 4 months to 92 years (mean 30 years, median 32.5 years); 109 samples came from pediatrics. Only 8 samples (4.2%) (4 children, 4 adults) tested positive. 5/8 samples tested positive to amoxicilloyl, 6/8 to penicilloyl V, 3/8 to penicilloyl G; 3/8 samples were positive for all three allergens. One further sample was equivocal.
Conclusion: The prevalence of positive sIgE to penicilloyl V, penicilloyl G and amoxicilloyl in our cohort was 4.2%. This is lower than previously reported [1].

Our results are limited because of the lack of any follow-up data for our cohort. However, we have confirmed the low prevalence of positive samples in children and adults with a history of suspected penicillin allergy. The utility of serological screening for penicillin allergy detects only a small proportion of penicillin allergic patients, however, this low cost test allows approximately 1/25 patients in this cohort to avoid further drug allergy testing which is not risk-free. Skin tests and drug challenge remain the gold standard in diagnosing penicillin allergy.

1: PMID: 23599125

© 2015 The Authors

Poster Discussion Session PDS 6

Innate immune responses

286
Per a 10 potentiates Th2 polarization via OX40L upregulation and CD40 cleavage on dendritic cells

Agrawal, K; Kale, SL; Arora, N
CSIR-Institute of Genomics and Integrative Biology, Allergy and Immunology Section, New Delhi, India

Background: Serine protease allergen from *Periplaneta americana* leads to Th2 polarization in murine model of allergic airway inflammation. The aim of the present study was to gain insights by which proteases can cause Th2 polarization in murine model.
Method: Role of protease activity was studied in a mice model of allergic airway inflammation developed by intranasal administration of Per a 10 or heat inactivated Per a 10 (ΔPer a 10). Cytokine in BALF, IgE level in sera, cell surface marker expression and mRNA levels of cytokines in lungs were analysed. Bone marrow derived dendritic cells were generated from Balb/c mice and stimulated with Per a 10 or ΔPer a 10. Cell surface markers and cytokines in supernatant were analyzed by flow cytometry and ELISA respectively. Stimulated DCs were co-cultured with naïve T cells to assess cytokine profile.
Results: Per a 10 immunized mice had significantly higher levels of IL4, IgE and reduced IL12p70, whereas the response was attenuated in ΔPer a 10 immunized group. Per a 10 immunized mice had higher level of OX40L at both transcript and protein level. CD40 expression was reduced in the lungs of Per a 10 immunized mice, however, there was no change at transcript level. IL12p35 transcript level was lower in the lung section of Per a 10 immunized mice. Level of IL12p35 and IL12p40 was increased in ΔPer a 10 exposed mice as compared to Per a 10 immunized mice. Per a 10 immunized mice had higher level of IL23p19 mRNA. BMDCs pulsed with Per a 10 had reduced expression of CD40 and lower IL12p70 secretion as compared to ΔPer a 10. OX40L expression was higher in Per a 10 stimulated BMDCs. TNFα and IL6 levels were significantly higher in Per a 10 stimulated BMDCs supernatant as compared to ΔPer a 10. Per a 10 pulsed BMDCs had higher level of IL4 and IL13 in the supernatant of DC-T cell co-culture.
Conclusion: Protease activity of Per a 10 leads to Th2 polarization by CD40 cleavage, OX40L upregulation on dendritic cells and lower IL12p35 thereby limiting IL12p70 secretion.

287
Platelet activating factor contributes to vascular leak in acute dengue infection

Jeewandara, KC[1,2]; Gomes, L[1]; Gutowska-Owsiak, D[2]; Waithe, D[2]; Paranavitane, SA[1]; Shyamali, NA[3]; Ogg, GS[1,2]; Malavige, GN[1,2]
[1]Centre for Dengue Research, Faculty of Medical Sciences, University of Sri Jayawardanapura, Nugegoda, Sri Lanka; [2]MRC Human Immunology Unit, NIHR Biomedical Research Centre, Weatherall Institute of Molecular Medicine, Oxford, United Kingdom; [3]Department of Medicine, Faculty of Medical Sciences, University of Sri Jayawardanapura, Nugegoda, Sri Lanka

Objectives: Severe dengue infections are characterized by capillary leakage due to increased vascular permeability. The exact immunopathogenic mechanisms leading to increased vascular permeability are unknown. We report the use of a human umbilical vein endothelial cell (HUVEC) in-vitro model in evaluating the effect platelet activating factor (PAF) on microvascular permeability and its use in dengue.
Methods: PAF levels were initially assessed in 25 patients with acute dengue infection to determine if they were increased in acute dengue. Serial PAF values were assessed in 36 patients. The effect of the PAF on endothelial tight gap junction protein zona occludens 1(ZO-1) expression was assessed by using a PAF agonist PAF antagonists, dengue patient serum and rupatadine which is a selective long acting PAF receptor antagonist. We also assessed the effect of PAF,its antagonist and rupatadine on transendothelial electrical resistance (TEER).
Results: PAF levels were significantly higher in patients with acute dengue ($n = 25$; $P = 0.001$) when compared to healthy individuals ($n = 12$). In serial blood samples of patients ($n = 36$), PAF levels rose just before the onset of the critical phase. PAF levels were significantly higher in patients with evidence of vascular leak throughout the course of the illness when compared to those with milder disease. A dose dependant significant reduction in the ZO-1 expression was seen in the HUVEC monolayers with increasing levels of PAF from 100 ng/ml ($P = 0.04$) to 1000 ng/ml (0.001) and with dengue serum ($P = 0.01$). The effect of PAF and dengue serum on ZO-1 was partially reversed with PAFR antagonist with a maximum level of antagonism observed at 500 ng/ml. Serum from patients with dengue significantly downregulated expression of tight junction protein, ZO-1 ($P = 0.004$),in HUVECs. This was significantly inhibited ($P = 0.004$) by use of a PAF receptor (PAFR) blocker. Rupatidine significantly increased the expression of ZO-1 ($P = 0.03$) compared to dengue serum alone in HUVEC cells. Serum from dengue patients also significantly reduced TEER and this reduction was also significantly ($P = 0.02$) inhibited by prior incubation with the PAFR blocker and Rupatadine.
Conclusion: PAF is likely to be a cause of vascular leak in dengue infection. This *in vitro* model may provide insights in assessing novel safe and effective therapeutic targets in preventing vascular leak in severe dengue infection.

288
The C1Q regulatory dendritic cell marker is a potent inhibitor of allergic inflammation

Mascarell, L; Airouche, S; Jain, K; Nony, E; Baron-Bodo, V; Moingeon, P
Stallergenes SA, Antony, France

Background: Besides its well-known role in complement activation, C1Q was recently identified as a regulatory dendritic cell (DCreg) marker. Specifically, the increased expression of C1Q in peripheral blood mononuclear cells (PBMCs) from grass pollen allergic patients correlates with clinical improvement following allergen immunotherapy (AIT). Herein, we investigated the role and mode of action of C1Q in downregulating allergic inflammation.
Methods: Ovalbumine (OVA) or birch pollen (BP) sensitized BALB/c mice received an intraperitoneal administration of C1Q before each of four aerosol allergen challenges. As controls, OVA or BP-sensitized mice received either PBS, dexamethasone (DEX) or heat-denatured C1Q. Airway

© 2015 The Authors

hyperresponsiveness (AHR), inflammatory cell (*i.e.* eosinophils and type 2 innate lymphoid cells (ILC2s)) infiltrates in bronchoalveolar lavages (BALs), as well as Th2/Treg responses in the lungs were assessed to evaluate the efficacy of C1Q therapy. In selected experiments, mice were treated by a monoclonal antibody depleting plasmacytoid dendritic cells (pDCs) before allergen challenge. The impact of C1Q was also assessed *in vitro* on inflammatory cytokine production by serum free cultured human DCs including monocyte-derived DCs (MoDCs), myeloid DCs (mDCs) as well as plasmacytoid DCs.

Results: C1Q but not its heat-denatured form was as efficient as DEX to treat OVA-or BP-induced allergic asthma in mice, with a dramatic reduction in both AHR, eosinophil and ILC2 infiltrates in BALs as well as Th2 cytokine production (*i.e.* IL5 and IL13) by specific Th2 cells in the lungs. Despite this strong anti-Th2 activity, C1Q had no impact on levels of OVA-specific seric IgEs nor on frequencies of IL10/Foxp3$^+$ regulatory T cells in the lungs. Interestingly, depletion of pDCs in OVA sensitized mice abolished the capacity of C1Q to reduce AHR and eosinophil infiltration in BALs, demonstrating that pDCs are critically involved in the anti-inflammatory activity of C1Q. *In vitro* experiments in human models confirmed that C1Q significantly dampens pDC activation, whereas it has no impact on mDCs or MoDCs maturation.

Conclusion: We report on a strong inhibitory activity of C1Q against allergic inflammation, which involves pDCs. These findings esthablish that the C1Q molecule induced in regulatory DCs during AIT can mediate a direct anti-inflammatory activity independently of the induction of regulatory T cell responses.

290
Natural IgG, IgM and IgA anti-Gal antibodies in sera of healthy humans and patients with primary hypogammaglobulinemia

Chovancova, Z[1]; Hamanova, M[2]; Chmelikova, M[2]; Thon, V[1]; Lokaj, J[1]
[1]Faculty of Medicine, Masaryk University, St. Anne's University Hospital in Brno, Department of Clinical Immunology and Allergy, Brno, Czech Republic; [2]Faculty of Medicine, Masaryk University, Department of Clinical Immunology and Allergy, Brno, Czech Republic

Background: Naturally occurring antibodies to a variety of endogenous and exogenous antigens are commonly detected in healthy individuals. The target of the most abundant xenoreactive natural antibodies in humans is the alpha-Gal epitope (Galalpha1-3Galbeta1-4GlcNAc-R). These antibodies were formerly detected as agglutinins against rabbit red blood cells. They are continuously produced throughout the life, presumably as an immunological response to gut microbiota.

Method: Quantitative determination of IgM, IgG and IgA anti-Gal antibodies by newly designed enzyme immunoassay (Human Anti-Alpha-Galactosyl IgM, IgG, IgA ELISA, BioVendor, Brno, Czech Republic) in cord blood samples obtained from 41 newborns during delivery, from these infants aged 6, 12 and 24 months and from their mothers, in sera from 346 healthy adult blood donors and in sera from 33 patients with common variable immunodeficiency (CVID), 30 patients with selective IgA deficiency (SIgAD) and 11 patients with selective IgM deficiency (SIgMD) was performed.

Results: The kinetics of the natural antibody formation in the first two years of life in principle corresponds with the kinetics of total immunoglobulins in all isotypes. The level of natural antibodies in children at the age of two years compared with their mothers is approximately 37% for IgM, 25% for IgG and 15% for IgA. Anti-Gal in sera from healthy blood donors showed wide interindividual variability, titers of IgM are significantly higher in females compared to males, donors with type B blood group have significantly lower all anti-Gal isotypes compared to donors with type 0 or A. In sera from patients with CVID, significantly lower levels of anti-Gal IgM, IgG and IgA were observed. In patients with SIgAD, IgA anti-Gal were not detected, but no significant differences in the IgM and IgG anti-Gal antibodies were observed. In patients with SIgMD, IgM anti-Gal were not detected, but serum levels of IgG and IgA were significantly lower compared to control group.

Conclusion: Determination of anti-Gal antibodies may broaden the spectrum of immunologic assays and can be used in detailed analysis of the changes in antibody production in patients suffering from hypogammaglobulinemia.

291
Induction of tolerogenic dendritic cells by flagellin is associated with up-regulation of TLR5 in patients with allergic asthma

Koh, Y-I[1]; Shim, J-U[1]; Kim, C-W[2]; Rhee, J-H[3]
[1]Chonnam National University Medical School/Hospital, Internal Medicine, Gwangju, Korea; [2]Inha University School of Medicine, Internal Medicine, Incheon, Korea; [3]Chonnam National University Medical School, Microbiology, Gwangju, Korea

Background: Bacterial flagellin, which activates TLR5, has a strong immunomodulatory activity. We reported that flagellin may suppress allergen-stimulated Th1 and Th2 responses through the induction of IL10-producing dendritic cells (DCs) and regulatory T cells in asthmatics. Here, we investigated whether the induction of tolerogenic DCs can be accompanied by alteration of TLR5 expression on DCs from allergic asthma patients.

Method: Peripheral blood mononuclear cells were obtained from patients with house dust mite-sensitive asthma and normal controls. CD14 + monocyte-derived DCs were prepared. Following a flagellin (FlaB) stimulation, cytokines were measured with ELISA and the expressions of HLA-G and TLR5 were determined by the FACS analysis.

Results: We prevously showed that baseline IL-10 production from DCs was lower in asthmatics compared to normal and then was increased to normal range following the FlaB treatment. In parallel with IL-10 production, TGF-beta production was lower in asthmatics and was increased to normal following the FlaB treatment. In contrast, baseline production of IL-6, IL-12, and IL-1beta was higher in asthmatics compared to normal and then was reduced after the FlaB treatment. The treatment with FlaB, but not with LPS, increased the expression of HLA-G on DCs in asthmatics. In parallel with IL-10 and TGF-beta production, in asthmatics, TLR5 expression was lower compared to normal controls and was increased to the normal range following the FlaB treatment. In normal controls, TLR5 expression was not changed by the FlaB treatment.

Conclusion: The expression of TLR5 on DCs and the production of anti-inflammatory cytokines were reduced in patients with asthma. The flagellin treatment may up-regulate the expression of TLR5 and then induce tolerogenic DCs in patients with asthma.

292
Decreased YKL-40 expression in bronchial epithelial cells and asthma models by double stranded RNA

Kwon, J-W[1]; Hong, Y[2]; Yang, S-R[3]; Jeon, SG[4]; Kim, Y-K[5]
[1]Department of Allergy and Clinical Immunology, Kangwon National University, Chuncheon-Si, Gangwon-Do, Korea; [2]Department of Internal Medicine, Kangwon National University Hospital, Chuncheon-Si, Gangwon-Do, Korea; [3]Department of Thoracic and Cardiovascular Surgery, Kangwon National University Hospital, Chuncheon, Korea; [4]Department of Life Sciences, Pohang University of Science and Technology (POSTECH), Pohang, Korea; [5]Department of Medicine and Institute of Convergence Medicine, Ewha Womans Medical Center, Seoul, Korea

Background: YKL-40, a member of the human chitinase-like protein family, is a key regulator of allergic inflammation, and its role is well described in asthma. Viral

© 2015 The Authors

infection has been suspected to be related with beginning of asthma. This study was conducted to evaluate the expression level of YKL-40 in human bronchial epithelial cells by double stranded RNA (dsRNA) and the expression level of YKL-40 in various asthma models using dsRNA.

Method: YKL-40 expressions were evaluated in BEAS2B treated with various stimuli such as LPS, polyIC (synthetic dsRNA), and SO_2. Then mouse asthma models were created through simultaneous airway sensitization to an allergen (OVA), and low or high doses of dsRNA or alum and inflammatory cytokines and BRP-39 (which is homologous to human YKL-40) were evaluated.

Results: The expresssion of YKL-40 mRNA, intracellular YKL-40, and secreted YKL-40 in BEAS2B were all decreased by dsRNA. The level of BRP-39 mRNA expression in BAL cells was lower in Th2 neutrophilic inflammation model (OVA + low dose dsRNA) than Th2 eosinophilic inflammation model (OVA + Alum) and Th1 neutrophilic inflammation model (OVA + high dose dsRNA), though it was not significant.

Conclusion: YKL-40 expression in bronchial epithelial cells was decreased by dsRNA. Although further studies are needed, the expression of YKL-40 might be different among asthma endotypes related with dsRNA stimulation.

293
Factors associated with release of platelet activating factor from dengue virus infected monocytes

Kamaladasa, A[1]; Gomes, L[1]; Ogg, G[2]; Malavige, G[1,3]
[1]University of Sri Jayawardenapura, Nugegoda, Sri Lanka; [2]University of Oxford, Headington, United Kingdom; [3]University of Oxford, Oxford, United Kingdom

Objective: Our previous studies showed that platelet activating factor (PAF) was increased in patients with acute dengue infection, and associated with disease severity. Lipopolysaccaride (LPS) has also shown to be elevated in patients with acute dengue. Therefore, we set out to determine whether dengue virus (DENV) infection results in PAF production by primary monocytes and if this was increased by LPS.

Methods: Monocytes from four healthy individuals were isolated with CD14 beads using MACS separating columns. Monocytes were then infected at a multiplicity of infection of one with DENV-3 serotype and incubated for 96 h with and without LPS levels that were reported in sera of patients with acute dengue. Monocytes were also incubated with varying concentrations of dengue immune sera to determine the possible effects of antibody dependant enhancement in producing PAF. Two biological replicates were done for all experiments. PAF levels and cytokines were measured at 24, 48 h, 72 and 96 h post infection (PI) using luminex assays and quantitative ELISA. Quantitative real time PCR was done to determine the DENV-3 viral loads in the culture supernatants.

Results: The PAF levels were significantly higher ($P = 0.005$) in DENV infected monocytes, co-cultured with LPS, when compared to monocytes co-cultured with LPS alone, at 24 h. The PAF levels in DENV infected monocytes, which were co-cultured with LPS gradually decreased to the levels that were detected in monocytes cultured with LPS alone at 72 h. However, monocytes which were infected with the DENV in the absence of LPS or uninfected monocytes did not produce PAF. DENV-3 viral loads were similar in monocytes infected with the virus alone and in monocytes infected with the virus in the presence of LPS. DENV loads did not correlate with PAF levels in culture supernatants. We also found that dengue antibodies at different concentrations appear to increase PAF production by monocytes.

Conclusion: LPS and low concentrations of dengue antibodies appear to augment production of PAF by DENV infected monocytes, which was unlikely to be due to an increase in viral loads. Since the increase in PAF in DENV infected monocytes was significantly higher than monocytes cultured with LPS alone, it is likely that LPS potentiates the effects of DENV on production of PAF.

294
Clinical experiences with canakinumab as a treatment for autoinflammatory disorders

Saperia, C[1]; Mcauley, P[1]; Raffaghello, J[1]; Haque, SF[1]; Sussman, G[1,2]
[1]Gordon Sussman Clinical Research, Toronto, Canada; [2]University of Toronto Faculty of Medicine, Toronto, Canada

Background: Patients seeking clinical treatment for urticaria, while presenting with elevated inflammatory markers, were found to be suffering from autoinflammatory disorders, such as Cryopyrin Associated Periodic Syndromes (CAPS). Patients were screened for, and/or began treatment with canakinumab.

Method: 17 patients identified for investigation presented with life-long cases of severe chronic urticaria, along with periodic fever, fatigue, joint pain and partial hearing loss. Patients ranged from 14- to 65-years-old (mean age 39.5). All patients showed elevated inflammatory markers commonly seen in patients with autoinflammatory disorders, including Serum Amyloid A (SAA) and C-reactive protein (CRP). Of the patient cohort, 12 patients (70%) presented with Muckle-Wells syndrome. Two patients (12%) presented with Familial Cold Autoinflammatory Syndrome and a history of treatment with Rilanocept. Two patients (12%) presented with undiagnosed periodic fever syndromes. One patient (6%) presented with Familial Mediterranean Fever. Of the initial cohort, a subset of five patients (38%) demonstrated a confirmed R260W mutation at the NLRP3 gene and a clinical diagnosis of Muckle-Wells syndrome. These patients began treatment with canakinumab. They received a treatment of 150 mg via sub-cutaneous injection every eight weeks. Total injections to date for individual patients range from three to five. The remaining 12 patients are currently being investigated for treatment with canakinumab.

Results: All five patients treated with canakinumab experienced remission within one week of their first injection. Patients with SAA levels >16 000 ng/mL (normal range 1000–5000 ng/mL) prior to treatment showed normal SAA levels within one week of their first injection.

Conclusion: Canakinumab treatment for patients with Muckle-Wells syndrome was associated with complete spontaneous remissions within one week of commencing treatment. Patients presenting with chronic urticaria should be carefully assessed for disguised auto-inflammatory disorders, through genetic testing and examination for elevated inflammatory markers.

295
Colabomycin E downregulates proinflammatory cytokines and chemokines in human monocytes/macrophages

Striz, I[1]; Cecrdlova, E[1]; Petrickova, K[2]; Kolesar, L[1]; Pospisil, S[2]; Sekerkova, A[1]; Svachova, V[1]; Petricek, M[2]
[1]Institute for Clinical and Experimental Medicine, Department of Clinical and Transplant Immunology, Prague, Czech Republic; [2]Academy of Sciences of the Czech Republic, Institute of Microbiology, Prague, Czech Republic

Background: Some of the streptomycete-derived antibiotics including macrolides are characterized by additional anti-inflammatory and immunomodulatory effects but these properties cannot be widely used due to a risk of bacterial resistance. The aim of our study was to assess the effect of cola-

© 2015 The Authors

bomycin E, a new manumycin family metabolite*, on the mRNA expression and release of proinflammatory cytokines and chemokines from THP-1 monocyte/macrophage cell line.

Method: The THP-1 cells were cultured in RPMI1640 with 10% fetal calf serum and then stimulated with TNF-α (20 ng/ml) under serum free conditions in the presence or absence of colabomycin E isolated and purified from the natural producer Streptomyces aureus SOK1/5-04 by extraction of post-fermentation medias and mycelia with organic solvents and multiple subsequent liquid chromatography purification steps and crystallization. Quantitative RT-PCR (SABiosciences) was used for the evaluation of 84 different gene expressions in TNF-α and colabomycin E stimulated cultures and compared to unstimulated cells. The concentrations of cytokines and chemokines in THP-1 culture supernatants were measured by ELISA or Luminex.

Results: Colabomycin E inhibited TNF alpha induced mRNA expression of several genes associated with proinflammatory responses including IL-1β, IL-6, TLR8, and MyD88. The effect was evident after 4 h and 8 h cultures and in some of the genes persisted for 24 h. Furthermore, colabomycin E downregulated TNF alpha induced IL-1β, IL-6 and chemokine CXCL8/IL-8 release in a dose dependent manner from 0.25 μM concentration. The secretion of IL-18 from THP-1 cells was only slightly upregulated by TNF alpha and not affected by colabomycin E.

Conclusion: We assume from our study that colabomycin E is a potent inhibitor of proinflammatory cytokines in human macrophages. Some of the potentially proinflammatory genes are downregulated on the level of transcription.

*Petrickova K et al. ChemBioChem 15:1334-45, 2014

Supported by IGA MZCR grant NT/13012-4 and by by MH CZ - DRO (Institute for Clinical and Experimental Medicine - IKEM, IN 00023001)

296
Two siblings with IRAK-4 deficiency: a novel homozygous mutation with different clinical presentations

Gokturk, B[1]; Casanova, J-L[2,3]; Picard, C[2,3,4]; Ayvaz, DC[5]; Reisli, I[6]
[1]Department of Pediatric Allergy and Immunology, Konya Training and Research Hospital, Konya, Turkey; [2]Laboratory of Human Genetics of Infectious Diseases, Paris Descartes University, Imagine Institut, Necker Branch, INSERM UMR1163, Sorbonne Paris Cité, Paris, France; [3]St. Giles Laboratory of Human Genetics of Infectious Diseases, The Rockefeller University, Rockefeller Branch, New York, United States; [4]Assistance Publique - Hôpitaux de Paris (AP-HP), Necker-Enfant Malades Hospital, Study Center for Primary Immunodeficiencies, Paris, France; [5]Department of Pediatrics, Division of Pediatric Allergy and Immunology, Hacettepe University Ihsan Dogramacı Children's Hospital, Ankara, Turkey; [6]Department of Pediatrics, Division of Pediatric Allergy and Immunology, Necmettin Erbakan University Meram Medical Faculty, Konya, Turkey

Background: Interleukin-1 receptor-associated kinase-4 (IRAK-4) deficiency is a rare innate-immunity disorder associated with increased susceptibility to invasive infections caused by pyogenic bacteria. Here we report two siblings with a novel homozygous mutation in *IRAK4 gene*.

Case 1: A 4-month-old girl from a Turkish consanguineous family was referred due to fever and change in consciousness. She was diagnosed as meningitis caused by *Pseudomonas aeruginosa* and hospitalized for a month. We have identified a new homozygous missense mutation in exon 3 of *IRAK4 gene* (c.161G>A, p.R54K, at protein level). She has spasticity, mental-motor retardation, is on intravenous immunoglobulin (IVIgG) treatment and antibioprophylaxis without any infections since last 8 months.

Case 2: A 14-year-old boy, brother of case-1 was diagnosed during family-screening owing the same homozygous mutation. He had a history of recurrent skin abscesses after minor trauma since infancy. He was diagnosed as Non-Hodgkin lymphoma at 27 month-old. He had one invasive infection *(Staphylococcus aureus)* and several non invasive infections *(Salmonella C1, Enterococcus feacalis, Candida albicans)* before chemotherapy. He had a history of recurrent upper respiratory tract infections and long lasting cough which resolved after antibiotic treatments. He was diagnosed as epilepsy at age of 10 and was put on antiepileptic treatment for 3 years. Since that, difficulties in learning and behavioral problems were started.

Conclusion: IRAK-4 deficiency should be considered in patients with invasive Pseudomonas infections and recurrent skin abscesses. Family screening is important to diagnose new cases with immunodeficiency. Case 2 is the first reported case of IRAK-4 deficiency with NHL.

297
Eicosapentaenoic acid attenuated lipopolysaccharide-induced autophagy in rat alveolar macrophages

Kim, S-H[1]; Jung, N-H[1]; Lee, B-H[1]; Lee, J-H[1]; Jun, JH[2]; Choi, JW[2]; Shin, J[2]
[1]Eulji Hospital Eulji University School of Medicine, Internal Medicine, Seoul, Korea; [2]College of Health Science, Eulji University, Bio-Medical Laboratory Science, Suongnam, Korea

Background: The omega-3 poly unsaturated fatty acid (w3-PUFA) eicosapentaenoic acid (EPA) plays an important role in regulation of inflammation and the innate immune response. Lipopolysaccharide (LPS)-induced autophagy involves in lung homeostasis. The role of EPA in lipopolysaccharide (LPS)-induced autophagy in alveolar macrophages (AMs), however, has not been well studied. We investigate the suppressive effect of EPA in LPS-induced autophagy in AMs.

Method: For investigating the incidence of LPS-induced autophagy in AMs, cells from rat lungs can be subjected to the transmission electron microscopy (TEM) analysis and western blotting analysis with light chain 3II (LC-3II).

Results: LPS induced LC-3II expression in a dose- and time- dependent manner in AMs. TEM analysis showed that two kinds of autophagic vacuoles were observed in LPS-stimulated AMs. EPA suppressed LPS-induced LC-3II expression in alveolar macrophages. EPA blocks the formation of autophagosomes and autolysomes induced by LPS in AMs.

Conclusion: Our data suggest that inhibition of LPS-induced autophagy by EPA can be involved in its beneficial anti-inflammatory properties.

298
Regulatory role of eosinophils in the preventive effect of P28GST, a recombinant enzyme form the schistosome helminth in experimental colitis

Capron, ML; Driss, V; Sarazin, A; Gatault, S; Delbeke, M; Dubuquoy, L; Desreumaux, P
U995 Inserm Université de Lille, Faculté de Médecine Pôle Recherche, Lille, France

Background: Epidemiological studies have suggested inverse relationships between the prevalence of helminth infections and the incidence of inflammatory diseases in developing countries. These parasitic infections inducing both Th2-type and regulatory immune responses, it is therefore proposed that they might control, at least partly, inflammatory diseases mediated by Th1/Th17 immune response, such as Crohn's disease. The aim of our study was

© 2015 The Authors

to evaluate the immuno-modulatory effects of one schistosome enzyme, the 28-kDa glutathione S-transferase (P28GST) in experimental colitis in mice.

Methods: BALB/c mice were immunized with P28GST in the presence of adjuvant prior to the induction of colitis by intrarectal administration of TNBS. Animals were killed 2 days post-TNBS to assess inflammation markers and myeloperoxidase (MPO). Immunological markers in colonic tissues were analysed by qRT-PCR. Cytokine profiles of spleen cells after activation with P28GST were measured by multiplex. Eosinophil depletion was obtained by treatment with anti-Siglec F and by using IL-5 deficient mice.

Results: Preventive treatment with P28GST significantly decreased inflammation, as shown by significant reduction of clinical and histological scores observed in TNBS- induced colitis. A decrease of MPO protein, TNF and IL-1β mRNA was associated to a polarization of the immune system towards a Th2 profile with increase of IL-13, and IL-5, associated with a dense eosinophil infiltration in the colon of P28GST-treated animals. Depletion of eosinophils by treatment with anti-Siglec-F mAb, or use of IL-5 deficient mice suggested the participation of eosinophils in colitis prevention by P28GST.

Conclusion: Our data reveal that preventive treatment with P28GST, a well-characterized parasitic enzyme, ameliorates inflammation in experimental colitis. They suggest that eosinophils participate in the protective effect, underlying their regulatory role. This recombinant helminth molecule, ready to use in humans, may represent a new immuno-regulatory strategy in inflammatory bowel diseases.

Funding: ANR 2011-RPIB 021-01

© 2015 The Authors

Poster Discussion Session PDS 7

Food allergy: diagnosis

300
Utility of allergen-specific IgE probability curves for supporting the diagnosis of food allergy

Sato, S[1]; Ogura, K[1]; Sato, Y[2]; Takahashi, K[1]; Kutsuwada, K[1]; Ebisawa, M[1]
[1]Clinical Research Center for Allergy and Rheumatology, Kanagawa, Japan; [2]Department of Biostatistics, Clinical Research Center Chiba University, Chiba, Japan

Background: Allergen-specific IgE (sIgE) measurements are useful in supporting the diagnosis of food allergy (FA). The aim of this study was to evaluate the clinical utility of risk- based assessment of FA from probability curves calculated by sIgE measurements.
Method: Serum samples were collected from 2072 patients with suspected FA (age: 0–22 years; hen's egg (HE): $n = 436$, cow's milk (CM): $n = 499$, wheat (W): $n = 626$, peanut (P): $n = 351$ and soybean (SB): $n = 160$). Diagnosis of FA was confirmed by the positive oral food challenge (OFC) and convincing histories within 6 months of serum collection. The sIgE testing was performed using the IMMULITE 2000 3 gAllergy sIgE assay (3g). Serum samples were drawn within 6 months from the initial visit (HE, CM and W) or 6 months after OFC (P and SB). Predicted probability curves were plotted using the results from logistic regression analysis.
Results: Of 2072 patients, 239 patients were diagnosed with FA (HE: $n = 149$, CM: $n = 123$, W: $n = 83$, P: $n = 136$, SB: $n = 38$). In all allergen, the sIgE levels in patients with FA were significantly higher than in food-tolerant patients (egg white: 95.8 IU_A/mL vs 15.7 IU_A/mL, milk: 52.3 IU_A/mL vs 1.39 IU_A/mL, wheat: 29.5 IU_A/mL vs 1.2 IU_A/mL, peanut: 61.4 IU_A/mL vs 6.1 IU_A/mL, soybean: 24.1 IU_A/mL vs 9.6 IU_A/mL, $P < 0.0001$). The 95% PPV as determined by 3 g probability curves were obtained for CM (100 IU_A/mL), W (114.8 IU_A/mL) and P (174 IU_A/mL), but could not be calculated for EW and SB.
Conclusion: Although many studies reported the use of probability curves for predicting various FA based on ImmunoCAP sIgE assay results, measurements of sIgE using 3 g can also be utilized for the same purpose. However, these probability curves should not be applied interchangeably between different assays.

301
Utility of whey specific IgE levels in evaluating clinical tolerance of milk allergy

Kuzume, K; Kuwabara, Y; Koizumi, M; Okamoto, M; Nishimura, K
Ehime Graduated School of Medicine, Toon, Japan

Background: The purpose of this study was to evaluate the utility of whey specific IgE levels in predicting clinical tolerance in patients with milk allergy.
Method: Thirty-seven (37) patients with milk allergy (25 boys and 12 girls, age 14 months to 17 years, median 4 years old) were examined for whey specific IgE levels using the IMMULITE 2000 3gAllergy (3gAllergy). In addition, specific IgE for cow′s milk, casein, and beta-lactoglobulin (BLG) were measured by 3gAllergy and ImmunoCAP specific IgE assays. All patients had been undertaking oral immunotherapy with milk and were categorized into 3 groups according to how much milk the patient could tolerate, group A, less than 10 ml ($n = 11$), group B, 10 ml to 100 ml ($n = 14$), and group C, 100 ml or more ($n = 12$). Since all data did not follow the Gaussian distributions, they were shown in (median (range minimum - maximum) and were analyzed by Spearman′s method or one-way ANOVA with Dunn′s multiple comparisons.
Results: The levels of cow′s milk specific IgE using 3gAllergy were significantly correlated to the levels using ImmunoCAP ($r = 0.9046$, $P < 0.0001$). Likewise, the casein and BLG specific IgE levels showed similar correlations: $r = 0.9557$, $P < 0.001$ and $r = 0.9441$, $P < 0.0001$, respectively. The whey specific IgE levels using only 3gAllergy were significantly correlated between milk, casein, and BLG ($r = 0.8907$, $P < 0.0001$, $r = 0.8602$, $P < 0.0001$, $r = 0.7575$, $P < 0.0001$, respectively).

The whey specific IgE levels with 3gAllergy in group A (31.2 UA/ml, (1.86–406)) were significantly higher than those in group C (3.5 UA/ml, (range 0.193–76.7), $P < 0.05$), although there were no significance between levels in group A and in group B (7.275 UA/ml, (1.89–11.3)) and between levels in group B and in group C. Levels of milk, casein, and BLG showed similar results. However, the whey specific IgE - total IgE ratio in group A (0.0268, (0.0049–0.0974), as well as the ratio in group B (0.0107 (0.0015 - 0.0550) were significantly higher than in group C (0.0022 (0.0002–0.0091)($P < 0.001$, $P < 0.05$, respectively). Also, the specific IgE - total IgE ratio of milk, casein and BLG with 3gAllergy showed the same results of whey. On the other hand, milk, casein, and BLG data using ImmunoCAP were not clear compared to the results of 3gAllergy.
Conclusions: The whey specific IgE levels using 3gAllergy is useful to evaluate the clinical tolerance of milk allergy, especially when considered influence of total IgE levels.

302
Identification of IgE-binding protein profiles of sunflower seeds (*Helianthus annuus* L.). Assessment of the clinical relevance

Martín-Pedraza, L[1]; González, M[2]; Berzosa, I[1]; López-Rodríguez, JC[1]; San Segundo-Acosta, P[1]; Abián-Saz, S[1]; Barderas, R[1]; Batanero, E[1]; Blanca, M[2]; Rodríguez, R[1]; Mayorga, C[2]; Villalba, M[1]
[1]Facultad Ciencias Químicas, Universidad Complutense de Madrid, Dpto. Bioquímica y Biología Molecular I, Madrid, Spain; [2]Allergy Unit, IBIMA, Regional University Hospital of Malaga, UMA, Málaga, Spain

Background: Foods constitute major antigenic inducers of allergy, being vegetables, most specifically dried fruits, seeds and nuts, responsible for most cases of allergy after the 15 years old. The aim of this study is to deep into the allergy to sunflower seeds (*Helianthus annuus* L.) which can trigger anaphylactic reactions, generalized urticaria, angioedema and oral allergy symptoms after ingestion. Among the allergens described in this source, storage proteins and nsLTPs are the main inducers. We want to establish the specific allergenic pattern of IgE recognition in a population of patients allergic to sunflower seeds compared to that of individuals with specific IgE against this food but exhibiting no symptoms.
Method: Sera from patients allergic to sunflower seeds were used in this work. Proteins were purified by means of two

© 2015 The Authors

chromatographic steps from the protein extract of roasted sunflower seeds: a gel filtration chromatography and a reverse phase HPLC. Fractions were tested by means of dot blot and immunoblotting. PAGE-SDS separated bands were excised and digested with trypsin. Mass spectrometry and fingerprinting were carried out obtaining 4 peptides sequences. Amino acid sequence alignments were performed in protein databases based on EST data and Blast system for searching similarities.

Results: IgE reactive profiles obtained by immunoblotting of sunflower seed extract distinguish two populations of allergic patients as Group I with symptoms and Group II without symptoms after ingestion of sunflower seeds. A band of around 12 kDa was recognized by 12 out of 19 patients of Group I in immunoblots. In contrast, patients of Group II do not recognize such protein, showing most of them bands of high molecular mass. The protein was purified, eluting from the last HPLC-column in 41% of acetonitrile. The allergen shows hydrophobic nature and a basic pI comprised between 8 and 9. Finally, this allergen was identified by mass spectrometry and fingerprint analysis as a 2S albumin.

Conclusion: A protein associated to allergic symptoms in a population of patients analysed was identified as a 2S albumin, the SFA-8 protein already reported as a storage protein in sunflower seeds.

303
Lettuce allergy is a lipid transfer syndrome-related food allergy

Muñoz García, E[1]; Luengo Sánchez, O[1]; Moreno Pérez, N[1]; Pastor- Vargas, C[2]; Cardona Dahl, V[1]
[1]Hospital Vall d'Hebron, Sección Alergia, Barcelona, Spain; [2]ISS-FJD, Immunology, Madrid, Spain

Background: LTP sensitization is by far the most common cause of food allergy in the Mediterranean basin, with peach allergy as the primary sensitizer in most cases. While some patients remain allergic only to one plant-derived food others tend to spread their clinical recognition to a wide variety of non-related plant foods and pollens. The later has been termed "LTP syndrome" and is a consequence of the high degree of IgE cross-reactivity between LTPs. Lettuce allergy has been recently reported as a common offending food in patients affected of LTP syndrome. The aim of the study was to investigate the frequency of LTP syndrome in a sample of lettuce allergic patients.

Method: We retrospectively determined sIgE to Pru p3 (ImmunoCAP-ISAC™) in a sample of 42 patients with a diagnosis of lettuce allergy, established on the basis of a clinical history and positive skin prick test (SPT) and/or serum-specific IgE (sIgE). Sensitization to Lac s1 had been previously assessed by immunoblotting and mass spectrometry analysis. Symptoms with other LTP- containing plant-derived foods, and the presence of cofactors were recorded. LTP syndrome was considered when patients were sensitized to Pru p 3 and had symptoms with >2 non-related plant-foods.

Results: Serum for sIgE to Pru p3 measurement was available from 30 patients. Subjects were predominantly females (70%), with a median age of 30.5 years (range16–60). The clinical symptoms to lettuce were frequently severe, 21/30 patients experienced anaphylaxis. In 53.3% of the cases a cofactor was implicated in the anaphylactic reaction. Oral allergy syndrome and urticaria/angioedema were the referred symptoms in 20% and 10% of the patients respectively. sIgE to lettuce was positive in 27 patients, with low median levels (0.99 kU/l;range 0.11–5.97). Regarding LTP sensitization, Lac s1 was positive in 80% of the patients studied and sIgE to Pru p3 was positive in all but one of the studied patients. Most patients (90%) had a previous history of allergic reactions to other plant-foods. All but one were sensitized to peach. Sensitization to pollens was found in 93% of the patients; 76.7% to plane tree and 60% to mugwort.

Conclusion: In our population lettuce allergy is mostly due to sensitization to LTP and seems to be driven by peach allergy. In the majority of cases lettuce is not the first offending food but becomes part of the LTP syndrome. Cofactors should be considered when studying allergic reactions to lettuce.

304
Performance of molecular diagnostic techniques *in vivo* and *in vitro* in gliadin allergy

Pascal, M[1,2]; Sanchez-López, J[2,3]; Garcia-Moral, A[2,3]; Muñoz-Cano, R[2,3]; Valero, A[2,3]; Bartra, J[2,3]
[1]Immunology Department, CDB, Hospital Clinic de Barcelona, Barcelona, Spain; [2]IDIBAPS, Universitat de Barcelona, Barcelona, Spain; [3]Hospital Clinic de Barcelona, Allergy Unit, Pneumology Department, ICT, Barcelona, Spain

Background: Food allergy to gliadin is related to anaphylaxis and urticaria in the context of a cofactor, being suspected diagnosis difficult. Other allergens have been described with a similar clinical pattern. Commercial whole extracts of wheat lack gliadin fraction, thus they are not valid for diagnosis. We sought to analyze the ability to detect specific gliadin IgE *in vivo* (intraepidermal test) and *in vitro*.

Method: The results of the intraepidermal test (Leti), ImmunoCAP and ImmunoCAP ISAC (ThermoFisher Scientific) of 16 patients diagnosed of gliadin food allergy by unequivocal clinical history and w5-gliadin specific IgE positive by ImmunoCAP (ThermoFisher Scientific) or intraepidermal test were analyzed.

Results: Of the 16 patients studied (56% male, median and range of age: 53 [28–69] years), 13% had recurrent acute urticaria, 69% anaphylaxis and 13% anaphylactic shock. In all cases the presence of cofactor (13% NSAIDs, exercise 44% or 44% both) was observed. Ninety four of patients were positive by ImmunoCAP, 50% by intraepidermal test and 13% by ImmunoCAP ISAC.

Conclusion: The diagnostic tools available for routine diagnosis confirm the difficulty to detect gliadin sensitization. The IgE to w5-gliadin by ImmunoCAP has a better diagnostic yield in these patients. The multiplex study could be considered the technique of choice to identify the culprit allergen in patients with complex clinical patterns, however at the moment is not a reliable technique for the diagnosis of food allergy to gliadin.

305
Profiles in peach allergy. A clinical study

Carrapatoso, IP; Faria, EA; Pereira, AC; Regateiro, FS; Todo-Bom, AM
Imunoallergy Department, Coimbra University Hospital Centre, Coimbra, Portugal

Background: Peach allergy is the most frequent fruit allergy in Mediterranean countries. Besides Pru p 3, other peach-derived allergens might contribute to the different clinical profiles observed. The aim of this study was to characterize, over a one-year period, the clinical profiles observed in patients allergic to peach, followed in a Food Allergy outpatient consultation in Central Portugal.

Method: Thirty patients (21 females), mean age 33 ± 17 years, with immediate reactions to peach were studied. The clinical manifestations of peach allergy, symptoms after ingestion of other foods, and inhalant allergies were recorded. Past history of emigration was registered. Skin prick tests (SPT) to peach, to inhalant allergens (GA2LEN battery), and to Pru p 3 and profilin were performed. Serum specific IgE (sIgE) to peach, to Pru p 3, to Pru p 4 (if pollen sensitisation), and to Bet v 1 (if tree pollen sensitisation) were determined. SPT and sIgE to other foods and to inhalant allergens were performed according to case history.

Results: Sensitisation to Pru p 3 was detected in 19 patients, to Bet v 1 in 10,

© 2015 The Authors

and to profilin in 5. Combined sensitisation to Pru p 3 and profilin was found in 3 patients and to Pru p 3 and Bet v 1 in 1. Clinical manifestations in patients sensitised to Pru p 3 were: anaphylaxis in 10, oral allergy syndrome (OAS) in 7, urticaria in 1, and 1 patient had OAS and urticaria. All the patients sensitised to Bet v 1 referred OAS after ingestion of peach. Concerning the patients only sensitised to profilin the symptoms were OAS. All patients were sensitised to other *Rosaceae* fruits and/or to nuts and reported similar symptoms after their ingestion. Twenty-two patients were sensitised to pollens. The remaining 8 patients without pollen sensitisation were all sensitised to Pru p 3 and reported anaphylaxis or urticaria after peach ingestion. All the patients sensitised to Bet v 1, except one, had lived outside Portugal, namely in Northern Europe.

Conclusion: OAS was the most frequent clinical presentation. Anaphylaxis was only associated with Pru p 3 sensitisation. Two main peach sensitisation profiles were identified: patients with peach allergy without pollinosis with severe clinical symptoms, and patients with pollen-peach OAS. Anaphylaxis occurred in 4 patients sensitised to pollens and Pru p 3 indicating that this association is not always protective. Frequent emigration to Northern Europe probably influenced Bet v 1 sensitisation.

306
Allergens involved in peanut and tree nut allergy - experience of a center in the north of Portugal

Sousa, MJ; Brosseron, L; Ferreira, AR; Moreira Silva, JP
Centro Hospitalar Vila Nova de Gaia/Espinho, EPE, Allergy and Clinical Immunology Department, Vila Nova de Gaia, Portugal

Background: Allergy to peanuts and tree nut (TN) is the leading cause of fatal allergic reactions in the United States (US). In Portugal, peanut and TN allergy prevalence is unknown. In the US, Ara h 1 to Ara h 3 are the most frequent allergens involved in peanut allergy (PA); Pru p 3 has been suggested as the primary sensitizer in patients with PA in Spain. The aim of this study was to evaluate the allergens involved in peanut and TN allergy in our Allergy Clinic.

Methods: Retrospective study of peanut and/or TN allergic patients referred to our Food Allergy Unit. Data regarding demography, atopy and suspect food were collected. Skin prick tests with commercial extracts [peanut, walnut, almond, hazelnut, pine nut, pistachio, cashew, soy, birch, non-specific Lipid Transfer Protein (nsLTP) and Profilin] and prick-to-prick tests with the suspect foods were performed. Specific IgE (sIgE) to the suspected food, rPru p 3, rCor a 8, rBet v 1, rPhl p 12, rAra h 1, rAra h 2 and rAra h 3 were determined by ImmunoCAP (Thermofisher®).

Results: Fourteen patients with peanut and/or TN allergy were included. Nine male, mean age 29.29 [±12.07]; all atopic. In 5 (35.7%) patients, ingestion of fresh fruit was responsible for the first clinical manifestations of food allergy, namely peach ($n = 2$), apple ($n = 2$) and cherries ($n = 1$). The remaining patients presented an allergic reaction to TN and/or peanut as the first food allergy episode. Six (42.8%) reported anaphylaxis. Ten (71.4%) patients were sensitized to Pru p 3 [1.31–19.7 kUA/L] and 8 (57.1%) to Cor a 8 [0.84–9.28 kUA/L], all also sensitized to Pru p 3. Five (35.7%) were sensitized to Phl p 12 [0.42–1.36 kUA/L] and 3 (21.4%) to Bet v 1 [2.33–3.25 kUA/L]. Two patients (14.2%) were sensitized to Ara h 2 [1.00–1.87 kUA/L] and 1 patient to Ara h 3 (0.36 kUA/L). Ten of the 12 patients with peanut and TN allergy were sensitized to Pru p 3; in 6, allergic reaction to peanut and/or TN was the presenting food allergy. The 2 patients with symptoms exclusively with peanut were sensitized to Ara h 2.

Conclusion: In our population, nsLTPs (Pru p 3, Cor a 8) were the primary sensitizer in patients with peanut and TN allergy. Our data suggest that reactions to both peanut and TN are associated with Pru p 3 allergy, so testing for nsLTP allergy is recommended in those patients. Sensitization to Ara h 2 was exclusively related to peanut allergy.

307
The epitopes that cause cross-reactions between peanuts and tree nuts

Maleki, SJ[1]; Teuber, SS[2]; Bublin, M[3]; Chruszcz, M[4]; Grimm, CC[5]; Cheng, H[5]; Hurlburt, BK[5]; Breiteneder, H[3]; Schein, C[6]
[1]Food Allergy, US Department of Agriculture, New Orleans, United States; [2]UC Davis School of Medicine, Davis, United States; [3]Medical University of Vienna, Vienna, Austria; [4]University of South Carolina, South Carolina, United States; [5]Food Allergy, US Department of Agriculture-Agricultural Research Center, New Orleans, United States; [6]Foundation for Applied Molecular Evolution, Gainsville, United States

Background: Many peanut allergic individuals also have allergies to tree nuts. Our previous work has shown that there are epitopes with different amino acid sequences, but similar physical and chemical properties are recognized by the same IgE molecule.

Method: Anti-Ara h 2 monoclonal antibodies were produced. They were epitope mapped and the binding sites shown by molecular modeling. Western blots were used to test the binding of these monoclonals to almond, cashew, peanut, pistachio, soy, green pea and walnut extracts and to purified Jug r 2 leader sequence, Jug r 1 and Ara h 2 and Ara h 6. Proteins in the reactive bands were identified by mass spectrometry. These monoclonal antibodies were tested for their ability to compete with IgE for binding to Ara h 2 by ELISA and histamine release assays.

Results: Searching the Structural Database for Allergic Proteins (SDAP) and empirical determination of the cross-reactive allergens in different nuts, revealed many potential IgE epitopes with similar physicochemical properties in nut allergens. Specific, anti-Ara h 2 monoclonal antibodies, made against surface exposed areas of native Ara h 2 recognized vicillins, conglutinins, and glycinins in multiple nuts. Four of the monoclonals were highly reactive with Jug r 2 leader sequence, and all recognized Jug r 1. Most of the monoclonals competed for IgE binding to Ara h 2 and prevented histamine release by Ara h 2 to different extents.

Conclusion: The presence of highly cross reactive, repeat peptide motifs is confirmed here and we produced monoclonal antibodies that inhibit IgE binding to these sequences.

308
The comparison of basophil activation test with other diagnostic tests to diagnose milk-allergy in children

Marseglia, A[1]; De Amici, M[1]; Caimmi, S[1]; Licari, A[1]; Trovamala, V[1]; Rundo, B[1]; Torre, C[1]; Nigrisoli, S[1]; Caimmi, D[2]; Marseglia, GL[1]
[1]Department of Pediatrics, Foundation IRCCS Policlinico San Matteo, Pavia, Italy; [2]CHRU de Montpellier, Allergy Unit and Cystic fibrosis Center, Montpellier, France

Background: Milk allergy is very common in children and its correct diagnosis is important to prevent reactions. Skin prick test (SPT) and specific IgE (sIgE) are very useful to diagnose milk allergy but limited in their predictive accuracy. When cases are not clear, oral food challenge (OFC) is requested even if it could represent a risk for children. The Basophil Activation Test (BAT) could represent a safer method that could be used instead of oral food challenges. Our aim was to compare performance of BAT with SPT and sIgE in the correct prediction of milk allergy.

Method: Milk-allergic ($n = 5$) and milk-sensitized but tolerant ($n = 36$) children underwent skin prick test (SPT) and specific IgE (sIgE) to milk have been evaluated for the analysis. The basophil activation test (BAT) has been performed by flow cytometry, and its diagnostic performance was evaluated in relation to allergy vs tol-

© 2015 The Authors

erance to milk. Four children have been excluded from the analysis because BAT resulted as "no responder". Statistical analysis was performed using the statistical software package Medcalc 9 (Frank Schoonjans, BE). A p-value ≤ 0.05 was considered statistically significant.

Results: The comparison between SPT, sIgE and BAT concerns the number of correct diagnosis of the tests. SPT and sIgE correct diagnosed in 16 cases (39%) while BAT in 30 cases (73%). Also the number of false positive is less for BAT: the wrong diagnosis resulted in 4 cases (9.76%) while for SPT in 11 cases (26.8%) and 22 cases (53.7%) for sIgE. The number of false negative is less for SPT that includes 2 cases (4.88%) while the wrong diagnosis of false negative resulted in 3 cases (7.3%) both for BAT and sIgE.

Conclusion: Our preliminary analysis shows that the basophil activation test correctly diagnosed milk-allergy in a good rate of cases. It seems to be a non-invasive and safe methods, alternative to oral food challenge, to confirm milk-allergy in children.

309
The use of basophil activation test to diagnose egg-allergy in children

Licari, A[1]; De Amici, M[1]; Marseglia, A[1]; Caimmi, S[1]; Trovamala, V[1]; Castagnoli, R[1]; Torre, C[1]; Nigrisoli, S[1]; Testa, G[1]; Caimmi, D[2]; Marseglia, GL[1]
[1]Department of Pediatrics, Foundation IRCCS Policlinico San Matteo, Pavia, Italy; [2]CHRU de Montpellier, Allergy Unit and Cystic fibrosis Center, Montpellier, France

Background: The oral food challenge (OFC) represents the current gold standard to diagnose food allergy even if this test is laborious and not without risk. Thus a safer method that could accurately diagnose food allergy, reducing the need of OFC, is desirable. Our aim was to assess the performance of basophil activation test (BAT) as diagnostic marker for egg-allergy, comparing it with skin prick test and specific IgE.

Method: Egg-allergic ($n = 8$) and egg-sensitized but tolerant ($n = 38$) children underwent skin prick test (SPT) and specific IgE (sIgE) to egg white and egg yolk have been evaluated for the analysis. The basophil activation test (BAT) has been performed by flow cytometry, and its diagnostic performance was evaluated in relation to allergy vs tolerance to egg. One children has been excluded from the analysis because BAT resulted as "no responder". Statistical analysis was performed using the statistical software package Medcalc 9 (Frank Schoonjans, BE). A p-value ≤ 0.05 was considered statistically significant.

Results: The difference between allergic and sensitized children has been evaluated for egg white and egg yolk, considering the three allergy tests. For SPT there was not a significant difference between the two groups, both for egg white ($P = 0.1905$) and for egg yolk ($P = 0.0734$). In allergic children, sIgE values were higher than in sensitized ones, both for egg white ($P = 0.019$) and egg yolk ($P = 0.0209$); also basophils showed increased expression of CD63 in allergic children and the difference with sensitized children was statistically significant for egg white ($P = 0.0025$) and for egg yolk ($P = 0.0018$). Egg allergy and tolerance status, based on OFCs, were the reference points to evaluate the diagnostic performance of BAT on ROC-curve analysis. The analysis showed the best cut-off at 26.5 (area under the curve = 0.845; sensitivity = 75; specificity = 89.19, $P = 0.0001$) for egg white and at 6.51 (area under the curve = 0.855; sensitivity = 100; specificity = 64.9, $P = 0.0001$) for egg yolk. A comparison for the best cut off between allergy tests has been performed. The area under the ROC curve for BAT was superior to that for other allergy tests.

Conclusion: The basophil activation test seems to be a non-invasive and safe methods, alternative to oral food challenge, to confirm egg-allergy in children.

310
SPINK5 variants increase risk of food allergy in a paediatric cohort

Ashley, S[1,2]; Martino, D[1]; Tan, T[3]; Martin, P[1]; Koplin, J[1]; Peters, R[1]; Dharmage, S[4]; Saffery, R[1]; Ellis, J[1]; Allen, K[1]; HealthNuts Team
[1]Murdoch Childrens Research Institute, Parkville, Australia; [2]Monash Institute of Medical Research-Prince Henry's Institute of Medical Research, Monash University, Clayton, Australia; [3]Department of Immunology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Malaysia; [4]Centre for Epidemiology and Biostatistics, The University of Melbourne, Parkville, Australia

Background: Increasing evidence suggests reduced skin barrier permeability may predispose to food allergy, via transcutaneous sensitization. The aim of this study was to test whether single nucleotide polymorphisms (SNPs) in and around *SPINK5*, a gene implicated in the maintenance of skin barrier function, were associated with IgE-mediated food allergy in infants. We used a tag-SNP approach to test for associations between *SPINK5* variants, challenge-proven food allergy and trans-epidermal water loss (TEWL) measures. We hypothesized that mutations in and around *SPINK5* were associated with higher TEWL measures and food allergy in early childhood.

Method: 57 SNPs, tagging variation ($r^2 \geq 0.8$) across a 263 kb genomic region incorporating *SPINK5* were successfully genotyped in 721 individuals drawn from a cohort of 12-month old infants phenotyped for clinical food allergy using skin prick testing and oral food challenges (the HealthNuts study). TEWL was measured using the Tewameter® TM300, recognised as the most accepted measure of TEWL. Data were analysed using multinomial logistic regression and ANOVA under three different models

clinical food allergy ($n = 372$) vs non-atopic controls ($n = 122$); clinical food allergy vs food sensitized but tolerant individuals ($n = 247$); food sensitized v non-atopic controls. Adjustments were made for ethnicity and current eczema.

Results: Variants rs1432975 & rs17641748, both downstream of *SPINK5* were associated with an increased risk of clinical food allergy (OR = 1.51, 95% CI = 1.035–2.20, $P = 0.033$; OR = 1.47, 95% CI = 1.032–2.088, $P = 0.033$) among those who are sensitized. In individuals genotyped for these food allergy associated SNPs we observed significantly higher TEWL readings ($P = 0.0055$ & $P = 0.0320$) in those with the minor allele homozygous genotype compared to those with wild type genotype.

Conclusion: Our data suggest that *SPINK5* variants may adversely affect skin barrier integrity and the risk for food allergy. This is concordant with emerging data that suggests sensitization to food allergens via the cutaneous route predisposes to food allergy. Preserving the integrity of the skin barrier in individuals with these mutations may be a logical preventative strategy.

© 2015 The Authors

Poster Discussion Session PDS 8

Atopic dermatitis and other skin conditions

311
Dairy and non-dairy beverage consumption for childhood atopic dermatitis

Hon, KL[1]; Poon, CWT[2]; Tsang, YC[1]; Pong, NH[1]; Chow, CM[1]; Leung, TF[1]
[1]Department of Paediatrics, The Chinese University of Hong Kong, Hong Kong, China; [2]Faculty of Health Sciences, The University of Macau, Macau, China

Background: Many parents practice empirical dietary avoidance and supplementation, and seek healthcare advice if consumption of dairy and non-dairy beverages may be beneficial or detrimental in childhood atopic dermatitis (AD). We investigated if frequency of consumption of different beverages were associated with disease severity and quality of life of pediatric AD patients.
Method: Parent-reported weekly and daily frequency of different beverages consumption were recorded in pediatric consecutive AD patients, and disease severity (evaluated by Nottingham Eczema Severity Score, NESS), Children Dermatology Life Quality Index (CDLQI), skin hydration (SH), transepidermal water loss (TEWL), blood pressures, resting heart rate, and body mass index were evaluated.
Results: AD was associated with worse quality of life than miscellaneous non-eczema skin diseases ($P < 0.001$). Fewer AD patients drank fresh milk (trend, $P = 0.062$) but more drank miscellaneous beverages (such as Chinese herbal tea and soymilk, $P = 0.030$) when compared with non-eczema patients. In AD, NESS correlated with CDLQI (rho = 0.66, $P < 0.001$), and reduced SH (rho = -0.32, $P < 0.001$), whereas CDLQI correlated with a higher resting pulse rate (rho = 0.25, $P = 0.003$). Multiple logistic regression showed male (odds ratio: 0.44, 95% CI: 0.20–0.97; $P = 0.042$) and fresh milk drinking (odds ratio: 0.42, 95% CI: 0.20–0.93; $P = 0.031$) were independent factors associated with less severe disease. Moderate-to-severe impairment of CDLQI was associated with NESS (odds ratio: 1.48, 95% CI: 1.28-1.71; $P < 0.001$) and pulse rates (odds ratio: 1.05, 95% CI: 1.02–1.08; $P = 0.002$) but not with reported habits of beverages consumption. Concerning cardiovascular health in AD patients, frequency of formula milk consumption was associated with pulse rate (rho = 0.17, $P = 0.044$), and soft drink consumption was associated with higher systolic pressure (rho = 0.18, $P = 0.044$).
Conclusion: This study is important in providing evidence for parental/patient guidance. Pediatric AD patients who reported more fresh milk consumption had less severe disease. There was no correlation between consumption of non-dairy beverages with disease severity or quality of life, but frequency of soft drink consumption correlated with systolic pressure. It seems reasonable to advice children to drink fresh milk instead of other beverages. Soft drinks should be consumed in moderation for optimal cardiovascular and metabolic health.

312
Fullerene C60 reduces the allergic inflammation in AD mouse model

Shershakova, N; Bashkatova, E; Purgina, D; Kamishnikov, O; Andreev, S; Khaitov, M
NRC Institute of Immunology FMBA of Russia, Moscow, Russian Federation

Background: Atopic dermatitis (AD) is an allergic disorder of the skin. Fullerene C60 has the unique electronic properties making it an attractive candidate for diagnostic, therapeutic and theranostic applications. The main purpose of our research was to assess of fullerene C60 therapeutic effect in a mouse model of AD.
Method: New efficient method for producing a water-soluble fullerene C_{60} has been developed. AD experimental model was induced by the epicutaneous sensitization of BALB/c mice with ovalbumin (OVA). Fullerene C60 was administrated epicutaneously (EC) and subcutaneously (SC). OVA-specific antibodies were assessed by ELISA. Splenocytes cytokine production upon OVA *in vitro* stimulation was detected by ELISA. Specimens of skin from OVA patch areas were removed for histological examination immediately after the last EC application with allergen.
Results: It was shown that OVA-specific IL-4 and IL-5 levels were significant decreased in groups treated with water-soluble fullerene C60. The OVA-specific IL-12 level was significantly higher in EC C60-treated group. The ratio of OVA-specific IgG1/IgG2a was decreased in the same group. The histologic analysis of skin samples from the sites of allergen application showed that C60-therapy by both EC application and SC administration improved the histologic picture.
Conclusion: Taken together, these results demonstrate that the water-soluble fullerene C60 exhibits a significant anti-inflammatory effect in a mouse model of AD, especially via EC application, and possesses a high therapeutic potential.

313
Water-soluble forms of fullerene C_{60} as a promising drug for a treatment of allergic inflammation

Purgina, D; Bashkatova, E; Shershakova, N; Khaitov, M; Andreev, S
NRC Institute of Immunology FMBA, Moscow, Russian Federation

Background: Fullerene C_{60} can be considered as a perspective scaffold for drug design since it possesses a uniquely structure, chemical properties and good cell-penetrating ability. The main problem associated with its use is the lack of water solubility. Previously, we have founded that a number of amino acid derivatives of fullerene attenuate inflammatory DTH reaction in mouse model. An addition, it was shown an unmodified fullerene attenuates IgE-mediated allergic reactions including anaphylactic manifestations. The objective of this work was to provide a facile and biocompatible method for producing of non-toxic aqueous fullerene dispersion (nC_{60}) applicable for a treatment of allergic diseases (as allergy, atopic dermatitis).
Method: The aqueous dispersions of fullerene (nC_{60}) were prepared by simple mixing the C_{60} solution in low toxic N-methylpyrrolidone (NMP) with aqueous solution of natural amino acid as stabilizing agent followed by exhaustive dialysis [1]. The nC_{60} obtained was thoroughly characterized by physico-chemical methods. Effects of the nC_{60} were investigated in a mouse model of atopic dermatitis induced by epicutaneous sensitization of

© 2015 The Authors

BALB/c mice with ovalbumin, where nC_{60} samples administrated epicutaneously and subcutaneously.

Results: Obtained nC_{60} are clear solutions of red-brown color with a pH 5.8–6.8, and concentration of fullerene in nC_{60} are about 100 mg/L, however the dispersion may be concentrated to 800–1000 mg/L without changing of its characteristics. Particle size measured by DLS method appeared to average between 100 and 120 nm with polydispersity index (PI) of 0.17–0.37. Experiment on a mouse model of atopic dermatitis showed that the nC_{60} was able to reduce a production of IL-4 and IL-5 cytokines and to increase the IL-12 level.

Conclusion: This work suggests a new attainable and viable approach to produce stable aqueous fullerene solutions without the need for ultrasonic treatment and suitable for biomedical applications, including a treatment of allergic diseases.

[1] Andreev S., Purgina D., Bashkanova B., Garshev A., Maerle A., Andreev A., Osipova N., Shershakova N., Khaitov M. Study of fullerene aqueous dispersion prepared by novel dialysis method. Simple way to fullerene aqueous solution. Fullerenes Nanotubes and Carbon Nanostructures, 2015, DOI:10.1080/1536383X.2014.998758.

314
Lateral upper arm as measuring site for transepidermal water loss in infants

Berents, TL[1,2]; Carlsen, KCL[1,3]; Mowinckel, P[1,3]; Skjerven, HO[1,3]; Kvenshagen, B[4]; Rolfsjord, LB[1,5]; Carlsen, K-H[1,3]; Gjersvik, P[1,2]
[1]Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [2]Department of Dermatology, Oslo University Hospital, Oslo, Norway; [3]Department of Paediatrics, Oslo University Hospital, Oslo, Norway; [4]Department of Paediatrics, Østfold Hospital, Fredrikstad, Norway; [5]Department of Paediatrics, Innlandet Hospital, Elverum, Norway

Background: Skin barrier function may be assessed by measuring transepidermal water loss (TEWL) on the volar forearm. In infants, atopic eczema (AE) is often located on the lateral upper arm, possibly being the first detection site for increased TEWL typical for the disease. This site is easily accessible and not affected by excess heat or humidity caused by the infant flexing its lower arm.

Objectives: To compare TEWL measurements on the lateral upper arm and the volar forearm in infants.

Methods: In 167 infants, recruited from a general population in south-east Norway, TEWL measurements were performed on the lateral upper arm and volar forearm, using a DermaLab USB®, under defined humidity and temperature. The mean of three measurements from each site was used in the analysis. The infants were diagnosed with either no eczema ($n = 110$), possible AE ($n = 28$) or AE ($n = 29$).

Results: In infants with no eczema, mean TEWL was 7.7 g/m^2 h^{-1} (95% CI 7.1, 8.4) on lateral upper arm and 9.3 (8.4, 10.2) on the volar forearm. In infants with AE, mean TEWL was 11.0 g/m^2 h^{-1} (9.7, 12.4) on the lateral upper arm and 12.8 (11.1, 14.6) on the volar forearm. Mean TEWL was significantly higher in infants with AE compared to possible AE and no eczema, both on the lateral upper arm ($P < 0.001$) and the volar forearm ($P = 0.02$).

Conclusion: Although TEWL measurements on the lateral upper arm may differ from those on volar forearm, both sites appear appropriate in assessing TEWL in infants with and without eczema.

315
The plasma gelsolin levels in atopic dermatitis: effect of atopy and disease severity

Eke Gungor, H[1]; Sahiner, UM[1]; Karakukcu, C[2]; Sahiner, N[3]; Altuner Torun, Y[4]; Patiroglu, T[5]
[1]Department of Pediatrics, Pediatric Allergy and Immunology Unit, Kayseri Education and Research Hospital, Kayseri, Turkey; [2]Department of Biochemistry, Kayseri Education and Research Hospital, Kayseri, Turkey; [3]Department of Dermatology, Kayseri Education and Research Hospital, Kayseri, Turkey; [4]Department of Pediatrics, Pediatric Hematology Unit, Kayseri Education and Research Hospital, Kayseri, Turkey; [5]Medical Faculty, Research Hospital, Department of Pediatrics, Pediatric Immunology Unit, Erciyes University, Kayseri, Turkey

Background: Gelsolin is an actin-binding protein with several cellular functions including apoptosis and is reported to have an anti-inflammatory effect. We aimed to determine plasma gelsolin (pGSN) levels in children with atopic dermatitis (AD).

Method: The diagnosis of AD was made according to Hanifin and Rajka criteria. The disease severity was scored by objective SCORAD index by the same allergist. Skin prick testing (SPT), total IgE, eosinophil counts were analyzed. The pGSN levels were determined using ELISA technique.

Results: Children aged between 0.5 to 3.0 years were included to the study. The children with AD (AD; $n = 84$) were analyzed in two groups according to the presence (AD+/Atopy+; $n = 54$) or absence of SPT positivity (AD+/Atopy-; $n = 30$). The comparisons were made with a healthy control group matched for age and sex ($n = 81$). The median (interquartile range) of pGSN levels measurements in AD+/A+, AD+/A- and control groups were 267 μg/ml (236–368), 293 (240–498) and 547 (361–695) respectively ($P < 0.001$). The difference between control group and AD subgroups remained significant after Bonferroni correction ($P < 0.001$). Correlation analysis failed to reach significance with the disease severity (Objective SCORAD index).

Conclusion: This is the first study investigating the association of pGSN levels with AD and disease severity. pGSN levels decreased in AD. These findings suggest that gelsolin may have a role in the disease process in AD patients. It may also be considered as a potential therapeutic agent becasue of its anti-apoptotic and anti-inflammatory effects.

316
Has genetic or acquired filaggrin loss influenced the immune-mediated skin inflammation in severe AD?

Gaspar, K[1,2]; Dajnoki, Z[2]; Mocsai, G[2]; Kapitany, A[2]; Szegedi, A[1,2]
[1]Department of Dermatology, University of Debrecen, Debrecen, Hungary; [2]Department of Dermatological Allergology, University of Debrecen, Debrecen, Hungary

Background: Much evidence was published over the last decade about inflammatory cytokines can impair skin barrier. In contrast, the opposite question if skin barrier alterations affect keratinocyte (KC) immune responses remains less studied. We sought to investigate if genetic or acquired filaggrin (FLG) loss have different effect on the immune-mediated skin inflammation characteristic to atopic dermatitis (AD).

Method: Paraffin-embedded skin biopsies of severe FLG mutant and wild type AD patients and control subjects were analysed by immunohistochemistry. Slides were digitalized by whole slide imaging that allowed us to quantify and compare the studied protein levels by Pannoramic Viewer software and correlations were also calculated. Skin barrier structure protein FLG was detected to demonstrate the level of FLG loss. Although the selection of the two AD patient groups was made by matching clinical severity (SCORAD), histological severity markers (Ki67) and epidermal thickness were also investigated. T cells and dendritic cells were detected by using antibodies specific for CD3 and CD11c surface proteins. Some of the inflammatory Th2-polarisation cytokines (TSLP, IL-33) and chemokines (CCL27), characteristically expressed by KCs in AD patients, were also immunostained.

Results: Compared with control subjects, in both AD groups, FLG levels were found significantly lower. Between the levels of proinflammatory cytokines, severity markers and numbers of $CD3^+$ and $CD11c^+$cells of the two AD groups no sig-

© 2015 The Authors

nificant differences were found. Epidermal thickness and IL-33 levels ($P = 0.1017$) and epidermal thickness and $CD3^+$ cell count ($P = 0.1116$) showed strong correlation. Statistically significant correlations were found between IL-33 levels and $CD3^+$ cell count ($P = 0.0107$). Statistically significant correlations were found between TSLP and CCL27 levels ($P = 0.0255$) and TSLP and IL-33 expression ($P = 0.0028$).

Conclusion: Our findings suggest that immune mediated skin inflammation show the same innate and adaptive immune cell and KC derived cytokine and chemokine content in severe AD patients with acquired or genetically determined FLG loss, which may indicate that genetic FLG mutation in KCs does not influence the immune function of these cells in different manner. Results of the correlations highlighted the importance of IL-33 cytokine since its levels were correlated with one parameter of severity markers (epidermal thickness), KC derived cytokine (TSLP) and T cell count.

317
A panel of biomarkers for disease severity in atopic dermatitis

Thijs, JL[1]; Nierkens, S[2]; Herath, A[3]; Bruijnzeel-Koomen, CAF[1]; Knol, EF[1,4]; Giovannone, B[1]; de Bruin-Weller, MS[1]; Hijnen, D[1]
[1]Dermatology & Allergology, University Medical Center Utrecht, Utrecht, Netherlands; [2]U-DAIR and Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht, Netherlands; [3]MedImmune Biotech, Cambridge, United Kingdom; [4]Department of Immunology, University Medical Center Utrecht, Utrecht, Netherlands

Background: Promising new treatments for atopic dermatitis (AD) are currently investigated. The question is whether they are more effective than established treatments. However, comparing the results of different trials in AD is difficult because of the large number of different clinical outcome measures that have been used. Therefore, there is an urgent need for valid, reliable and objective severity measures of AD that allow comparison of clinical trials and epidemiological studies.

A great variety of serum biomarkers for disease severity in AD has been reported. The most frequently reported biomarkers include serum ECP, serum IgE, serum IL-2R, and serum TARC/CCL17 levels. Correlations between these biomarkers and disease severity showed large differences between publications.

The aim of this study was to explore if a panel of biomarkers shows a better correlation with disease severity compared to individual biomarkers.

Method: Using a multiplex approach we investigated 31 potential biomarkers for AD in sera from 17 adult patients diagnosed with severe AD. Blood was taken at admission to the hospital and after two weeks of treatment. Patients were treated with potent topical corticosteroids. Severity of AD at the time of blood sampling was assessed by using the Six Area Six Sign Atopic Dermatitis (SASSAD) score.

Results: All patients showed significant clinical improvement. SASSAD scores decreased from 36.9 at baseline to 8.0 after two weeks of treatment. Of the 31 markers studied, seven showed a statistically significantly decrease during treatment. This included TARC, MDC, IL-22, PARC, sIL-2R, sE-selectin and IL-16. Using stepwise regression analysis with cross validation, we found a combination of biomarkers ($n = 4$) that shows a strong correlation to disease severity ($r^2 = 0.856$).

Conclusion: In AD a combination of serum biomarkers was found to correlate better with disease severity than a single biomarker in this complex disease. We suggest to use this set of biomarkers for monitoring disease severity in future clinical studies to improve study comparability. In addition, we showed that serum IL-22 levels correlate with disease severity in AD patients.

318
Risk factors influencing eczema herpeticum in atopic dermatitis children

Myong Soon, S[1]; Jin Wook, C[2]; Sung Won, K[3]; Yun Ha, H[3]; Sung Wone, K[2]
[1]Gumi CHA University Hospital, Pediatrics, Gumi, Korea; [2]Busan St. Mary's Hospital, Pediatrics, Busan, Korea; [3]Busan St. Mary's Hospital, Busan, Korea

Background: Even though eczema herpeticum(EH) is serious and increasingly prevalent disease, just a few studies have investigated a large number of patients with EH. Unfortunately, the risk factors for EH are not yet obvious. So we investigated the relationship among the clinical factors, the allergic laboratoy features and serum 25-hydroxyvitamin D_3 levels in a group of the AD children depending on the presence of EH.

Method: This cross-sectional study was carried out on 380 patients aged 1 to 18 years between January 2012 and November 2014. A retrospective data collection through individual medical record, depending on the presence of EH, AD children were divided into two groups- with EH (EH+) and without EH (EH-).

Results: The male gender was related to the presence of EH (OR, 2.56; 95% CI, 1.1 9–5.53, $P = 0.01$), but age and 25-$(OH)D_3$ levels were not related. There is statistically significant association between the status of vitamin D and the presence of EH ($P = 0.04$). Serum total IgE and ECP levels were higher in the EH+ group, but serum total eosinophil count levels were higher in the EH- group. In a multivariate analysis after adjusting for age, gender, and 25-$(OH)D_3$, the relation between allergic markers (total IgE, ECP and total eosinophil count) and the presence of EH was not significant ($P > 0.05$). There was a statistical difference between two groups in the skin culture ($P < 0.01$).

Even after adjusting for age, gender, and 25-$(OH)D_3$, the correlation between the positive result of skin cultures and the presensce of EH was significant ($P < 0.01$), and MRSA was related to the development of EH (OR, 0.19; 95% CI, 0.04–0.92, $P = 0.03$). But even after adjusting for age, gender, and total IgE, the association between allergen sensitization and the presence of EH was not significant ($P > 0.05$).

Conclusion: We did not find an apparent relationship among allergic markers, 25-$(OH)D_3$ and allergen sensitization and the presence of EH.

However, we have identified the male gender, the positive result of skin cultures, and MRSA, as risk factors influencing EH in AD children.

319
Cluster subcutaneous allergen-specific immunotherapy in moderate/severe atopic dermatitis: a Brazilian experience

Bassani, C; Kopke, R; Dracoulakis, M; Silva, B; V, P-A; Aun, WT; Mello, JF; Tanno, LK
Hospital Servidor Público Estadual de São Paulo, Allergy and Clinical Immunology, São Paulo, Brazil

Background: The subcutaneous allergen-specific immunotherapy (SCIT) has been shown as a potential treatment in patients with atopic dermatitis (AD), however, the results are still controversial up to now. Therefore, the aim of our study was to access the efficacy of cluster SCIT in Brazilian patients with moderate/severe AD.

Method: We analyzed 40 patients with diagnosis of moderate/severe AD based on the SCORAD under follow-up in the Allergy Department of our Hospital between 2012 to 2014. The specific-immunotherapy was indicated based on the dust mite specific-IgE (*Dermatophagoides pteronyssinus* and/or *Blomia tropicalis*) and clinical relevance of these allergens after 3 months of follow-up without changes in the AD severity. All the patients underwent cluster SCIT and were evaluated every 03 months considering the SCORAD and the need of systemic and topical medications during the first year. The follow-up was proceeded by the same group of physicians.

© 2015 The Authors

Results: Of all 40 patients, 50% were women and the mean age was 12.4 years. Thirty-five (87%) patients had AD associated with other atopic diseases; 100% with allergic rhinitis and 14% with asthma. The median of SCORAD in the first evaluation was 38.9 and decreased to 5.1 after one year of SCIT ($P < 0.001$). None of the patients had significant adverse reactions during the SCIT. All of them were in use of systemic anti-histaminic drugs in the first evaluation (70% more than twice per day); but after one year, 5% of those patients were in use of these drugs at the time of the last evaluation ($P < 0.001$). Thirty-five patients (88%) were in use of topical corticosteroids (23% twice per day) before the SCIT and eight (20%) were in use of the same drugs once per day in the last evaluation ($P < 0.001$).

Conclusion: This first Brazilian experience on achieving the effect of cluster SCIT in AD, showed that the house-dust mite SCIT is effective in the management of moderate/severe AD, to reduce the SCORAD and the need of both systemic and topical medications, but larger controlled prospective multicentric studies shall respond to the question whether AD alone may be an indication of cluster SCIT.

320
Dihomo-g-linolenic acid prevents the development of atopic dermatitis through prostaglandin D_1 production in NC/Tnd mice

Tanaka, A; Amagai, Y; Oida, K; Matsuda, H
Tokyo University of Agriculture and Technology, Tokyo, Japan

Background: Atopic dermatitis (AD) is a chronic and relapsing skin disorder with pruritic skin symptoms. We previously reported that dihomo-g-linolenic acid (DGLA) prevented the development of AD in NC/Tnd mice. In the current study, we attempted to investigate the mechanism of preventive effect of DGLA on AD development in NC/Tnd mice.

Method: w? > The clinical outcomes of NC/Tnd mice that were given diets containing DGLA, arachidonic acid, or eicosapentaenoic acid were compared. In addition, lipid mediator contents in the skin in each group were quantified. Furthermore, lipid mediators released from RBL-2H3 mast cells under the DGLA treatment was examined.

Results: Only DGLA containing diet suppressed the development of dermatitis *in vivo*. By quantifying the 20-carbon fatty acid-derived eicosanoids in the skin, the application of DGLA was found to upregulate PGD_1, which correlated with a better outcome in NC/Tnd mice. Moreover, we confirmed that mast cells produced PGD_1 after DGLA exposure, thereby exerting an inhibitory effect on IgE-mediated degranulation.

Conclusion: These results suggest that oral administration of DGLA causes preventive effects on AD development in NC/Tnd mice by regulating the PGD_1 supply.

This work was supported by a joint research fund from Suntory Wellness Ltd., (Osaka, Japan).

321
Extracts from the fruit of *Dillenia indica* rich in betulinic acid may be useful to treat psoriasis

Correia, MR[1]; da Silva, J[2]; Clarinda, MM[1]; Freitas, AF[1]; Ourique, F[3]; Kviecinski, MR[2]; Grupo de Desenvolvimento em Tecnologia Farmacêutica
[1]Universidade do Sul de Santa Catarina - UNISUL, Curso de Graduação em Farmácia, Tubarão, Brazil; [2]Universidade do Sul de Santa Catarina - UNISUL, Programa de Pós-Graduação em Ciências da Saúde, Palhoça, Brazil; [3]Universidade Federal de Santa Catarina, Departamento de Bioquímica, Florianópolis, Brazil

Background: *Dillenia indica* (Dilleniaceae) is an evergreen tree found abundantly in the coastal region of southern Brazil where it is known mostly as "elephant apple". Macerations containing the fruit are used popularly to treat topically skin inflammations and contusions. The fruit of *Dillenia indica* is striking, a big pendulous globose capsule rich in betulinic acid. Antinflammatory and immunomodulatory activities were already previously attributed to betulinic acid. This study started an evaluation about the antipsoriatic activity of the hydroethanol (HE) and ethyl acetate (EA) extracts from the fruit of *Dillenia indica* correlating data to the content of betulinic acid in each extract.

Method: The content of betulinic acid was measured by high performance liquid chromatography. The antioxidant activity was measured using the thiobarbituric acid species method *in vitro*. The antipsoriatic activity was assessed using the Perry psoriasis tail model in rats. The psoriatic lesions were induced and the rats divided in 4 groups ($n = 12$): a non-treated control, a group treated with HE and other with EA (50 mg/ml) and a control group treated with clobetasol 0.5 mg/ml. Treatments were administered (1 ml) every 24 h for 7 days. After, 50% of animals were euthanized for histology of tails. The remaining animals were kept to compare the reduction in lesion length (cm) and the time required for healing.

Results and conclusion: The content of betulinic acid found in EA was 20 times higher than that found in HE (107.6 and 4.6 mg/g, respectively). The extracts had strong antioxidant activity. They caused total prevention of oxidation of egg yolk lipids by AAPH 2.5% at concentrations raging from 0.02 to 2 μg/ml. The most promising antipsoriatic activity was caused by EA being associated with the higher content of betulinic acid. The extent of psoriatic lesions and the time to healing were slightly reduced when the animals were treated with HE (~ 15%). But these parameters were reduced by 50% when animals were treated with EA. The activity of about 10% superior than clobetasol's. Histological analyses of tails indicated that EA induced orthokeratosis and reduced the inflammatory infiltrate when compared to the non-treated control.

322
A role of type 2 innate lymphoid cells in a murine model of atopic dermatitis-like skin lesions induced by *Aspergillus fumigatus*

Park, AL[1]; Yoon, J[2]; Lee, E[2]; Yu, J[2]
[1]Asan Medical Institute, Asan Medical Center, Seoul, Korea; [2]Asan Medical Center, University of Ulsan College of Medicine, Pediatrics, Seoul, Korea

Background: Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease mediated by Th2 cells in acute phase. Type 2 innate lymphoid cells (ILC2s) have a role in initiating Th2 cell. We investigated a role of ILC2s in a murine model of AD-like skin lesions by topical application with a crude extract of *Aspergillus fumigatus* (*Af*).

Method: We applied *Af* extract (40μg) to the dorsal skin of BALB/c mice 5 times a week repeatedly with an interval of 2 weeks. Clinical score and transepidermal water loss (TEWL) were assessed and histology was examined. The levels of interferon (IFN)-γ, IL-13, IL-17 in skin draining lymph node (LN) and immunoglobulin E (IgE) in serum were measured using ELISA. The ILC2s of Lin^- $CD25^+$ $IL\text{-}33^+$ cells in the skin were analyzed using flow cytometry.

Results: The clinical score and TEWL increased in mice applied with *Af* (*Af* group), compared with control group. Histologic findings showed that epidermal and dermal thickness, and eosinophilic and mast cell infiltration in the skin of *Af* group. The levels of total IgE were increased in the serum of *Af* group. Moreover, *Af* group showed increased levels of IL-13 in the supernatant from culture of skin draining LN stimulated with *Af*. The populations of Lin- CD25 + IL-33 + cells were increased in the skin of *Af* group.

Conclusion: This study suggests that ILC2s have a role in Th2-mediated skin inflam-

© 2015 The Authors

mation in a murine model of AD-like skin lesions induced by *Af*.

324
Evaluation of cyclosporine-sparing effects of polyunsaturated fatty acids in the treatment of canine atopic dermatitis

Müller, M-R[1]; Linek, M[2]; Röthig, A[3]; Löwenstein, C[4]; Mueller, RS[1]
[1]LMU Munich, Centre for Clinical Veterinary Medicine, Munich, Germany; [2]Tierärztliche Spezialisten, Hamburg, Germany; [3]Justus-Liebig-University Giessen, Klinik fuer Kleintiere, Giessen, Germany; [4]Tierärztliche Gemeinschaftspraxis Dr. David, Dr. Krützfeld, Frankenthal, Germany

Background: A good treatment option for canine atopic dermatitis is oral cyclosporine with proven good efficacy. However, this drug is cost-intensive and gastrotinestinal aderse effects are common, although rarely severe enough to discontinue treatment. A treatment alternative is the supplementation with polyunsaturated fatty acids (PUFA) which are less effective, but safe and in dogs on glucocorticoids have been shown to reduce the needed dose of the latter, when administered concurrently.
Method: A randomised, double-blinded, placebo-controlled multicentre trial was conducted in 36 dogs with atopic dermatitis to evaluate the cyclosporine sparing effect of polyunsaturated fatty acids. Dogs were stable on their individual cyclosporine dosage and received either a combined omega-3/omega-6 fatty acid product or placebo orally for 12 weeks. Dogs were examined monthly and the Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) was determined by a clinician. Pruritus, quality of life, global condition and coat quality were scored by the owner. If the dog's CADESI-03 and/or pruritus score improved by at least 25% compared to the previous visit, the cyclosporine dosage was decreased by approximately 25%. If the scores deteriorated by at least 25%, the cyclosporine dosage was increased by the same percentage.
Results: The median daily cyclosporine dosage per kilogramm body weight decreased in the active group from 3.8 mg to 2.8 mg and in the placebo group from 3.7 mg to 3.4 mg from the beginning to the end of the study. The difference between the two groups was significant ($P = 0.009$). The improvement in median pruritus score from inclusion to completion was significantly more prominent in the active group than in the placebo group ($P = 0.04$). There was no significant difference in CADESI-03 changes between both groups ($P = 0.38$).
Conclusion: The results of this study indicate a cyclosporine-sparing effect of omega-3/omega-6 fatty acids supplementation in dogs with atopic dermatitis.

325
Some epidemiological features of atopic dermatitis in Armenia

Sargsyan, A
SRI of Epidemiology, Virology and Medical Parasitology after A.B. Aleksanyan, Yerevan, Armenia

Background: During the recent years atopic dermatitis shows an increased incidence both among children and adults. The incidence reaches 15 or more cases per 1000 people of population. A genetic predisposition of autosomal dominant type is observed to the disease, there's an impact of a climate change, nutrition and allergic anamnesis. There haven't been conducted any epidemiological researches about AtD in Armenia and only data obtained in other countries is at our disposal.
Method: The aim of this study was to investigate several epidemiological factors in Armenia, the role of the latter in AtD development and possible impact on the course of the disease.

An epidemiological study has been conducted based on the example of one of the children hospital of Yerevan for the period of years 2008–2012. The materials of study are the medical records of the hospitalized children diagnosed with AtD.
Results: During 5 years there have been identified 347 hospitalization cases with AtD diagnosis. Out of those there are 197 boys (57%) and 150 girls (43%). According to our data only 137 (40%) out of total 347 cases had weighed family anamnesis. Among those the prime relationship (mother, father, both parents) has been noted at 58.4%; secondary relationship was applicable to 14% and 27% of patients had a particularly weighed hereditary predisposition (relatives of first and second levels altogether). Two hundred and six patients (60%) had no positive allergic anamnesis (history). Fourty six out of total 347 patients were hospitalized in serious condition, 275 - in moderate, 21 - in satisfactory and 5 - in remission condition (to clarify the diagnosis). We have compared the average amount of days that patients with severe and moderate course of the disease, spent in hospital. As far as these 2 indicators are not particularly distinguished, a conclusion can be made that the amount of days spent in hospital does not depend on the severity of the disease. Thus it was found that there were mostly boys among reported cases of AtD of patients before the age of 17. Family allergic history (anamnesis) has been observed among 40% only. Given the evidence of history it can be stated that exacerbation is more frequently observed during late autumn-winter and late spring-beginning of summer periods. From the data obtained it follows that greater number of patients comes from the regions, where dry and moderately dry, cold and temperate mountainous climate is predominant.

© 2015 The Authors

Poster Discussion Session PDS 9

Urticaria and angioedema

326

Involvement of ascarid nematodes not diagnosable by standard parasitological methods in spontaneous urticaria

Viñas, M[1]; Postigo, I[2]; Martínez, J[2]; Ibero, M[1]
[1]Hospital de Terrassa, Unitat d'Al·lèrgia, Terrassa, Spain; [2]Facultad de Farmacia UPV/EHU, Microbiología, Inmunología y Parasitología, Vitoria, Spain

Background: Nematode infections (Toxocarosis and Anisakiosis), both those highly prevalent and classically located in tropical and subtropical regions of the world and those of universal distribution, have become a growing health problem in industrialized areas such as the United States and Europe.

Because the *Toxocara* or *Anisakis* larvae do not grow or multiply in the accidental human host, in which they are widely distributed throughout all tissues of the body in the first case or primarily the stomach and duodenum in the case of *Anisakis*, direct identification of the parasite by standard parasitological methods is very difficult.

Objective: The aim of this study was the diagnosis of these parasites based on indirect identification, mainly by immunological techniques and their involvement in urticaria of unknown origin.

Method: Skin prick test, total IgE, specific IgE determination by CAP-FEIA system, eosinophil cationic protein, *Toxocara canis* larval excretory-secretory antigen to detect specific IgG and IgE antibodies by ELISA, IgG and IgE antibodies by FEIA using Anisakis simplex antigen and by ELISA using rAni s 1 and rAni s 7, Western blotting using *Toxocara canis* larval excretory-secretory antigen.

Results: The prevalence of parasitism by *Anisakis simplex* and *Toxocara canis* was high: 24% in the general population and 44% in allergic population. The allergic patients with urticaria had higher prevalence of parasitism than the control group. The determination of IgE and IgG antibodies increases more diagnostic sensitivity.

Conclusion: The possibility today of using individual antigens that allow us to establish the molecular diagnosis, the identification of different immunoglobulin isotypes targeted against different antigens could establish a much more robust and effective basis in both the diagnosis and prognosis of certain types of parasitosis that are difficult to identify. From an allergology standpoint, these parasites could be the cause of unusual hypersensitivity reactions.

327

Prevalence, patients' profiles and management in chronic spontaneous urticaria – the ETNA study

Thenie, C[1]; Amsler, E[2]; Augustin, M[1]; Pinton, P[1]
[1]Novartis Pharma AG, Rueil-Malmaison, France; [2]Tenon Hospital, Paris, France

Background: Chronic Spontaneous Urticaria (CSU) is a chronic inflammatory skin disease lasting for more than 6 weeks. Epidemiological data in France about CSU are very limited and not recent.

Objective: The primary objective of ETNA is to determine the rate of patients suffering from CSU with an insufficient response to anti-histamines anti-H1 (anti-H1) at standard dose (x1-licensed dose) and elevated dose (x4-off label dose) among patients consulting a dermatologist, an allergist or a physician neither dermatologist nor allergist, but known to manage CSU. Secondary objectives among others are to determine the patient profile and the management of CSU.

Method: Retrospective, non-interventional study performed by dermatologists, allergists (GPs) and internists, working at hospital, in private practice, or both. Descriptive analysis: means, standard deviation, median, minimal and maximal values, for quantitative variables, and frequencies, percentages and bilateral 95% confidence intervals for qualitative variables.

Results: A total of 116 physicians included 1504 patients from March to September 2014: 59 of the practitioners (51.3%) were office-based, 25 (21.7%) in hospital, and 31 (27%) in both practices.1259 patients were fully eligible for the analysis: 703 (56.1%) were included by 57 allergists, 465 (37.1%) by 53 dermatologists, and 85 (6.8%) patients by 5 internists. Among these patients, 79.5% had CSU and 20.5% had chronic inducible urticaria. Mean age of CSU patients was 45.2 ± 15.6 years and 658 (67.9%) were women. CSU was diagnosed since 48.1 ± 75.9 months on average. Five hundred and ninety two CSU patients (66.3%) presented only superficial wheal whereas 38 CSU patients (4.3%) presented angioedema alone and 263 (29.5%) associated to the wheals. During their disease, the treatment received were anti-H1x1 for 807 (80.6%) patients and anti-H1x4 for 146 (14.6%) patients. Two hundred and 252 patients (25.2%) received oral corticosteroids for CSU treatment. Among all patients suffering from CSU, the rate of CSU patients with an insufficient response to anti-H1x1 was 51.0% (95%CI[48.0–54.1]) and the rate of CSU patients having an insufficient response to anti-H1x4 was 8.2% (95%CI[6.5–9.9]).

Conclusion: The data of the ETNA study emphasize a strong medical need for CSU, despite anti-H1 treatment. A significant rate of patients could not be even managed with anti-H1x4 (off label dose). CSU remains a disease with a strong burden of disease and high unmet needs.

328

Differential medical resource consumption and direct healthcare costs of patients with adult onset chronic idiopathic urticaria determined by antihistamine doses needed to control the disease

Parisi, CA; Ritchie, C; Petriz, NA; Morelo, CB
Hospital Italiano de Buenos Aires, Medicine Department, Servicio de Clínica Médica, Sección Alergia Adultos, Ciudad Autónoma de Buenos Aires, Argentina

Background: Scarce studies address the issue of resource consumption and direct healthcare costs of patients with diagnosis of adult onset chronic idiopathic urticaria (CIU).

The objective of the study was to estimate the resource consumption and direct costs of chronic idiopathic urticaria (CIU) patients sort by antihistamine doses needed to control the disease as an indirect marker of disease severity.

Method: All the patients with diagnosis of adult onset CIU and members of Hospital Italiano de Buenos Aires HMO (IHHMO) were included in the study. IHHMO provides comprehensive medical and health services through two main hospitals and 24

© 2015 The Authors

medical office buildings to over 160 000 members primarily located in the urban areas around the Autonomous City of Buenos Aires, Argentina. All hospitalizations, drug prescription, outpatient episodes, consultations with general or specialists medical practitioners, and investigations/tests in the 12 months before the inclusion in the study were considered to estimate resource consumption and direct healthcare costs. Patients were stratified according to how many doses per day of antihistamine were needed to control the disease. We separate patients in 3 groups:

a patients that used antihistamines on demand;
b one dose per day;
c ≥2 doses per day.

Second quarter 2014 costs were obtained from IHHMO sources and converted to US Dollars (November 2014).

Results: One hundred and thirty four patients were included. 39.5% of patients were on group a; 48.5% on group b and 12% on group c. Patients on group c had significantly more visits to the specialists per year than group a and b ($P < 0.01$) as well as more frequent use of systemic corticosteroids ($P = 0.01$), montelukast ($P < 0.01$) and treatment adverse events ($P < 0.001$). Mean cost per year of patients in group a was USD 227 (95% CI 184–353); in group b was USD 416 (95% CI 282–650) and in group c was USD 1134 (95% CI 916–2119).

Conclusion: This is the first study performed in Argentina that evaluated the cost of CIU considering and indirect approach to disease severity. This information provides important data for the economic analysis of the disease Argentina, where this type of information is need.

329
Clinical characteristics of 197 patients with chronic spontaneous urticaria in the Bulgarian population

Valerieva, A; Staevska, M; Petkova, E; Kralimarkova, T; Lazarova, T; Dimitrov, V; Popov, T
Clinic of Allergy and Asthma, Medical University of Sofia, Sofia, Bulgaria

Background: Chronic spontaneous urticaria (CSU) is a worldwide-spread disease defined by the presence of hives and/or angioedema (AE) for a duration longer than 6 weeks. The prevalence of this disorder is known to be up to 1% of the general population. The purpose of the study was to contribute to a better understanding of the clinical characteristics and epidemiology of CSU patients in Bulgaria.

Method: This study has included 197 patients with CSU evaluated in our clinic between 2006 and 2012. All patients were analyzed for clinical features, symptom score, demographic and anthropometric characteristics, ASST, H. pylori (antigen in feces), NSAIDs intolerance, anti-thyroid autoantibodies, hematological and biochemical indices, ESR. We divided the patients into two major groups: I-difficult to maintain severe CSU (67 patients) and II-light to mid-severe CSU (130 patients).

Results: We found a median age of 36.5 in the Ist group against 45.9 in the IInd ($P < 0.0001$). The age of onset of the disease was 35.2 in the Ist group against 44.4 in the IInd ($P < 0.0002$). The studied population consisted of 72% women. AE was present in 61.42% of our patients and 41.12% had signs of DPU. A positive ASST was found in 56.85% of all patients. There was no association between ASST(+) and symptom score, gender, age, history of atopy, and age of onset of the disease. The frequency of NSAIDs intolerance was 17.26% (8.96% in the Ist vs 21.54% in the IInd group, $P = 0.027$). NSAIDs intolerant patients had positive thyroiditis markers in 41.67% vs 23.12% ($P = 0.022$). Thyroiditis autoantibodies were found in 15.15% (gr.I) and in 33.08% (gr.II) ($P = 0.008$). A strong association of ASST(+) and H. pylori infection was found (42.73% vs 15.29%, $P < 0.0001$). Among hematological indices we found significant differences between both groups in: monocyte count ($P < 0.0001$), hemoglobin rate ($P = 0.027$) and ESR ($P = 0.005$).

Conclusion: We performed the first study in Bulgaria with such a sample size. Based on our findings: younger patients have more severe CSU; NSAIDs intolerance is more frequently seen in the group of light CSU; patients with NSAIDs intolerance have positive markers for thyroiditis with a significantly higher frequency; ASST(+) patients have H. pylori infection with a higher prevalence. A strong association between monocyte count, hemoglobin rate and ESR, and the severity of the disease was found which may be considered as the laboratory expression of a low-grade systemic inflammation.

330
Clinical feature of chronic idiopathic urticaria in adult Kuwaiti patients

Arifhodzic, N; Radhakrishna, P; Al-Ahmad, M; Al Enezi, A
Ministry of Health, Allergy Center, Kuwait, Kuwait

Background: Chronic Idiopathic Urticaria (CIU) is a common disabling disorder, characterized by recurrent episodes of transient itchy wheals. Frequently CIU is associated with angioedema (AE). In majority of patients Chronic Urticaria is idiopathic (CIU), since triggering of mast - cell degranulation in majority of patients with CIU has not been identified

Method: Medical records of outpatient allergy consultations due to diagnosis of CIU were retrospectively reviewed to collect demographic and clinical data, and urticarial activity score. Two hundred and two adult patients were recruited during 2013. All patients were followed up at the Allergy Clinic in the Al Rashed Allergy Centre.

Results: With respect to gender there was no significant difference in the mean age of patients (F: 29 ± 12; M: 36 ± 14 year). Female patients prevailed (F/M ratio = 1:2.5) in all ages, but this was most significant in young age group. Only 6.6% of patients were above the age of 60. Duration of the disease before visiting Allergy Clinic was longer than expected in both gender ($P < .285$). Clinical evaluation of disease severity was based on Urticaria Scale Score (USS) (0–15) during the week before the first visit to our Centre as a sum of; a: number of wheals, b: number of episodes, c: wheals size, d: duration of wheals and e: pruritus (each: 0–3). USS ranged 6–10 in majority of patients ($P < .010$). Angioedema was common, present in more than 50% of patients. Atopy was not found to play a significant role. Associated dermographism was common in both genders. This was more frequent in female patients, although the difference was not statistically significant ($P < .537$).

Conclusion: The obtained results showed that CIU in Kuwaiti patients is more frequent in females of younger age. We had more severe forms of CIU, with a longer duration and late referral to an allergist.

331
Histamine release test correlates with disease activity in chronic spontaneous urticaria

Berti, A[1]; Yacoub, MR[1]; Skov, PS[2]; Casati, L[1]; Colombo, GM[1]
[1]IRCCS San Raffaele Scientific Institute, Milan, Italy; [2]University Hospital of Southern Denmark, Odense, Denmark

Background: Chronic spontaneous urticaria (CSU) is defined by the presence of urticaria lasting more than 6 weeks, in which appearance of lesions is not triggered by an identifiable external factor. CSU may be autoimmune (IgG autoantibodies against the FcεRIα receptor or against IgE) or idiopathic (without a known etiology). We aimed to evaluate the role of Histamine Release (HR) test in relation to autologous serum skin test (ASST), autologous plasma skin test

© 2015 The Authors

(APST, detecting histamine releasing plasma factors) and disease activity.

Method: We studied 20 patients with CSU, where all other forms of chronic urticaria were excluded, by medical history and by diagnostic work up according to EAACI guidelines. The physical examination also assessed disease activity by UAS (Urticaria Activity Score) and questionnaires on quality of life (VAS, DLQI, CU-Q2oL). The patients underwent *in vivo* assessment of ASST and APST and *in vitro* determination of HR from healthy subjects' basophils incubated with patients serum.

Results: 60% (12/20) of patients has positive ASST and APST, 5% (1/20) has only positive ASST and 20% (4/20) has only positive APST. Only 15% (3/20) shows negative ASST and APST. HR test was positive in 45% (9/20) of patients has positive results. HR and ASST were concordant in 50% (10/20) of cases. In contrast, 35% (7/20) of patients with positive ASST has negative HR test.

Of the 16 patients with positive ASST and/or APST, 75% (12/16) has moderate-severe UAS. Nine patients with positive HR test have a moderate-severe UAS ($P = 0.04$) and QoL negative impact.

Conclusion: Our work is the first that correlates HR test results with ASST/APST and activity disease. Although HR test do not inform on the real etiology of the CSU, it may be complementary to ASST/APST, if skin test provide doubtful results or if they cannot be assessed. Interestingly, HR test statistically correlates with disease activity assessed by UAS, suggesting a potential role in prediction of disease severity and stratification of patients. Finally, we concluded for an "idiopathic" urticaria in only 15% of our patients, despite of 41% reported in literature. If these results will be confirmed by larger studies, HR test may be useful to better characterize patients with CSU.

332

Urticaria, angioedema and D-dimer at the emergency room

Cancian, M[1]; Dako, S[1]; Vettore, G[2]; Baldo, D[2]; Mormando, G[2]; Mezzocolli, I[2]; Bendo, R[1]; Senter, R[1]; Fabris, F[1]
[1]DIMED-Clinica Medica 1, General Hospital and University of Padua, Padova, Italy; [2]Emergency Medicine, General Hospital and University of Padua, Padova, Italy

Background: The complexity of the scenario at the Emergency Room (ER), where acute urticaria-angioedema syndrome (UA) represents about 0.7% of admissions, pushes to perform several blood analyses to assess clinical conditions of patients. D-dimer evaluation plays a critical role among these assays, and increased levels suggest the opportunity to rule out lung thromboembolism by expensive and even hazardous diagnostic investigation. The aim of our study was:

1 To establish the prevalence of high D-dimer levels in patients with UA admitted at the ER
2 To investigate the possible presence of deep venous thrombosis (DVT), well known to be the first cause of pulmonary thromboembolism, in patients with an increase in D-dimer plasma concentration
3 To verify the relationship between D-dimer levels and UA activity score.

Method: Thirty-five patients admitted at the ER of the General Hospital-University of Padua with UA underwent physical examination as well as an assessment of the urticaria/angioedema activity score (UAS/AAS) and a panel of emergency blood analyses, including D-dimer evaluation. In all patients with D-dimer levels higher than normal (referral range 0–225 μg/L), a lower limbs venous ultrasonography was performed and they were re-evaluated after 72–96 h from discharge for physical examination and blood testing.

Results: The overall mean value of D-dimer resulted 623.65 μg/L ± 945.88 DS, with increased levels detected in 43% (15/35) of patients (1316.86 μg/L ± 1126.18 DS; range 319–4210 μg/L). We found a correlation between D-dimer levels and UAS or AAS ($P = 0.027$ / $r = 0.38$ and 0.018 / 0.53, respectively), and all the patients with plasma concentrations >500 μg/L had the highest activity scores. Lower limbs venous ultrasonography resulted negative, and ruled out deep venous thrombosis in all of them. Paralleling clinical improvement after steroid and antihistamine therapy, D-dimer levels significantly decreased after 72 to 96 h from discharge ($P = 0.003$) in all but in one patient, who remained fully symptomatic at follow up.

Conclusions: The increase of D-dimer is restricted to the acute phase and is not predictive for DVT in urticaria-angioedema. The role of the coagulation and fibrinolysis pathway in UA needs further study to be clarified: based on present data, indeed, it might be involved in the pathogenesis of the disease or, on the contrary, simply represent an epiphenomenon.

333

Improvement of sleep in patients with chronic idiopathic/spontaneous urticaria (CIU/CSU) treated with omalizumab: results of three randomized, double-blind, placebo-controlled clinical trials

Spector, S[1]; Antonova, E[2]; Trzaskoma, B[2]; Omachi, TA[2]; Zazzali, JL[2]
[1]California Allergy and Asthma Medical Group, Inc., Los Angeles, United States; [2]Genentech, Inc, South San Francisco, United States

Background: Symptoms of chronic idiopathic/spontaneous urticaria (CIU/CSU) can interfere with sleep. Omalizumab reduces CIU/CSU disease activity; patient-reported data on sleep were collected in omalizumab phase III CIU/CSU studies.

Methods: Subjects were randomized to every-4-week dosing of placebo (PLB) vs omalizumab (OMA) 75 mg, 150 mg, 300 mg (ASTERIA I, $n = 318$, 6 doses and ASTERIA II, $n = 322$, 3 doses); or PLB vs OMA 300 mg (GLACIAL, $n = 335$, 6 doses). Urticaria Patient Daily Diary (UPDD; sleep interference question), Chronic Urticaria Quality of Life Questionnaire (CU-Q_2oL; sleep problems dimension), and a Medical Outcomes Study Sleep Scale (MOS-SS; sleep problem index II [SPI-II]) were used to assess sleep. Higher negative numbers indicate greater improvement. Treatment change from baseline was compared with placebo change from baseline using ANCOVA t-test, adjusted for baseline score and weight.

Results: At Baseline, patients reported substantial sleep impairment. By Week 12, sleep improved in all treatment arms, and OMA300-treated patients demonstrated greatest improvements. Relative to placebo, improvements were observed with OMA300 in mean sleep scores for all measures, from Baseline to Week 12, in ASTERIA I, ASTERIA II, and GLACIAL, respectively: UPDD: −9.6 ($P < 0.001$), −9.2 ($P < 0.001$), −9.2 ($P < 0.001$); CU-Q_2oL: −30.2 ($P = 0.052$), −33.3 ($P < 0.001$), −29.4 ($P < 0.001$); MOS-SS SPI-II: −18.1 ($P = 0.025$), −14.8 ($P = 0.029$), −19.0 ($P = 0.029$). Change was also significant, compared with placebo, for UPDD with OMA150 (ASTERIA I): −7.1 ($P = 0.044$), but change was not significant with OMA75 or OMA150 in the other studies. Sleep continued to improve in OMA300 between weeks 12 and 24 in ASTERIA I [as measured by UPDD: −10 ($P = 0.009$)] and GLACIAL [as measured by UPDD: −9.4 ($P < 0.001$), CU-Q_2oL: −30.2 ($P < 0.001$), and MOS-SS SPI-II: −19.6 ($P = 0.003$)].

Conclusions: In all three studies, CIU/CSU patients treated with OMA achieved improvements in sleep, with improvements

© 2015 The Authors

greatest in patients treated with OMA300. Only OMA300 demonstrated statistically significant improvements in all three sleep measures.

334
'Urticaria': an app to optimize patients' management

Spranger, O[1]; Murth, S[2]
[1]Global Allergy and Asthma Patient Platform (GAAPP), Vienna, Austria; [2]ALYSIS Technic Support, Vienna, Austria

Background: Patients with urticaria are often not diagnosed and treated appropriately. Patients quality of life is severely affected and management of the disease should, therefore, be prompt and involve close cooperation between patient and physician. According to the 2009 EAACI/GA2LEN/EDF/WAO guidelines, the most important diagnostic procedure is to obtain a thorough history including all possible eliciting factors and significant aspects of the nature of the urticaria. In addition, as symptoms frequently change in intensity during the course of a day, overall disease activity is best measured by advising patients to document 24-h self- evaluation scores for several days.

Smartphone applications (apps) have been increasingly identified as a novel platform for dissemination of healthcare related information and for patients self management. These systems are suitable to be used by the patients themselves to identify warning signs and report events (Clinical diary).

Method: In occasion of the World Urticaria Day (1st Oct, 2014), GAAPP launched "Urticaria", the first app for patients with urticaria worldwide. The app is freely available for download from Google Play and Itunes. It is designed to be easy to use and includes:

1 Information on urticaria in plain language (forms, treatment, patient role in the management)
2 Early diagnosis questionnaire
3 Urticaria control test
4 7-Day Urticaria Activity Score questionnaire
5 Angioedema Activity Score
6 Clinical diary (with easy to complete information on wheals, redness, swelling, itch, drugs, stylelife, etc)

"Urticaria" is available in English and German. Russian and Spanish versions are being beta tested.

A Photo Gallery tool will be also included. Patients will be able to photograph the wheals, store them in the Photo Gallery, in order to show them to the doctor at the next visit.

Results: The ultimate goal of the app is to help disease understanding by patients and to provide a tool that may facilitate patient/doctor communication. Since its launch, the app was downloaded 626 times.

Conclusion: "Urticaria" is the very first app for patients with this condition. It is designed to be comprehensive and clear. Although further studies are needed to assess the validity of apps as aid for patients self management (Chochrane, Nov 2013), "Urticaria" is a unique opportunity to provide patients with tools aimed at improving their quality of life, based on the indications of international guidelines.

335
Pattern of hospitalizations due to angioedema between 2009 and 2013 - a retrospective study of 201 cases

Cosme, J[1]; Spínola, A[1]; Branco-Ferreira, M[1,2]; Pereira-Barbosa, M[1,2]
[1]Hospital de Santa Maria, CHLN, E.P.E., Lisboa, Portugal, Serviço de Imunoalergologia, Lisboa, Portugal; [2]Faculdade de Medicina de Lisboa, Lisboa, Portugal

Background: Angioedema (AE) is clinically characterized by self limiting episodes of oedema of the subcutaneous and mucosal tissue that can occur with or without urticaria. The epidemiologic characteristics and hospitalization pattern of AE in its isolated form have not been well described.

Objective: To profile hospital admissions for AE in a central Lisbon hospital (Centro Hospitalar Lisboa Norte) with respect to clinical and epidemiological characteristics.

Methods: The admissions between 2009 and 2013 with a diagnosis grouped under the broad categories of AE (ICD-9-CM codes 995.1 and 277.6) were retrospectively analysed. Data regarding age, sex, location of the oedema, cause, time course of the hospitalization, associated disorders and outcomes were collected. The study protocol was approved by the Centro Hospital Lisboa Norte Ethics Committee.

Results: Of a total of 290 hospital admissions with an AE ICD-9 code during the study period, only 201 admissions (165 patients) were included on our study. 51% of the hospitalizations occurred in the Allergy Immunology department (the hospitalizations were distributed by 23 hospital departments). The mean annual angioedema admission rate was 8.6 per 10 000 admissions. The median age was 53 years. 53% of the admissions were in female, 73% in white and 96% in adults. The *average length of hospitalization* due to angioedema was 6.1 days. With regard to the admissions: in 57% the AE was the cause for the admission, in 27% a complication that happened during the admission and 16% were for short-term prophylactic treatment before an invasive procedure. In 4% there was laryngeal edema. The causes of angioedema were: hereditary angioedema (HAE) (36%), angiotensin converting enzyme inhibitor (ACEi) induced angioedema (26%), angioedema associated with thrombolysis (rtPA) (11%), angioedema induced by nonsteroidal anti inflammatory drugs (4%) and other (23%). The tongue was involved in 27% and 41% in ACEi and rtPa induced angioedema respectively. In ACEI-induced angioedema the majority of patients were prescribed with an ACEi for 1 to 5 years. In rt-PA-related AE 64% of the patients were under an ACEi.

Conclusions: The mean annual angioedema admission rate was 8.6 per 10 000 and the majority in the Allergy Immunology department. Laryngeal oedema happened only in 4% of the hospitalizations. The main cause of hospitalization was HAE followed by ACEi induced AE. AE can be a complication after rtPA therapy.

336
Plasmapheresis in cold urticaria

Stankeviciute, R[1]; Chomiciene, A[1,2]; Blaziene, A[1,2]; Zilenaite, E[1]; Griskevicius, A[3]; Audzijoniene, J[3]
[1]Faculty of Medicine, Clinic of Infectious, Chest Diseases, Dermatovenerology and Allergology, Vilnius University, Vilnius, Lithuania; [2]Center of Pulmonology and Allergology, Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania; [3]Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania

Background: Non-sedating H1 antihistamines are the first line treatment for all forms of urticaria, however, some patients with severe urticaria are unresponsive even to high doses of antihistamines. Plasmapheresis is one of the second line treatment choices, but there are only a small number of studies exploring its clinical efficacy. The aim of the study was to determine the effect of plasmapheresis in severe cold urticaria.

Method: We reviewed the records of patients diagnosed with cold urticaria who underwent plasmapheresis and interviewed patients regarding the long term effect of this treatment.

Results: 21 patient with cold urticaria resistant to conventional treatment, who underwent plasmapheresis between 2010–2014 year were studied. The mean age of the patients was 38.4 ± 12.738 years and 18 (85.7%) of them were females. The duration of the disease ranged from 2 to 156 months. 12 (57.1%) patients together with urticaria experienced angioedema and 1 patient (4.8%) had experienced anaphy-

© 2015 The Authors

laxis after bathing in the cold water. 2 (9.6%) patients had positive serum cryoglobulins. All the patients were previously treated with antihistamines and some of them received short courses of oral corticosteroids, H2 antagonists and antibiotics. 20 (95.6%) patients indicated clinical improvement of initial symptoms by the end of the plasmapheresis course. It was possible to contact 4 patients by phone and they were asked about the long term effect of plasmapheresis. One patient still has a complete symptom resolution for more than 3 years, 2 patients had reduced temperature thresholds, but the treatment was effective temporary (lasting 4–6 months), 1 patient did not experience any relief of the symptoms.

Conclusion: Plasmapheresis could be used as additional treatment for cold urticaria.

© 2015 The Authors

Poster Discussion Session PDS 10

Cellular and molecular allergy mechanisms

337
Specific and combined effects of two food contaminants on Th2 cell differentiation in an *in vitro* model

Joly-Condette, C[1]; Anton, PM[1]; Gay-Quéheillard, J[2]; Chango, A[1]; Delayre-Orthez, C[1]
[1]Institut Polytechnique LaSalle Beauvais, Nutrition and Health department, EGEAL Unit, Beauvais, France; [2]PERITOX Unit, Université de Picardie Jules Verne, Amiens, France

Background: The prevalence and incidence of allergy have increased in last decades. One hypothesis is the presence in the food matrix of compounds that could increase susceptibility to develop allergies. Among them, there are pesticide residues and neoformed compounds resulting from the Maillard reaction occurring during food processing. Both types of contaminants are found in the diets of young children who are particularly vulnerable because of the immaturity of their immune system. The impact of these contaminants on the development of allergies remains poorly studied. In this context, this study is aimed at determining if the Nε-carboxymethyllysine (CML), a neoformed compound present in infant formulas and chlorpyrifos (CPF), an organophosphate pesticide, can change the Th cell differentiation *in vitro*.
Method: Human naive CD4 + cells isolated from PBMC were cultured in the presence of activating beads and interleukin-4 (IL-4) in the absence (control condition) or presence of 100 μM of CML or CPF, alone or in mixture. Analysis of the phenotype of the cells was performed by flow cytometry using specific markers and by the study of the expression of genes of interest (TBX21 and GATA3) by qPCR.
Results: CD4 + cells stimulated in the presence of 100 μM CML have greater proliferation, notably with a doubled number of living IL4(+) T cells, as compared to the control condition ($P < 0.05$). CD4 + cells stimulated in the presence of 100 μM CPF have a different pattern of response characterized notably by an increased cell death and a decrease in living IL4(+) T cells (21% vs 82% in the control condition, $P < 0.05$). The effect of a mixture of both contaminants resulted again in an increased cell death and a strong decrease in the number of living IL4(+) T cells (9% vs 82% in the control condition). The expression of GATA3 was significantly higher in CD4 + cells stimulated in the presence of 100 μM CML ($P < 0.01$ as compared to control condition) and CD4 + cells stimulated in the presence of both contaminants ($P < 0.05$ as compared to control condition).
Conclusion: These results show that food contaminants such as pesticides or newly formed compounds may disrupt the differentiation of Th cells, causing a possible change of the predisposition to develop allergies.

338
Evolution of the IgE response to house dust mite allergen molecules in childhood

Posa, D[1]; Hofmaier, S[1]; Hatzler, L[1]; Rohrbach, A[1]; Panetta, V[2]; Bauer, CP[3]; Hoffman, U[3]; Forster, J[4]; Zepp, F[5]; Schuster, A[6]; Stock, P[7]; Wahn, U[1]; Grabenhenrich, L[8]; Keil, T[8,9]; Lau, S[1]; Chen, K[10]; Resch, Y[10]; Vrtala, S[10]; Valenta, R[10]; Matricardi, PM[1]
[1]Charité-Universitätsmedizin Berlin, Pediatric Pneumology and Immunology, Berlin, Germany; [2]L'altrastatistica srl, Consultancy & Training, Biostatistics Office, Rome, Italy; [3]Technical University of Munich, Pediatrics, Munich, Germany; [4]St Josefs Hospital, Pediatrics St Hedwig, Freiburg, Germany; [5]Johannes Gutenberg University Medical Centre, Pediatrics and Adolescent Medicine, Mainz, Germany; [6]Heinrich-Heine-University, Pediatric Cardiology and Pneumology, Düsseldorf, Germany; [7]Altonaer Kinderkrankenhaus, Paediatric Pneumology & Immunology, Hamburg, Germany; [8]Institute for Social Medicine, Epidemiology and Health Economics, Charité - Universitätsmedizin Berlin, Berlin, Germany; [9]Institute of Clinical Epidemiology and Biometry, University of Würzburg, Würzburg, Germany; [10]Medical University of Vienna / Center for Pathophysiology, Infectiology and Immunology, Division of Immunopathology, Department of Pathophysiology and Allergy Research, Vienna, Austria

Background: We have recently shown that the IgE response to grass pollens in early childhood involves progressively a growing number of molecules ("molecular spreading"). We aimed this study to investigate the development of the IgE response to house dust mite allergen molecules in childhood.
Method: We examined the sera of 820 children participating in the German Multicentre Allergy Study, a birth cohort born in 1990. Blood samples were collected at 1, 2, 3, 5, 6, 7, 10, 13 and 20 years of age. Sera with IgE antibodies to an extract of *Dermatophagoides pteronyssinus* (≥0.35 kU/L) were further tested for the presence of IgE to Der p 1, Der p 2, Der p 4, Der p 5, Der p 7, Der p 10, Der p 11, Der p 14, Der p 15, Der p 18, Der p 21, Der p 23, Clone 16 in the context of the MeDALL chip. The propensity to become sensitised to many molecules has been expressed with a "Spreading Factor" (SF), defined as the number of new molecules obtained/year.
Results: Over 150 children had IgE antibodies to *Dermatophagoides pteronyssinus* at least once up to 13 years of age. The molecules most frequently recognized by IgE (i.e. at least at one time point in over 50% of the mite-sensitised children) were Der p 2, followed by Der p 1 and Der p 23. Among mite-sensitised children, an elevated heterogeneity in the development of IgE response to individual allergenic molecules was observed. A minority of mite-sensitised children responded to only one molecule for the whole observation period, while many others underwent a weak or strong process of "molecular spreading", especially in the first decade of life. so that the "spreading factor" varied from 0 to >4 in the examined population.
Conclusion: The evolution of the IgE response against mites is extremely heterogeneous, and the molecular spreading phenomenon can be observed in most, but not all mite sensitised children. The heterogeneity of molecular sensitization profiles in mite allergic children may partially explain the heterogeneity in their response to allergen immunotherapy. This hypothesis deserves to be tested in clinical trials.

339
Histamine production by human neutrophils

López, C[1]; Bellido, V[1]; Alcañiz, L[1]; Vega, A[1]; Chacón, P[1,2]; Ventura, I[1]; Aroca, R[1]; Monteseirín, J[1,3]
[1]Hospital Universitario Virgen Macarena, UGC-IC Alergología, Sevilla, Spain; [2]Cell Signaling Department, Centro Andaluz de Biología Molecular y Medicina Regenerativa/Consejo Superior de Investigaciones Cientificas (CABIMER/CSIC), Sevilla, Spain; [3]Facultad de Medicina, Universidad de Sevilla, Departamento de Medicina, Sevilla, Spain

Background: Histamine is an important mediator in the development of allergic reactions. Only a small subset of human cell types is able to produce histamine. No previous studies have shown that human neutrophils are among them. The present

© 2015 The Authors

work was undertaken to analyze whether human neutrophils produce histamine, and to determine what agonists are involved in histamine production by human neutrophils.
Method: The expression of histidine decarboxylase in human neutrophils was established by quantitative PCR, Western blotting, and flow cytometry analysis. The activity of the enzyme was determined by ELISA, which measured histamine in the culture supernatant of neutrophils stimulated with a set of classical agonists.
Results: Human neutrophils are *bona fide* histamine-producing cells. Neutrophils store ~ 0.29 pg/cell and release ~ 50% of the histamine content in an antigen-dependent manner and on stimulation with other neutrophil agonists. Basal expression of histidine decarboxylase, the rate-limiting enzyme in histamine production, is higher in neutrophils from patients with allergies than from healthy.donors. Our results cannot be ascribed to cell contamination for several reasons. LPS failed to induce histamine release by basophils, whereas it induced histaminerelease by neutrophils; and we did not detect basophils, monocytes, or lymphocytes in our neutrophil preparations. Eosinophils, albeit detected, were only 0.001–0.004% of the final cell population, and they did not store or release histamine on antigen or LPS stimulation.
Conclusion: Antigens to which patients with allergies were sensitized stimulated release of histamine from neutrophils. These observations represent a novel view of neutrophils as possible source of histamine in the allergic diseases.

340
Binding of retinoic acid to the major cow milk allergen Bos d 5 has an immunosuppressive effect on subsequent immune responses

Hufnagl, K; Wagner, S; Roth-Walter, F; Hofstetter, G; Jensen-Jarolim, E; Comparative Medicine
Messerli Research Institute of the University of Veterinary Medicine, Medical University and University Vienna, Comparative Medicine, Vienna, Austria

Background: Cow milk allergy is one of the most common food allergies in infants, but mechanisms of allergic sensitization and resulting Th2 type response are not yet identified. The major cows milk allergen Bos d 5 is a member of the lipocalin family and able to bind several ligands in its intramolecular pocket, among them the active Vitamin A metabolite retinoic acid (RA). Recently, we could show that Bos d 5 acts immunomodulatory depending on its load with siderophore-iron complexes. Here, we investigated the capacity of Bos d 5 complexed with RA to polarize immune cells towards Th2.
Method: Dose-dependent quenching of the autofluorescence of Bos d 5 by incubation with RA was measured spectrofluorimetrically. Activated human peripheral blood mononuclear cells (PBMCs) of 9 healthy donors were incubated with the "empty" milk allergen (apo-Bos d 5) or Bos d 5 loaded with RA (holo-Bos d 5). CD3 + , CD4 + and CD8 + T-cell subsets were analysed by FACS. Measurement of IFN-γ, IL-10 and IL-13 cytokines was done by ELISA in supernatants after 48 h of stimulation.
Results: Spectrofluorimetric titration revealed a dissociation constant (K_D) of 6.1 μM for the ligand RA to Bos d 5 in accordance with literature. Incubation of PBMCs with apo-Bos d 5 for 48 h did not affect the composition of CD3 + , CD4 + and CD8 + positive cells, but strongly upregulated IFN-γ, IL-13 and IL-10 ($P < 0.001$) levels. In contrast, stimulations with holo-Bos d 5 led to a decrease in CD4 + positive cells and to a pronounced decrease in all three cytokines (IL-10, $P < 0.05$). This phenomenon was dependent on the Bos d 5/RA complex, as treatment with RA alone only slightly enhanced numbers of all T-cell subsets and did not influence cytokine levels.
Conclusion: Our data suggest that holo-Bos d 5, with RA in its pocket, has a pronounced immunosuppressive effect on the CD4 + T-cellular and cytokine level. Proper loading of this major cows milk allergen may thus protect from subsequent allergic immune responses to it. The causative factors leading to insufficient load of milk proteins remains to be determined.

342
Neutrophils from allergic asthmatic patients produce and release metalloproteinase-9 upon direct exposure to allergens

Bellido Linares, V; Lopez Ruiz, C; Ventura, I; Vega, A; Chacon, P; Chamorro, C; Aroca, R; Puente, Y; Monteseirin, J
Hospital Universitario Virgen Macarena, UGC Intercentros de Alergologia, Sevilla, Spain

Background: Asthma is characterized by airway inflammation and remodelling in which matrix metalloproteinases (MMPs) play an important role. MMP-9 is the major MMP found in bronchoalveolar lavage fluids and bronchial biopsies from patients with allergic asthma after challenge, where it correlates with the count of neutrophils and macrophages. However, the cellular sources of MMP-9 in the inflammatory condition have not yet been clearly identified. This work was undertaken to analyse whether neutrophils may be a source of MMP-9 in the allergic asthma condition upon allergen challenge.
Method: Neutrophils from allergic asthmatic patients were *in vitro* stimulated and the levels of MMP-9 release were measured in the cell culture supernatants using enzyme-linked immunosorbent assay (ELISA) and zymography.
Results: We show that MMP-9 is released neutrophils but not eosinophils from allergic asthmatic patients in response to allergens to which the patients were sensitized. Neutrophils also released MMP-9 in response to anti-Ig E Abs, and agonist Abs against FcRI, FcRII/CD23 and galectin-3. Inhibitors of transcription and translation, actinomycin D and cycloheximide, partially cancelled this process, suggesting that MMP-9 is also de novo synthesized in response to stimuli.
Conclusion: These data demonstrate that the exposure of neutrophils to allergens leads to generation of MMP-9, which may then lead to remodelling in asthma.

343
Generation of allergen-specific T-cell stimulator cells to investigate coinhibitory pathways in allergy

Rosskopf, S; Jutz, S; Leitner, J; Candia, MR; Neunkirchner, A; Pickl, WF; Steinberger, P
Medical University of Vienna/Center for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Vienna, Austria

Background: More than 25% of the population suffers from IgE-mediated immune reactions (allergies) and its related symptoms. T cells have a crucial role in initiating and promoting allergies and their responses are tightly regulated by numerous activating and inhibitory signals. Currently, there is limited knowledge regarding the role of inhibitory pathways in allergen-specific T cells.
Method: To address this issue we have generated a novel type of an engineered, modular antigen-presenting cell that can present allergenic peptides on MHC class II molecules to T cells from allergic individuals. The major birch and mugwort pollen allergens Bet v 1 and Art v 1 were used as model allergens in our studies. The human K562 cell line was transfected to stably express HLA-DR1 or HLA-DR7 and fusion proteins of the invariant chain with allergenic peptides were expressed to endogenously load the MHC II molecules. Co-cultivation experiments with T cell reporter cell lines expressing allergen-specific T cell receptors were performed to compare external peptide with endogenous MHC II loading, to evaluate MHC loading enhancers MLE and MHC acid stripping

© 2015 The Authors

and also to investigate the antigen processing pathway. By expressing coinhbitory molecules on these cells we will be able to investigate their ability to down-modulate the response of allergen-specific CD4 + T cells in stimulation assays. Furthermore, we have established a flow cytometry-based proliferation assay to analyze the influence of coinhibitory molecules on allergen-specific human T cells. PBMCs of individuals allergic to cats, house dust mites, birch pollen, grass pollen and mugwort pollen were stimulated with the respective allergen extracts. The effects of costimulatory pathways were assessed using blocking antibodies to PD-1, PD-L1, CTLA-4, BTLA and LAG-3.

Results: Experiments with T cell reporter cell lines showed that overexpression of HLA-DM and addition of MLE increased the proliferatory capacity of exogenously loaded allergen-specific T cell stimulator cells. The flow cytometry-based proliferation assay indicated that blockade of PDL-1 strongly increased the proliferation of allergen-specific CD4 + T cells in response to allergen extracts.

Conclusion: Our system of stimulator cells is a promising tool to study the role of coinhibitory pathways in allergen-specific CD4 + T cells. Preliminary results showed that the PD-1 pathway has an important function in limiting T cell responses to allergens.

344
The fungal allergen Alt a 1 skews immune cells to Th2 response

Garrido-Arandia, M[1]; Gómez-Casado, C[1]; Roth-Walter, F[2]; Cubells-Baeza, N[1]; Pacios, L[3]; Singer, J[4]; Jensen-Jarolim, E[2]; Díaz-Perales, A[1]
[1]Center for Plant Biotechnology and Genomics, Technical University of Madrid, Madrid, Spain; [2]Comparative Medicine, Messerli Research Institute of the University of Veterinary Medicine, Vienna, Austria; [3]Department of Natural Resources and Systems, ETSI Montes, Technical University of Madrid, Madrid, Spain; [4]Comparative Immunology and Oncology, Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Background: Alt a 1 is a protein from *Alternaria alternata* spores, responsible for chronic asthma. It has been claimed that its crystal structure (a unique β–barrel found exclusively in fungi) has no equivalent in the Protein Data Bank.

Our purpose is to show that Alt a 1 has structural relationship with lipocalins, and thus the ability to bind iron-siderophore complexes which regulate its immuno-modulatory properties.

Method and results: We used *in silico* procedures consisting of three distinct structural alignment methods to identify the structural relationship between Alt a 1 and lipocalins. Absorbance measurements were employed to measure the ability of Alt a 1 to bind compounds that act as siderophores (quercetin) with K_d estimated in the range of μM.

The immuno-modulatory properties of Alt a 1 were characterized by incubation with human peripheral blood mononuclear cells and found to depend on uncomplexed or iron-siderophore (catechols) complexed forms. Uncomplexed Alt a 1 led to an increase in CD4 + cells and was capable of skewing the immune response by inducing apoptosis and production of IL13 (Th2) by CD4 + cells, whereas Alt a 1 in combination with iron-siderophore complex decreased the number of CD4 + but not of. CD8 + cells.

Conclusion: Our results are in accordance with previously published data on Bet v 1 and Bos d 5, and may provide the basis in the development of new immunotherapeutic strategies for Alt a 1.

345
Correlation between allergic rhinitis prevalence and immune responses of children in Ulsan, Korea: nested case-control study

Lee, J[1,2]; Sim, CS[1,2]; Oh, I-B[2]; Kim, A[2]; Kim, Y[1,2]
[1]Ulsan University Hospital, University of Ulsan College of Medicine, Occupational & Environmental Medicine, Ulsan, Korea; [2]Environmental Health Center, University of Ulsan College of Medicine, Ulsan, Korea

Background: This study aims to investigate the correlations between allergic rhinitis (AR) prevalence and immune responses for elementary school children in an industrial city, Ulsan of Korea and to identify major environmental risk factors associated with AR prevalence.

Method: A nested case-control study for evaluating the relationship between AR and its related cytokines was conducted for 339 schoolchildren living in different urban environments who were selected from a elementary student cohort that surveyed from 2009 to 2011 in Ulsan of Korea. Data on the physician-treated prevalence in the past 12 months and potential risk factors of AR were collected by a questionnaire from the 339 cases and controls. The logistic regression analysis was carried out with propensity score matched data (n = 180) to assess the influences of cytokines (IL-13, IL-33, IL-4 and IL-5) on AR prevalence and to determine which environmental factors affected AR.

Results: In univariate analysis, the AR prevalence was influenced by family history of AR (mother and sibling), environmental factors (odor condition and irritated symptom of air pollution), and indoor allergens (D. farinae and D. pteronyssinus). The t-test demonstrated that eosinophils, Immunoglobulin E (IgE), and interleukins (IL-13 and IL-5) were statistically significant different according to treatment of allergic rhinitis for the last 12 months. The results of the multiple logistic regression analysis showed that statistically significant association between several factors (such as irritated symptom of air pollution (OR: 4.075, CI: 1.735–9.568), IL-13 (OR: 0.825, CI: 0.734–0.928), odor condition (OR: 2.409, CI: 0.908–6.389), and AR history of siblings (OR: 2.217, CI: 0.999–4.921)) and the prevalence of AR was found after adjusting confounders.

Conclusion: These results suggest that the AR prevalence is significantly associated with cytokines level, genetic condition, and outdoor environmental factors. Although the living in polluted area and the genetic condition can contribute to the increased risk of childhood AR, cytokines level should be considered as important factor for treatment of AR in the last 12 months.

346
Dermatophagoides farina promote LPS induced inflammation by the functional mimicry with MD-2 in the human middle ear epithelial cell

Youn, CK[1]; Kim, YH[2]; Jung, SC[2]; Ji-Eun, L[2]
[1]Division of Natural Medical Science, Chosun University, Gwang-ju, Korea; [2]Department of Otorhinolaryngology, Head & Neck Surgery, Chosun University, Gwang-ju, Korea

Background: While otitis media with effusion (OME) is a well-known disease entity of a chronic inflammatory disease of the middle ear space characterized by the accumulation of fluid, but allergic otitis media is still not well- recognized. Previous investigations have suggested that the composition of the inflammatory substrate in the effusions of allergic otitis media is similar to the late-phase allergic response seen elsewhere in the respiratory tract, such as in asthma and in allergic rhinitis. In this study, we aimed to determine whether the prior treatment of Der f can effect on the inflammatory response induced by the subsequent LPS infection and which signaling pathway is involved.

Method: Primary human middle ear epithelial cells (HMEEC) were exposed to Der f crude body extract, LPS or both in different sequences, and the magnitude of each immunologic response produced by the HMEEC was compared. The mRNA expression level of mucin gene (MUC) 2, 5A, 5B, 8, GM-CSF, TNF-a, TLR 4 and MD 2 were evaluated by using real-time polymerase chain reaction (qRT-PCR). The MUC proteins level before and after knocking out the TLR 4 and MD2 via siRNA transfection were assessed by Western blot analysis. Accordingly, the

© 2015 The Authors

involved cell signaling pathway was evaluated by Western blot analysis and confocal microscopic image.

Results: The inflammatory response of cytokines (GM-CSF, TNF-a) and the expression of MUC 2, 5A, 5B and 8 were augmented by the pretreatment of Der f followed by LPS, however, sequential treatment of HMEEC with LPS and Der f or adding together at the same time did not induce the same amount of response. Moreover, lower dose of LPS was enough to elicit inflammatory response by the pretreatment of Der f. The MUC expression was decreased by prior knockdown of TLR4 with siRNA but not by the MD2-siRNA. The MUC proteins level were increased by the pretreatment of Der f followed by LPS and decreased by the treatment of SB203580 (p38 inhibitor) and Bay (NF-kB inhibitor). The nuclear factor kB (NF-kB) translocation was demonstrated in the pretreatment of Der f followed by LPS condition.

Conclusion: Theses results suggest that Der f may act as a substitute for MD2 and make a strong augmentative response to the subsequent LPS infection. There was an increase in p38 and NF-kB activation within human middle epithelial cells, suggesting an important role for the development of OME in patients with concealed allergy airway sensitization.

347

Immunomodulatory effects of long-term low strength static magnetic field exposure on human T lymphocytes function *in vitro*

Turuntas, V[1]; De Luka, SR[2]; Trbovich, AM[2]; Vasilijic, S[3]
[1]University Hospital Foca, Pediatrics, Foca, Bosnia and Herzegovina; [2]Department of Pathological Physiology, School of Medicine, University of Belgrade, Belgrade, Serbia; [3]Institute of Medical Research, Military Medical Academy, Belgrade, Serbia

Background: Unlike electromagnetic fields, for health effects from exposure to static magnetic fields (SMF), few studies have been carried out. Most of them are related to acute, high strenght SMF, measured in several units of tesla (T), trying to explain possible adverse health effects of exposure to magnetic resonance imaging. For effects of long term, low strength SMF, in the millitesla (mT) range, especially on immune cells, there is a just a few studies. Noboy has studied *in vitro* possible different biological effects of north (N) and south (S) magnetic pole so far. Only the N pole was used in all previous studies. The aim of this study was to investigate *in vitro* effect of long-term exposure of low strength SMF on human T lymphocytes proliferation, allostimulatory function of proffesional antigen-presenting dendritic cells (DC) and production of Th1, Th2 and Th17 interleukin and proinflammatory cytokine TNF-α.

Method: Cultures of allogenic purified T lymphocytes and human monocyte-derived dendritic cells (DC) induced to mature by lipopolysaccharide, were continuously exposed to 5 mT north and south pole of SMF. The samples were from healthy donors. Supernatants were assayed to quantify IFN-γ, IL-4, IL-17 and TNF-α by ELISA. T lymphocytes proliferation was induced with phytohemagglutinin (PHA).

Results: South pole of SMF increased release of IFN-γ (almost 50% compared to control) in DC culture supernatants, and not affected the level of IL-4 and IL-17. North pole of SMF produced no significant changes in cytokines level. SMF didn′t affect allogenic lymphocytes proliferation, but PHA stimulated lymphocytes proliferation was affected, dose and SMF pole dependently. Interestingly, SMF significantly increased levels of proinflammatory cytokine TNF-α in some cases, and decreased in others.

Conclusion: This is the first time in literature that has been shown *in vitro* different biological effect of N and S magnetic pole, what is unexplainable from the physics point of view. Not only this study will help better understanding effects of SMF on immune system, but also this selective effect on Th1 immune response could have potential therapeutic effects in different immune/allergy disorders.

348

Bixin inhibits *Blomia tropicalis*-induced Th2 cytokine production by PBMC from atopic patients

Meza, C[1]; Ocampo, Y[2]; Hernandez, L[1]; Martinez, B[1]; Franco, L[3]; Marrugo, J[1]
[1]University of Cartagena, Institute of Immunological Research, Cartagena, Colombia; [2]University of Cartagena, Doctorado en Ciencias Biomédicas, Cartagena, Colombia; [3]University of Cartagena, Facultad de Ciencias Químicas y Farmacéuticas, Cartagena, Colombia

Background: Bixin is the principal carotenoid from annatto (achiote), a pigment extracted from the seeds of Bixa Orellana L., which is widely used in Latin America as food color. Bixin has been shown antioxidant, antineoplastic and anticlastogenic properties. Although the immunomodulatory effect of other carotenoids such as curcumin is well documented, the effect of Bixin on the production of Th2 cytokines in atopic patients has not been determined. In the present study, we evaluated the effect of Bixin on the production of IL-5 and IL-13 in peripheral mononuclear blood cells (PBMC) from allergic patients in response to house dust mites.

Method: Freshly isolated PBMC of five atopic patients with asthma and/or allergic rhinitis with positive skin prick test and specific IgE to B. tropicalis (Bt), with a mean age of 41.8 years (19–65) were cultured in RPMI-1640 (10% FBS and penicillin-streptomycin 1%) in the presence of different concentrations of Bixin (25–1.56 μg/mL), and stimulated with PHA/PMA, calcium ionophore, Bt extract (10 μg/mL) and IL-2r, for 72 h and 7 days. After these times, supernatants were collected and levels of IL-5 and IL-13 were measured by ELISA. Cytotoxicity and cell proliferation were evaluated by MTT and incorporation of CFSE by flow cytometry, respectively. All subjects consented to participate in the study. Statistical analysis was performed using lineal regression and one way ANOVA, p values below 0.05 were considered significant.

Results: Our results showed that Bixin reduced significantly the production of IL-5 and IL-13 by PBMC from atopic patients ($P < 0.005$) stimulated with allergen (Bt) and non-specific stimulus (PHA, PMA, Calcium Ionophore). The inhibitory effect of Bixin in IL-5 production by Bt stimulated PBMC was partially reversed by IL-2r. Bixin showed no cytotoxic or inhibitory effect on cell proliferation at tested concentrations.

Conclusion: Bixin could be an important modulator of the TH2 type immune response. Its use in the diet might have an influence in the prevalence of allergic diseases.

© 2015 The Authors

Poster Discussion Session PDS 11

Origins of asthma

349

Association between BclI polymorphism of glucocorticoid receptor gene and obesity in patients with bronchial asthma

Kmyta, V[1]; Orlovskiy, V[2]; Prystupa, L[1]
[1]Sumy State University, Medical Institute, Internal Medicine Department Postgraduate Education, Sumy, Ukraine; [2]Sumy State University, Medical Institute, Family Medicine Department, Sumy, Ukraine

Background: It was reported in the literature that the BclI polymorphism of glucocorticoid receptor (GR) gene was associated with the accumulation of visceral fat, which is a risk factor for atherosclerosis, cardiovascular diseases, obesity and bronchial asthma (BA). The objective of this investigation was to analyze possible association between BclI polymorphism of GR gene and obesity in patients with BA.
Method: 188 patients with BA and 95 apparently healthy adult individuals have been examined. We measured body mass, height, body mass index (BMI), and determined the ratio of fat centralization (RFC). The determination of Bcl1 polymorphism (rs41423247) was performed by means of polymerase chain reaction with subsequent analysis of restriction fragment length polymorphism (according to the instructions of Fleury I. et al.) with modifications.
Results: Among patients with BA, normal body weight (NBW) was found in 50.5% of individuals, overweight - 15.4%, obesity - 34%. In the control group 76.8%, 20%, 3.2% respectively ($P = 0.001$). The patients with BA had higher BMI parameter, than the individuals in the control group (27.2 ± 0.44 kg/m^2 vs $23.5 \pm .29$ kg/m^2; $P < 0.001$). The obtained data showed that BMI values didn't significantly differ in carriers with different genotypes for BclI polymorphism in the control group ($P = 0.91$). However, dependence between BclI polymorphism and BMI parameters was found in patients with BA: G/G genotype carriers had higher BMI (31.3 ± 0.74 kg/m^2), than representatives with others genotypes. Comparing the groups, we found out that BMI values didn't differ significantly in C/C and C/G genotypes carriers. However, G/G homozygotes among BA patients had higher BMI, than those in the control group: 31.3 ± 0.74 kg/m^2 vs 23.1 ± 0.61 kg/m^2 ($P = 0.0001$).

Thus, the analysis of genotypes distribution depending on the ratio of fat centralization in patients with asthma showed a statistically significant difference ($\chi^2 = 25.5$; $P = 0.001$). It was found out in the patients with BA that G/G homozygotes have higher ratio of fat centralization, than the major allele homozygotes or heterozygotes.
Conclusion: Genotypes distribution for BclI polymorphism in patients with BA showed a statistically significant difference between patients with different BMI and RFC unlike the control group.

350

Genetic variants regulate the promoter activity of PTGDR

García-Sánchez, A[1]; Marcos-Vadillo, E[2]; Sanz, C[3]; García-Solaesa, V[4]; Moreno-Rodilla, E[1,4]; Muñoz-Bellido, F[4]; Macias, E[4]; Isidoro-García, M[2,5]; Dávila, I[1,4]
[1]Universidad de Salamanca, Departamento de Pediatría, Salamanca, Spain; [2]Complejo Asistencial Universitario de Salamanca, Servicio de Bioquímica Clínica, Salamanca, Spain; [3]Universidad de Salamanca, Departamento de Microbiología, Salamanca, Spain; [4]Complejo Asistencial Universitario de Salamanca, Servicio de Alergia, Salamanca, Spain; [5]Universidad de Salamanca, Departamento de Medicina, Salamanca, Spain

Background: PTGDR, a receptor of PGD2, has been proposed as a candidate gene in allergy. The objective of this work was to interrogate the contribution of PTGDR SNPs to PTGDR expression in cell culture.
Method: Four PTGDR reporter constructs of 700 bp were created by PCR amplification of genomic DNA from homozygous subjects who had the following alternative haplotypes (positions -613, -549, -441 and -197): CTCT, CCCT, TCCT and CCCC. The fragments were cloned in the pGL3-basic vector. The cloned regions were confirmed by sequencing.
Results: The haplotype with the CTCT sequence (-613C, -549T, -441C, and -197T) was significantly associated with the lowest reporter activity, while the rest of haplotypes, CCCC, CCCT and TCCT, showed a remarkable reporter activity increase of 3 magnitude orders. CTCT: 0.0 ± 0.0; CCCC: 3.11 ± 0.17; CCCT: 3.06 ± 0.17; TCCT: 3.08 ± 0.24 logRLU, (P -value< 0.0001.
Conclusion: The findings presented in this study suggest a differential PTGDR promoter activity according to genetic variants. Response to new treatments based on PTGDR modulation could depend on the genetic background in allergic patients.

351

A polymorphism in the *TBX21* gene is associated with inadequate response to common asthma treatment in children

Banic, I[1]; Rijavec, M[2]; Plavec, D[1]; Korosec, P[2]; Turkalj, M[1]
[1]Children's Hospital Srebrnjak, Zagreb, Croatia; [2]University Clinic for Respiratory and Allergic Diseases Golnik, Golnik, Slovenia

Background: Inhaled corticosteroids (ICS) in combination with long-acting beta-adrenoreceptor agonists (LABA) are commonly used for asthma control and the level of response to such treatment is characterized by high intra-individual repeatability and high inter-individual variability with up to 50% of patients with asthma having poor or even no response to treatment. Polymorphisms in *TBX21*, a gene important for the biological action of corticosteroids, could be associated with treatment response in asthmatics. Our aim was to determine treatment outcomes in children with asthma in association with *TBX21* (rs991008) genotype.
Method: We recruited 365 children with asthma and clinically assessed their health status and treatment outcome over 4 years at 3 time points: at the point of diagnosis, after 3 years and after 4 years. Genetic material was extracted from peripheral whole blood samples and these were then genotyped for rs9910408 polymorphism in the *TBX21* gene.
Results: 96 children were treated with combination therapy (ICS+LABA) continuously for at least 4 years. When treatment success was assessed by changes in lung function parameters (Maximum expiratory flow at 50%, MEF50), the frequency of GG genotype was significantly higher in bad responders ($P = 0.0409$) compared to the AA genotype. This genotype related response was even more evident when comparing patients with moderate and bad response to treatment with patients with good response: the frequency of GG geno-

© 2015 The Authors

type was also significantly higher in moderate and bad responders vs good responders ($P = 0.0152$). The levels of response to treatment were defined as relative changes in MEF50 between clinical visits (assessments) at 3 time points.

Conclusion: Our results showed that inadequate levels of response to treatment with ICS in combination with LABA in children with asthma, assessed as changes in lung function parameters (MEF50), are associated with a polymorphism in the *TBX21* gene.

352
The influence of obesity on the gene expression in obese asthma patients

Gruchała-Niedoszytko, M[1]; Niedoszytko, M[2]; van der Vlies, P[3]; Małgorzewicz, S[1]; Niedoszytko, P[4]; Jassem, E[2]; Górska, A[2]
[1]Clinical Nutrition, Medical University of Gdansk, Gdansk, Poland; [2]Allergology, Medical University of Gdansk, Gdansk, Poland; [3]Genetics, University Medical Center Groningen, Groningen, Netherlands; [4]Rehabilitation, Medical University of Gdansk, Gdansk, Poland

Background: Obesity significantly impairs asthma control. Patients with asthma and obesity present more severe symptoms and increased medication use. The aim of this study was to evaluate genes which are differently expressed the peripheral blood of the asthmatic patients with obesity asthmatic, patients with normal body mass and obese patients. Secondly we wanted to know if the analysis of the gene expression in the peripheral blood may be helpful in the differential diagnosis of obese patients who present symptoms similar to asthma.

Method: The group of 17 patients with asthma (6 obese and 9 normal weight) and 13 obese patients where asthma was excluded were studied. Whole genome gene expression analysis was performed on RNA samples isolated from all blood cells in whole blood using the Illumina Human HT-12_V3_expression arrays. Data analysis was performed using GeneSpring.

Results: Comparison of the gene expression profiles in patients with asthma and obesity vs patients with asthma and normal body mass revealed a log2 fold change difference >2 in gene expression was found in 19 transcripts. A significant difference corrected for multiple testing was found in 6 of transcripts analysed. The study on the gene expression profile comparing patients with obesity without asthma and asthmatic patients with normal body mass revealed a $\log_2$ fold change difference >2 in gene expression was found in 23 transcripts. A significant difference corrected for multiple testing was present in all 23 entities.

The analysis of the genes differently expressed revealed the common group of transcripts in both comparisons which may be related to the increased body mass (PI3, LOC100008589, RPS6KA3, LOC441763, IFIT1, LOC100133565).

Conclusion: The results of our study show significant differences in the gene expression among patients with obesity and asthma as well as patients with obesity od normal weighr patients. Such an analysis may be an useful tool in the differential diagnosis of symptoms in obese patients with asthma.

353
The effect of PIV-3 infection on miRNA profile in nasal epithelial cells from patients with nonsteroidal anti-inflammatory drugs (NSAIDs) exacerbated respiratory disease

Pawelczyk, M[1]; Globinska, A[1]; Olszewska-Ziaber, A[1]; Moskwa, S[2]; Kowalski, ML[1]
[1]Department of Immunology, Rheumatology and Allergy, Medical University of Lodz, Lodz, Poland; [2]Inter-Faculty Department of Microbiology and Laboratory Medical Immunology, Medical University of Lodz, Lodz, Poland

Background: MicroRNAs are small non-coding RNA molecules, which negatively regulate expression of many genes, including those associated with immunological response to infections. NSAIDs Exacerbated Respiratory Disease (NERD) is a complex asthma phenotype associated with chronic rhinosinusits and nasal polyps. Viral infections are regarded as important factors in the etiology of NERD and in the pathogenesis of asthma/rhinosinusitis exacerbations in these patients.

The aim of the study was to compare miRNA expression profile in patients with NERD as compared to healthy subjects and to assess whether miRNA profile can be modified in response to infection with virus PIV-3.

Method: Nasal scrapings were collected from 3 patients with NERD and from 3 healthy individuals in order to set primary epithelial cell cultures. Epithelial cells were either infected with PIV-3 (0.5 MOI) or treated with medium. The profile of 2087 miRNAs was assessed 48 h post infection (miRCURY LNA™ microRNA Array, Exiqon, Denmark).

Results: Out of 588 detected miRNAs, three (miR-4417, miR-203a, miR-222-3p) were significantly downregulated ($P = 0.03$) in epithelial cells of patients with NERD. Moreover, in patients with NERD expression of five miRNAs (miR-4762-5p, miR-1185-2-3p, miR-4687-3p, miR-3121-3p, miR-4421) was significantly higher ($P < 0.01$) after infection with PIV-3 when compared with cells treated only with medium. In contrast, in healthy individuals the miRNAs expression profile was not changed in response to viral infection.

Conclusion: The results of miRNA profiling allowed for an initial selection of candidate genes for further analysis aiming at identification of miRNAs associated with NERD and being modified in response to viral infections.

354
Leptin and adiponectin polymorpisms in obese and lean asthmatics

Ozbek, O[1]; Koksal, B[1]; Atac, B[2]; Yazici, C[3]
[1]Pediatric Allergy, Baskent University Faculty of Medicine, Ankara, Turkey; [2]Medical Biology, Baskent University Faculty of Medicine, Ankara, Turkey; [3]Biostatistics, Baskent University Faculty of Medicine, Ankara, Turkey

Background: Increase in the development of asthma in obese individuals is an area of growing research. Active adipose tissue secretes adipokines, such as leptin (pro-inflammatory) and adiponectin (anti-inflammatory). 3′UTR A/G and -2549 A/G leptin gene polymorphisms have been found to be related with asthma and the latter also with BMI. Adiponectin -11377 CC homozygot form has been defined to be a risk for obesity. The aim of this study was to evaluate the polymorphisms of leptin and adiponectin in children with asthma and/or obesity and their relations with leptin and adiponectin levels.

Method: 402 children were enrolled in study. Asthma was defined according to GINA criteria and children with a BMI above the 95th percentile for age and sex were defined as obese. Patients were investigated in four groups. Group 1: Obese children with asthma ($n = 76$) Group 2: Lean children with asthma ($n = 105$) Group 3: Obese children without asthma ($n = 96$), Group 4: Lean healthy children ($n = 125$). Serum leptin and adiponectin levels were measured by ELISA. Genomic DNA was amplified by polymerase chain reaction (PCR).

Results: TT polymorphism for leptin and CC polymorphism for adiponectin were more frequent than other polymorphisms in all groups. Frequency of leptin and adiponectin polymorphisms did not show difference between groups. No association was found between leptin/adiponectin polymorphisms and leptin/adiponectin levels. Mean leptin levels of obese asthmatics were significantly higher (13.1 ± 9.1) than lean asthmatics (3.7 ± 4.4) ($P < 0.001$). Serum adiponectin levels of lean asthmatics (16 ± 7.1) were significantly higher than obese asthmatics (12.1 ± 6.9) and lean healthy children (13.2 ± 5.9) ($P < 0.001$ and $P < 0.05$, respectively). FEV_1/FVC ratio was posi-

© 2015 The Authors

tively correlated (Spearman's rho: 0.322, $P < 0.01$) with adiponectin levels and FEF_{25-75} levels were inversely correlated with serum leptin levels in obese asthmatics (Spearman's rho: −0.234, $P < 0.05$).
Conclusion: No association was found between leptin/adiponectin polymorhisms and leptin/adiponectin levels. Further investigations including larger study groups are needed. Not only leptin but also adiponectin may have effect on airways in obese asthmatic children.

355
Th17 cell subsets, steroid sensitivity, and asthma severity

McAlees, JW; Lewkowich, IP
Division of Immunobiology, Cincinnati Children's Hospital Medical Center, Cincinnati, United States

Allergic asthma is an inflammatory lung disease classically characterized by airway eosinophilia, airway hyperresponsiveness (AHR) and a maladaptive Th2 dominated immune response to normally innocuous environmental aeroallergens. However, there is a growing appreciation that the Th2 paradigm does not explain the full spectrum of asthma severity. Indeed, individuals with severe asthma often display neutrophilic inflammation, suggesting that mild and severe disease may be regulated by different processes. Consistent with this, human and animal studies correlate asthma severity and IL-17A levels, and we have found that IL-17A acts to enhance the biological activity of IL-13. However, a number of mouse studies also describe a protective role for Th17 cytokines, suggesting a complex relationship between IL-17A and asthma severity.

In the current study we hypothesize that the role of IL-17A in allergic asthma may be related to its cellular source. Recently, in both humans and animals, two functionally distinct IL-17A-producing CD4 + T cell populations were identified. "Regulatory" Th17 (rTh17) cells control normal homeostatic barrier function at mucosal sites while "pro-inflammatory" Th17 (pTh17) cells arise under inflammatory conditions. Using a mouse model of asthma, we show that in A/J mice, airway delivery of HDM drives severe AHR and selective expansion of pTh17 cells. In contrast, similar treatment in C3H/HeJ mice drives mild, AHR associated with lower IL-17A production and expansion of rTh17 cells. Assessment of steroid sensitivity demonstrated that in C3H mice both AHR and IL-17A production were completely abrogated by steroids. In contrast, steroid exposure had no impact on either AHR or IL-17A production in A/J animals. Interestingly, IL-13 production was similarly reduced in both A/J and C3H mice, suggesting that A/J mice can respond to steroids. Supporting a link between asthma severity, steroid sensitivity and the type of Th17 cell response, *in vitro*-skewed pTh17 cells were steroid resistant and factors from these cells demonstrate increased capacity to synergize with IL-13. In contrast, *in vitro*-skewed rTh17 cells were highly sensitive to inhibition via steroids, and factors produced by rTh17 cells demonstrate reduced synergism with IL-13. Collectively, these data suggest that different types of Th17 cells may differentially contribute to asthma pathogenesis.

356
Is adenoidectomy and/or tonsillectomy a risk factor for allergic diseases and asthma seen in adult patients?

Akinci, N[1]; Soyyigit, S[1]; Celebi Sozener, Z[1]; Mungan, D[1]; Kose, SK[2]; Misirligil, Z[1]
[1]Department of Respiratory Medicine, Division of Immunology and Allergic Diseases, Ankara University School of Medicine, Ankara, Turkey; [2]Department of Biostatistics, Ankara University School of Medicine, Ankara, Turkey

Background: Removing the tonsils and adenoids which have roles in humoral and cellular immune system in childhood was asserted to increase the risk of allergic diseases in adulthood. However there is lack of data on this subject. Our objective was to search the relationship between adenoidectomy and/or tonsillectomy in childhood and allergic diseases in adulthood to evaluate the relative risks.
Method: A survey which questioned the history of adenoidectomy and/or tonsillectomy was given to patients who were followed in our department between January-June 2014 with diagnoses of asthma, allergic rhinitis, urticaria-angioedema, drug allergy, food allergy and venom allergy and accepted to participate in the research. Demographic and clinical information of adenoidectomized and/or tonsillectomized patients and the relationship & risk ratios between atopy, asthma, allergic rhinitis and other allergic diseases were studied.
Results: 510 (F/M: 379/131) patients were included in the study; 248 having asthma, 205 with rhinitis, 82 having drug allergy, 24 having food allergy, 14 with venom allergy and 73 with urticaria. Sixty five (12.7%) of these patients had tonsillectomy or adenoidectomy. Among them, 41 had asthma, 33 had allergic rhinitis and 28 had other allergic diseases. There was no relation between history of atopy and tonsillectomy or adenoidectomy ($P = 0.129$). However there was a positive relation between presence of asthma and having history of tonsillectomy or adenoidectomy under the age of 15 ($P = 0.020$). The risk of asthma was determined to be increased by 1.96 times if adenoidectomized or tonsillectomized (CI:1.14–3.36). No association was found between atopic and non-atopic asthmatic patients in relation to adenoidectomy or tonsillectomy ($P = 0.46$). There was no relationship between allergic rhinitis or other allergic diseases and adenoidectomy or tonsillectomy.
Conclusion: Our data show that having tonsillectomy/adenoidectomy in younger ages may increase the risk of asthma development in adulthood.

357
Basophils membrane expression of Th2 epithelial cytokines receptors in asthma: relationship with phenotype and severity

Boita, M; Nebiolo, F; Raie, A; Bussolino, C; Fornero, M; Bucca, C; Heffler, E; Rolla, G
Medical Science, University of Turin, Turin, Italy

Background: Basophils, together with mast cells and eosinophils, are effector cells most often associated with airway inflammation in patients with asthma and a marker of basophil activation and differentiation (CD203c) has been related to asthma exacerbation. Basophils can have also an important role in the initiation of Th2 inflammation, as these cells may be activated directly by tissue cytokines (IL-25, IL-33 and TSLP) released by epithelial cells in response to damage signals from allergens, bacterials, virus or pollutants. Airway epithelial damage is frequently observed in asthma and tissue cytokines have been reported to be overexpressed in bronchial biopsies of asthmatic patients. We investigated whether IL-25, IL-33 and TSLP receptors expression levels on basophil membrane are associated with the severity and control of asthma.
Method: 20 patients with asthma (12 mild/moderate, 8 severe), 15 with eosinophilic and 5 with non eosinophilic asthma have been enrolled in the study. Basophils membrane expression of IL25 R, TSLP R and IL33 R was analyzed before and after IgE, fMLP, LPS, LTA-SA (Lipoteicoic Acid from S. Aureus) stimulation.
Results: TSLP R and IL25 R expression was significantly increased in eosinophilic compared to non-eosinophilic asthma at baseline (TSLP R: 5.4 ± 3 vs 2.1 ± 1.7, $P = 0.01$; IL25 R: 13 ± 6.7 vs 5.9 ± 2.5, $P = 0.01$) and after IgE stimulation (TSLP R: 5.9 ± 2.7 vs 2.6 ± 1.4, $P = 0.02$; IL25 R: 34.2 ± 15.5 vs 19.2 ± 3.4, $P = 0.05$). Atopy and severity of asthma were associated with lower (9.9 ± 4.3 in atopic vs 18.1 ± 7.3 non atopic, $P = 0.02$) and

© 2015 The Authors

higher (8.8 ± 4.3 in mild/moderate vs 15.6 ± 7.8 in severe, $P = 0.05$) IL25 R expression respectively. IL33 R was not detectable.

Conclusion: The high expression of epithelial cytokines (IL-25 and TSLP) receptors on basophils of patients with eosinophilic asthma suggests the important role of tissue cytokines in driving Th2 immunity. Our data suggest that IL25R expression could be used as biomarker of asthma severity.

358
B cell activating factor (BAFF) in severe asthma: a possible regulator of eosinophilic inflammation

Samitas, K[1,2,3]; Malmhäll, C[1]; Rådinger, M[1]; Lu, Y[1]; Deák, T[1]; Semitekolou, M[2]; Gaga, M[3]; Sjöstrand, M[1]; Lötvall, J[1]; Bossios, A[1]
[1]Krefting Research Centre, Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [2]Cellular Immunology Laboratory, Division of Cell Biology, Biomedical Research Foundation of the Academy of Athens, Athens, Greece; [3]Athens Chest Hospital "Sotiria", 7th Respiratory Medicine Dept. and Asthma Center, Athens, Greece

Background: Asthma is a chronic inflammatory airway disease, in which many cells and cellular elements play a role. Allergic asthma is characterized among others by eosinophilia, a hallmark of asthma pathogenesis, and IgE produced by B cells. B cell activating factor (BAFF) is a key regulator of B lymphocyte development required for B cell survival, differentiation and maturation. Earlier studies have shown that BAFF is increased in the airways upon allergen challenge. However, its role in the pathogenesis of asthma, and severe asthma in particular, remains unclear.

The aim of this study was to examine BAFF levels in patients with asthma of varying severity and investigate its role in the development of eosinophilic inflammation using a murine model of allergic inflammation.

Method: BAFF levels were measured by means of ELISA in the bronchoalveolar lavage fluid (BALF) of non-atopic healthy controls (HC; $n = 14$) and patients with mild-moderate (MMA; $n = 11$) and severe asthma (SA; $n = 19$). Ovalbumin (OVA)-sensitized BALB/c mice were exposed intranasally to OVA or PBS for 5 days. In another set of experiments, OVA sensitized mice received *i.n.* instillations of BAFF receptor (BAFFR)-Ig fusion chimeric protein, 1 h prior to OVA exposure for 5 days. Lung tissue was harvested 24 h after final allergen exposure in both models. Cytospins of lung single-cell suspension were stained with May-Grünwald Giemsa for eosinophil count and BAFF levels in lung cell lysate were analyzed.

Results: BAFF is significantly increased in the BALF of severe asthmatics relative to healthy controls (SA: 36.62 pg/ml, MMA: 31.63 pg/ml and HC: 20.35 pg/ml, $P < 0.01$ SA vs HC). OVA exposure result in lung eosinophilic inflammation (lung eosinophils: 41.3% in OVA/OVA, 4.3% in OVA/PBS, $P < 0.05$) BAFF is increased in the lung in OVA sensitized mice after allergen challenge compared to control mice (BAFF: 16998 pg/g lung tissue in OVA/OVA, 3867 pg BAFF/g lung tissue in OVA/PBS, $P < 0.05$). Blocking BAFFR in the lung *in-vivo* decrease eosinophilic airway inflammation (lung eosinophils: 49.62% in OVA/OVA with control protein, 38.43% in OVA/OVA with BAFFR-Ig fusion, $P < 0.05$).

Conclusion: Our data support the concept that BAFF may play a role in severe asthma pathogenesis partly through the regulation of eosinophilic inflammation.

359
Airway and systemic inflammation in obese children with asthma

Vezir, E[1]; Misirlioglu, ED[1]; Toyran, M[1]; Civelek, E[1]; Capanoglu, M[1]; Demirel, F[2]; Ozguner, M[3]; Karakus, E[4]; Kocabas, CN[5]
[1]Pediatric Allergy and Clinic Immunology, Ankara Children's Hematology Oncology Education and Research Hospital, Ankara, Turkey; [2]Pediatric Endocrinology, Ankara Children's Hematology Oncology Education and Research Hospital, Ankara, Turkey; [3]The Bone Marrow Transplant Unit, Ankara Children's Hematology Oncology Education and Research Hospital, Ankara, Turkey; [4]Patology, Ankara Children's Hematology Oncology Education and Research Hospital, Ankara, Turkey; [5]Pediatric Allergy and Clinic Immunology, Mugla Sitki Kocman University Faculty of Medicine, Mugla, Turkey

Background: Obesity and asthma are common conditions and associated, but the mechanisms of the association has not been comprehensively described in children. The purpose of the present study was to compare airway and systemic inflammation in obese and nonobese astmatic children and controls.

Method: In this prospective, cross-sectional study obese (OA) ($n = 46$) and nonobese (NOA) ($n = 45$) children with asthma, and obese (OC) ($n = 26$) and nonobese (NOC) ($n = 29$) healthy controls were evaluated. Induced sputum and fasting blood samples were analysed for inflammatory markers. Patients who had ≥3% eosinophils in sputum are defined as "eosinophilic asthma". Between-group differences in spirometric values, induced sputum cell counts, and biomarkers of inflammation. [Compleman3(C3), Compleman4(C4), C-Reactive Protein (CRP)] and vitamin D in blood were measured.

Results: Patients in the obese asthma group had higher C3 (median: 121 mg/dl vs 139 mg/dl $P < 0.001$), C4 (median:20.2 mg/dl vs 25.5 mg/dl $P < 0.001$) and CRP(median:0.15 mg/dl vs 0.37 mg/dl $P < 0.001$) levels than NOA groups. Eosinophilic asthma was more prevalent among NOA (66.7%) vs OA (47.6%) but the difference was not statistically significant. Patients in NOA and AO groups were not different in terms of the distrubituon of cells in sputum, spirometric parameters and vitamin D levels.

Conclusion: Obesity was not associated with significantly altered airway inflammation in asthmatic children. Biomarkers of systemic inflamation were higher among OA patients than NOA patients.

360
Changes in total and specific IgE following treatment with mebendazole in patients with persistent asthma and IgE to Ascaris lumbricoides

Qualizza, R[1]; Incorvaia, C[1]; Manoli, S[1]; Maraschini, A[2]
[1]Istituti Clinici di Perfezionamento, Allergy Department, Milano, Italy; [2]IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico, Diagnostic Services Department, Milano, Italy

Background: Recent studies have reported that infection from Ascaris lumbricoides may be associated with high IgE levels and asthma. This study aims at evaluating the changes in total and Ascaris-specific IgE, as well as in control of asthma, following anti-helminthic therapy in patients with asthma poorly controlled with standard drug treatment.

Method: Eight adult patients with persistent asthma and high levels of total and Ascaris-specific IgE antibodies were included in the study. Though they had been treated by associations of inhaled corticosteroids and long-acting beta-agonists, they continued to present asthma exacerbations and were referred to us for parasitological evaluation. All patients were treated by mebendazole one tablet 100 mg b.i.d for 3 days, based on two courses with a 20-day interval. After 6 and 12 months, in case of persistent positivity, 2 other courses of mebendazole were prescribed, repeated after 20 days.

Results: The mean value of total IgE increased after 6 months of treatment from 1380 to 2010 kU/L but decreased after 12 months to 930 kU/L. Similarly, Ascaris-specific IgE increased from 3.5 to 5.8 kU/L after 6 months and decreased to 1.3 kU/L after 12 months. Clinically, the mean number of exacerbations decreased from 2.75 before treatment to 0.37 after treatment with mebendazole.

© 2015 The Authors

Conclusion: These findings confirm the association between Ascaris infestation and uncontrolled asthma and show that antiparasitic therapy remarkably reduces the high levels of total and specific IgE for A. lumbricoides and achieves the clinical control of the disease.

361
Changes in IL-9, IL-17, and IFN-γ and dendritic cells and their TLRs of peripheral blood in asthmatic children with mycoplasma pneumoniae infection

Shao, L
Renji Hospital, Shanghai Jiaotong University School of Medicine, Allergy, Shanghai, China

MP infection in children with asthma led to a more severe allergic state compared with non-MP infected group. The infection rate of children with asthma was higher than the other groups, suggesting that being asthmatic is a predisposing factor for MP infection and that the infection itself is an important co-factor in the disease progress of asthma. The number of DCs and its expression of TLR2 and TLR4 were compared in 22 asthmatic patients with MP infection, 22 asthmatic patients without MP infection, and 17 normal children as control. The percentages of DCs in the peripheral blood of the three groups exhibited significant difference between asthmatic children with MP infection and control, and asthmatic children without MP and control ($P < 0.05$), whereas no difference was found between asthmatic children with and without MP infection. The asthmatic children with MP infection group showed an increase expression of TLR-2 and TLR-4 on DC($P < 0.01$). Asthmatic patients infected with MP showed that DCs and its TLRs (TLR-2 and TLR-4) may play an important role in the asthma pathogenesis with MP infection. The cytokines of the T cell subsets in asthmatic children with MP infection showed a significant increase of IL-9($P < 0.01$) and a decrease of INF-γ($P < 0.05$) post-MP infection, and the IL-17 level remained stable($P > 0.05$), indicating a shift of Th1/Th9 with the MP infection.

362
Monocyte and granulocyte expression of Toll-like receptor 4 and endotoxin binding in patients with moderate to severe asthma

Beloglazov, VA[1]; Popenko, YO[1]; Gordienko, AI[1]; DuBuske, LM[2,3]
[1]Crimean State Medical University, Simferopol, Ukraine; [2]Immunology Research Institute of New England, Gardner, MA, United States; [3]George Washington University, MFA, Washington, DC, United States

Background: Peripheral blood monocytes and granulocytes may have abnormal expression of TLR-4 and endotoxin-binding potential in patients with moderate to severe asthma (BA).

Methods: Twenty two patients with moderate to severe asthma were assessed includiung 8 males (36%) 14 (64%) females who received combination therapy with inhaled glucocorticosteroids (fluticasone propionate) and long-acting β2-agonist (salmeterol). Patients were divided into:

(a)Group 1–12 patients with moderate asthma with Asthma Control Questionnaire (ACQ) mean score 2.8 ± 0.3 and
(b)Group 2–10 patients with severe asthma with ACQ mean score 4.1 ± 0.2. The control group was 10 healthy donors.

Expression of TLR-4 on peripheral blood monocytes and granulocytes was measured using the monoclonal antibody Anti-Human CD284 (TLR-4), conjugated with phycoerythrin FITS (Clone HTA125, eBioscience Inc., USA). Endotoxin-binding potentials (ET-BP) of monocytes and granulocytes in peripheral blood were determined using a conjugate of LPS Escherichia coli with FITS assessed by flow cytometry.

Results: Levels of expression of TLR-4 on monocytes in patients with moderate and severe asthma was 2.5 fold greater than in C ($P < 0.01$) but the level of TLR-4 expression on granulocytes was less than in C ($P < 0.01$). Levels of expression TLR-4 on granulocytes in patients with severe asthma was 1.2 fold greater than in patients with moderate asthma ($P < 0.01$), but endotoxin-binding potential was similar to C ($P > 0.05$).

Conclusions: Increased stimulation of TLR-4 on monocytes by endotoxin may occur in moderate to severe asthma with normal endotoxin-binding potential and TLR-4 expression by granulocytes. This suggests that the impact of endotoxin in moderate to severe asthma may largely be monocyte dependent.

© 2015 The Authors

Poster Discussion Session PDS 12

Management of asthma

363

Bronchial thermoplasty: a therpeutic option for severe allergic uncontrolled asthma

Aubier, M[1]; Dombret, M-C[1]; Thabut, G[2]; Taille, C[1]; Marina, P[3]; Chanez, P[4]
[1]Faculté de Médecine Paris Diderot, Hôpital Bichat, Pneumologie A, Paris, France; [2]Faculté de Médecine Paris Diderot, Hôpital Bichat, Pneumologie B, Paris, France; [3]Inserm UMR-S 1152 Faculté de Medecine Paris Diderot, Paris, France; [4]Hôpital Nord, Maladies Respiratoires, Universté de la Mediterranée, Marseille, France

Background: Bronchial thermoplasty (BT) is a procedure involving delivery of controlled radiofrequency-generated activations via a catheter inserted into the bronchial tree through a flexible bronchoscope. This procedure aims to reduce airway smooth muscle (ASM) mass with the goal of diminishing bronchial constriction and ameliorating asthma symptoms. Published studies of BT have addressed patients over a broad range of asthma severity, from mild to severe, but not those at the extreme end of the severity spectrum. The current study was aimed at determining the effect of BT in uncontrolled patients with severe allergic refractory asthma.
Methods: Ten subjects with asthma who met the ATS workshop criteria for severe refractory asthma were recruited at Bichat University Hospital, Paris, France. All had uncontrolled asthma as assessed by an Asthma Control Test (ACT) score of $\leq$10 despite high-dose inhaled corticosteroid ($\geq$1000 μg fluticasone propionate per day) and long-acting beta-2 agonist with oral prednisone (7 patients) and leukotriene modifiers (6 patients). All patients had 3 or more severe exacerbations within the last 12 months. Patients who met the criteria (7 patients) received omalizumab for 6 months without successful clinical outcome. All patients underwent 3 sessions of BT (ALAIR, Boston Scientific, MA), separated by one-month intervals.
Results: One year after BT, important clinical improvement was noted in the majority of patients. The number of severe exacerbations requiring corticosteroid courses were reduced from 9.3 ± 6.3 in the year before BT to 0.8 ± 1.0 ($p \leq 0.0001$). An improvement in asthma control was also noted, with ACT scores of 9.1 ± 2.7 and 15.5 ± 4.3 ($p \leq 0.0001$) for the year before and after BT, respectively. Improvement in asthma quality of life was noted with AQLQ scores of 3.52 ± 0.9 and 4.68 ± 1.2 ($p \leq 0.001$) before and after BT, respectively. Five of the 7 patients (71%) who required oral corticosteroids (OCS) on a daily basis were able to stop completely; the 2 remaining patients decreased their OCS dose from 40 to 5 mg/day and from 60 to 20 mg/day, respectively. No significant change in FEV_1 was noted with pre-BT and post-BT FEV_1 values (% of predicted) of 64.9 ± 18.6 and 61.1 ± 16.0 (NS), respectively.
Conclusion: Our data suggest that BT could be an effective treatment for patients with severe refractory asthma, independent of their allergic status, particularly in patients who did not respond to omalizumab.

364

State of the Union - an assessment of current asthmatics, their demographics, treatment and outcomes in 153 practices across the UK

Price, D[1]; van Aalderen, W[2]; Bleecker, E[3]; Campbell, J[4]; Roche, N[5]; Skinner, D[1]; Thomas, V[1]
[1]Research in Real Life, Cambridge, United Kingdom; [2]Department of Paediatric Respiratory Medicine and Allergy, Emma Children's Hospital AMC, Amsterdam, Netherlands; [3]Center for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Winston-Salem, United States; [4]Center for Pharmaceutical Outcomes Research, University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences, Aurora, United States; [5]Respiratory and Intensive Care Medicine Department, University Paris Descartes, Paris, France

Background: Asthma is a chronic condition with significant health and economic consequences, which may be attributable to poor control. This study characterises patients with asthma from 153 UK primary care practices and evaluates their disease severity, control and treatment.
Method: Electronic practice data of patients with asthma were extracted from 153 UK practices between January 2010 and March 2013. Patients included in the analysis had clinician-diagnosed asthma (i.e. a diagnostic Read code for asthma compatible with UK Quality and Outcomes Framework) and were receiving current asthma therapy ($\geq$1 asthma prescription in the last 2 years). Eligible patients were sent asthma management questionnaires to capture patient-reported outcomes. Pooled practice and patient data were used to characterise patients in terms of control status (classified by the Global INitiative for Asthma [GINA] and Royal College of Physician three questions); risk status (according to severe exacerbation frequency, defined as the number of asthma related hospitalisations and courses of acute oral steroids in previous 12 months); treatment step (as classified by GINA); and smoking status (current, ex-smoker or non-smoker).
Results: 29 505 patients with asthma were evaluated (mean age [SD]: 49 years [21]), of which 46% and 49% had partially controlled and uncontrolled asthma, respectively; 16% experienced $\geq$1 severe exacerbation; and 84% were in GINA treatment steps 2 to 4.

	Step 1	Step 2	Step 3	Step 4
Uncontrolled asthma [n (% of total in step)]	1345 (32)	3933 (43)	3562 (49)	5226 (62)
$\geq$1 severe exacerbation [n (% of total in step)]	232 (6)	917 (10)	1251 (17)	2254 (27)

[Asthma treatment step, control and risk status]

Frequent reliever use was the main contributor to low numbers of patients with controlled asthma (78% using reliever $\geq$ twice / week). Non-smokers were more likely to achieve complete or partial asthma control (57%) and less likely to suffer $\geq$1 severe exacerbation (14%), than current (49% and 20%, respectively) and ex-smokers (36% and 20%, respectively; $P < 0.001$ for differences between smoking categories).
Conclusion: Few patients in routine UK primary care achieve complete asthma control and a considerable number of patients experience severe exacerbations. A high proportion of patients with asthma use their reliever frequently (i.e. $\geq$ twice / week)

© 2015 The Authors

despite inhaled corticosteroid prescriptions. Smoking (current and past) may reduce the probability of achieving asthma control and increase frequency of severe exacerbations.

365
Different reference equations and asthma control

Gomes Belo, J[1]; Martins, P[1,2]; Peralta, I[1]; Serranho, S[1]; Neuparth, N[1,2]; Leiria Pinto, P[1,2]
[1]Allergology and Clinical Immunology Department, Hospital Dona Estefania, Lisbon, Portugal; [2]CEDOC, NOVA Medical School / Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisbon, Portugal

Background: In the last decade several studies have questioned the use of fixed criteria in the interpretation of lung function tests (LFT). The *Global Lung Function Initiative* (GLI) proposed the use of unifying reference equations, based on the lower limit of normal (LLN) to define the presence of airway obstruction. Our aim was to compare the theoretical values obtained through the reference equations routinely used in our hospital's lung function laboratory (Equation 1) and those obtained with GLI's equations (Equation 2). The association of bronchial obstruction according to each criteria and the lack of control of asthma was also studied.
Method: 367 individuals with physician diagnosed asthma were evaluated through spirometry and performance of Asthma Control Tests (ACT). A comparison of the theoretical values of the equations using Bland-Altman's agreement test was also performed. The considered bronchial obstruction criteria were the following: FEV_1< 80%, FEV_1< /FVC 0.70, FEV_1 < LLN and FEV_1/FVC < LLN. The association of each of these criteria with the absence of asthma control (ACT < 20) was studied through the Chi-square test.
Results: Half of the individuals in our sample were males (50.1%), with a median age of 18 years old (p25-p75: 14–30 years old). Regarding Equation 1, 9% (n = 33) presented a FEV_1< 80%. As far as Equation 2 is concerned, 16.7% (n = 61) had a FEV_1< 80%, 15.8% (n = 58) had a FEV_1 < LLN, 20.8% (n = 76) had a FEV_1/FVC < LLN and 12% (n = 44) presented a FEV_1/FVC < 0.70. Regarding the ACT, 22.6% (n = 83) of the individuals lacked asthma control. The average of the differences between Equation 1 and equation 2 was 8.5% (± 1.96 SD: -5.4% to 22.4%). The absence of asthma control had significant associations with FEV_1< LLN (P = 0.003), FEV_1< 80% (P = 0.015) and FEV_1/FVC < LLN (P = 0.003) of the equation 2. No significant association was observed between the absence of asthma control and FEV_1< 80% of equation 1. An association was found between a FEV_1/FVC < 0.70 and an ACT score < 20 (P = 0.008).
Conclusion: The mean differences found between the two reference equations should be considered in the interpretation of the LFT. Although there is no available information regarding the suitability of the GLI equations to the Portuguese population, our results suggest that the use of the FEV_1 LLN is a useful criterion in the assessment of the degree of asthma control.

366
Influence of chronic rhinosinusitis diagnosis on asthma response to oomalizumab in severe uncontrolled asthmatics

Sintobin, I[1]; Vennera, MDC[2,3,4]; Holtappels, G[1]; Picado, C[2,3,4]; Mullol, J[3,5]; Bachert, C[1,6]
[1]Upper Airway Research Laboratory, Ghent University, Ghent, Belgium; [2]Department of Pneumology and Respiratory Allergy, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain; [3]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [4]Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias (CIBERES), Barcelona, Spain; [5]Rhinology Unit & Smell Clinic, ENT Department, Hospital Clínic de Barcelona, Barcelona, Spain; [6]Division of Ear, Nose, and Throat Diseases, Clintec, Karolinska Institutet, Stockholm, Sweden

Background: Omalizumab is a humanized anti-immunoglobulin E monoclonal antibody that has been approved as add-on therapy for the treatment of adults with moderate-to-severe (United States) or severe (Europe) allergic asthma, inadequately controlled after treatment with high-dose inhaled corticosteroids plus long-acting β-agonists. The clinical efficacy of Omalizumab has not only been shown in the treatment of severe uncontrolled asthma, but also in the treatment of chronic rhinosinusitis with nasal polyps (CRSwNP) and comorbid asthma. The aim of this analysis was to examine whether the diagnosis of chronic rhinosinusitis (CRS) influenced the asthma response to Omalizumab treatment.
Methods: This study retrospectively analysed data from 70 patients with severe, uncontrolled asthma treated with Omalizumab. In serum, specific Immunoglobulin E (IgE) to Staphylococcal enterotoxins (SE) and total IgE before the start of the treatment (basal total IgE) were measured by Immunocap. Asthma response was evaluated by physician's Global Evaluation of Treatment Effectiveness (GETE-score); GETE 0, 1 and 2 were considered as non-responders, GETE 3 and 4 as responders.
Results: The mean age of the patients was 54.4 years and 62% of them were female. 84.3% of the asthmatics responded to Omalizumab treatment. Within the total patient group, 55.7% had CRSwNP and 83.8% had antibodies to staphylococcal enterotoxins in their serum. Within the SE-IgE positive group, 85.7% were responders compared to 14.3% non-responders (P < 0.001). A higher SE-IgE concentration was found in patients with CRSwNP and a strong correlation between SE-IgE and basal total IgE was observed (R = 0.612, P < 0.001). The proportion of responders tended to be higher in CRSwNP compared to CRS without nasal polyps (CRSsNP) and both higher compared to no CRS (89.7% vs 85.7% vs 81.8%); without reaching statistical significance.
Conclusion: These results showed that a high proportion of severe uncontrolled asthma patients are sensitized to Staphylococcal enterotoxins and more than a half present comorbid CRS with nasal polyps. There is a trend towards a better asthma response in patients with antibodies to SE and in CRSwNP patients.

367
Evaluation of patients with uncontrolled asthma in a specialized severe asthma centre; the effect on asthma control, quality of life and health care utilization

van der Meer, AN[1]; Pasma, H[1]; Kempenaar, W[1]; Pelinck, J[2]; Schutten, M[3]; Storm, H[4]; ten Brinke, A[1]
[1]Medical Centre Leeuwarden, Pulmonology, Leeuwarden, Netherlands; [2]Medical Centre Leeuwarden, Psychology, Leeuwarden, Netherlands; [3]Medical Centre Leeuwarden, Physiotherapy, Leeuwarden, Netherlands; [4]Medical Centre Leeuwarden, Clinical Chemistry, Leeuwarden, Netherlands

Background: Severe asthma is an invalidating condition associated with increased health care utilization (HCU) and reduced quality of life. Evaluation of patients with severe asthma symptoms in specialized severe asthma centers is recommended to optimize therapy. In 2013, in our hospital a multidisciplinary team was iniated for evaluation of patients with uncontrolled asthma referred by pulmonologists. In a 1-day-visit patients were systematically evaluated (correct diagnosis, confounding factors and comorbidities). Based on clinical and inflammatory parameters (including induced sputum) an initial determination of asthma phenotype was made. Findings were discussed in our team and a personalized management plan aiming to improve asthma outcomes was provided to the patient and referring pulmonologist.

Aim: To investigate whether there was an improvement in asthma control (ACQ), quality of life (AQLQ) and HCU after our assessment.
Method: In this prospective cohort study all patients completed questionnaires (ACQ, AQLQ, HCU) at baseline, 6 and 12 months after the assessment.

© 2015 The Authors

Results: In the first 18 months 47 patients were evaluated (40% male, age 51 ± 14 yr, 28% OCS-dependent). 9% of the patients were incorrectly diagnosed as asthma, 19% difficult asthma and 51% severe asthma. The most prevalent contributing factors were rhinosinusitis (60%) and reflux (34%). 40% of patients were phenotypically identified as early-onset atopic asthma, 37% as adult-onset eosinophilic asthma and 5% as neutrophilic asthma. ACQ improved from 2.7 ± 1.1 at baseline to 2.1 ± 1.1 at 6 months and 2.0 ± 1.0 at 1 yr ($P = 0.012$). AQLQ improved from 4.4 ± 1.1 at baseline to 5.3 ± 1.0 at 6 months and 5.1 ± 1.2 at 1 yr ($P = 0.006$). After 1 yr the number of exacerbations was decreased from 3.5 (IQR 2–8) to 2 (IQR 0–3)/yr ($P = 0.003$), as were ER visits 5.5 (2–10) to 1 (0–3)/yr ($P = 0.011$). Hospital admissions decreased from 1 (0–5) to 0 (0–0)/yr ($P = 0.018$). The 12 patients with evaluable data at 1 yr had 27 admissions in the yr before assessment vs 1 in the next yr.

Conclusion: Patients with uncontrolled asthma who were systematically evaluated by a multidisciplinary team and provided with a personalized management plan showed a significant and clinical relevant improvement in asthma control, quality of life and health care utilization lasting up to 12 months. This implies that for these patients a one-time extensive characterization in a specialized severe asthma centre seems to be effective and is recommended.

368
Relationship of response to omalizumab and baseline FeNO: results from a post-hoc analysis of EXALT study

Manga, V[1]; Erpenbeck, VJ[1]; Rao, S[1]
[1]Novartis Pharma AG, Basel, Switzerland

Background: Fractional exhaled nitric oxide (FeNO) is used as an inflammation marker for asthma. Here we report outcomes of omalizumab treatment combined with optimised asthma therapy (OMA/OAT) vs OAT alone in sub-groups of asthma patients categorised by baseline FeNO.

Methods: EXALT was a 32-week, open-label study (NCT00264849) in patients with uncontrolled severe allergic asthma. In this post-hoc analysis, patient's baseline FeNO was categorised as high (≥19.5 ppb) or low (<19.5 ppb). Effect of OMA/OAT vs OAT alone on physician's global evaluation of treatment effectiveness (GETE) at Week 16 and on exacerbation rate at Week 32 were compared within each high and low FeNO categories.

Results: Of the 404 patients randomised, 72 and 79 patients with baseline FeNO data were available for the GETE analysis and exacerbation analysis, respectively. Patients with a GETE rating of 'excellent' or 'good' were defined as responders; those with a rating of 'moderate', 'poor' or 'worsening' were defined as 'non-responders'. At Week 16, significantly higher proportion of patients treated with OMA/OAT were evaluated as GETE responders compared with those treated with OAT alone (table) in both FeNO high and low levels. The mean exacerbation rates at 32 weeks in the OMA/OAT group were observed to be lower vs OAT alone, in both FeNO high and low categories (table).

	FeNO level at baseline	GETE	OMA/OAT	OAT	p-value*
GETE at Week 16, n (%)	<19.5 ppb	Responders	15 (58%)	0 (0%)	0.02
		Non-responders	11 (42%)	5 (100%)	
	≥19.5 ppb	Responders	24 (75%)	3 (33%)	0.02
		Non-responders	8 (25%)	6 (67%)	
Total exacerbations (n)	<19.5 ppb		19 ($n = 26$)	15 ($n = 7$)	N/A
	≥19.5 ppb		16 ($n = 35$)	11 ($n = 11$)	N/A
Mean exacerbation rate per 32-weeks	<19.5 ppb		0.75	2.23	N/A
	≥19.5 ppb		0.47	1.16	N/A

*P-values based on Cochran-Mantel-Haenszel test without stratifying FeNO group; N/A - not available

[Effect of omalizumab on GETE and exacerbations]

Conclusions: This post-hoc analysis from a maximum of 79 patients of the 404 patients randomized in the EXALT study demonstrated that significantly greater number of patients, were classified as responders to combination treatment with omalizumab vs optimized asthma treatment alone at Week 16. Additionally, the patients on omalizumab combination treatment showed lower exacerbation rates compared with those treated with optimized asthma treatment alone in both FeNO level groups at Week 32, confirming efficacy of omalizumab in both the high and low FeNO level groups.

369
Association between the dyspnea perception and the degree of bronchoconstriction in asthmatic children

Oh, HL[1]; Song, JH[2]; Koh, YY[1]; Suh, DI[1]
[1]Seoul National University College of Medicine, Pediatrics, Seoul, Korea; [2]Kyung Hee University Medical Center, Pediatrics, Seoul, Korea

Background: Dyspnea is one of the cardinal symptoms of asthma, but it is difficult to quantify clinically. A modified Borg scale has been successfully used to assess dyspnea in adult, but children can have comprehension difficulties with this scale. The object of our study is to assess the validity and clinical efficacy of the Pediatric Dyspnea Scale (PDS) and correlation with pulmonary function test and bronchial inflammation of the PDS.

Methods: We performed the metacholine challenge test to confirm the asthma in 49 children. Before the test, we checked the FeNO value. Serial FEV1 value and dyspnea rating using the modified Borg scale and PDS were recorded during provocation test. We compared the modified Borg scale, PDS and FeNO value between bronchial hyperresponsiveness positive group (FEV1 value decreased by 20% as compared with baseline control value, BHR(+) group) and bronchial hyperresponsiveness negative group(FEV1 decreased by20% as compared with baseline control value, BHR (-) group). We analyzed the correlations between modified Borg scale, PDS and percentage fall in FEV1 at the last step of the challenge(ΔFEV1%), FeNO value. A P value of< 0.05 was considered statistically significant.

Results: There was no significant difference for dyspnea scale between BHR(+) and BHR(-) group at baseline(Borg scale, $P > 0.9999$;PDS, $P > 0.9999$) and at the last step of the challenge(Borg scale, $P = 0.8238$;PDS, $P = 0.5408$). We did not find correlations between ΔFEV1% and dyspnea scale(modified Borg scale and PDS) (Borg scale, $r = 0.047$, $P = 0.750$; PDS, $r = 0.131$, $P = 0.370$). There was correlation between ΔFEV1% and dyspnea scale(modified Borg scale and PDS) at each step of provocation test(Borg scale, $r = 0.341$, $P < 0.001$;PDS, $r = 0.330$, $P < 0.001$). There was a significant difference for FeNO value between BHR(+) and BHR(-) group($P = 0.028$). We found correlations between dyspnea scale (PDS) and FeNO value in BHR(+) group ($r = 0.4024$, $P = 0.0248$).

Conclusions: The pediatric dyspnea scale had similar pattern to assess the dyspnea with the modified Borg scale. The pediatric dyspnea scale correlates with a decrease of pulmonary function. We presumed that dyspnea scale with BHR(+) group was related bronchial inflammation.

© 2015 The Authors

370
An identikit of patients admitted to emergency department for asthma exacerbation: a real-life study

Bussolino, C[1]; Losappio, L[2]; Heffler, E[1]; Carpentiere, R[3]; Cannito, CD[3]; Di Biase, M[2]; Raie, A[1]; Rolla, G[1]; Giorgis, V[1]
[1]Department of Medical Sciences – Allergy and Clinical Immunology, University of Torino, Turin, Italy; [2]Cardiology, University of Foggia, Foggia, Italy; [3]'Dimiccoli' Hospital, Emergency Department, Barletta, Italy

Background: Asthma is an important and common cause for emergency department (ED) visits. Most asthma-related ED visits represent a treatment or maintenance failure and are deemed to be preventable. A better characterization of the patients could help to improve their asthma management and reduce health care spending.

Aim: To examine the patient characteristics of adult patients admitted to Emergency Department of Dimiccoli Hospital, the only acute care general hospital of Barletta, a 90 000 inhabitants town of South Italy, with a diagnosis of acute asthma attack, focusing on previous diagnosis of asthma and current asthma therapy.

Method: A structured questionnaire was administered to all patients admitted to ED for acute asthma in one year period (Jan-Dec 2013). The ED interview assessed patient demographics, asthma history, atopy, chronic asthma medications, outpatient visits for assessing asthma control and spirometry in the last 12 months, previous ED visits for asthma attack in the last 12 months, and details of the current asthma exacerbation, including duration of symptoms. Data on oxygen saturation, ED course, and ED disposition were obtained by chart review.

Results: Two hundred and sixty patients were admitted to ED for acute asthma attack, 163 (62.7%) male and 97 (37.3%) female, mean age 39.9 (IC 95%: 36.6 - 43.2). Ninety seven patients (37.3%) were hospitalized following ED evaluation. Only 122 patients (46.9%) had received a previous diagnosis of asthma and 21 of them (18.4%) used short acting beta-2 agonists (SABA) only. Patients were admitted to ED within 6 h from the onset of asthma exacerbation in 23.1% of cases, between 6 and 24 h in 51.5% and after 24 from the onset of symptoms in the remaining 19.6%. None had used rescue medication according to written instructions. Among patients with known asthma, 64.8% had an asthma specialist visit and 49.2% performed spirometry in the previous 12 months. Determining factors for hospitalization were age (52.2 vs 32.6 ys, $P < 0.001$), previous diagnosis of asthma (54.6 vs 42%, $P < 0.05$), previous ED visits (14.4% vs 4.3%, $P = 0.004$), lower SaO2 (90.85 vs 96.4%, $P < 0.001$).

Conclusion: Half of patients admitted to ED for asthma did not have received a previous diagnosis of asthma, while a significant proportion of patients with previous diagnosis of asthma was not receiving adequate maintenance therapy and they had not received written instructions for self-management of asthma exacerbation.

371
The attitude towards the use of magnesium sulphate for acute asthma exacerbations among Turkish physicians: the current practice

Baccioglu, A[1]; Bakirtas, A[2]; Oner Erkekol, F[3]; Kalaycı, O[4]; Bavbek, S[5]
[1]Department of Pulmonary Diseases, Division of Immunology and Allergic Diseases, Kirikkale University Faculty of Medicine, Kirikkale, Turkey; [2]Department of Pediatric Diseases, Division of Allergy and Asthma, Gazi University Faculty of Medicine, Ankara, Turkey; [3]Clinic of Immunology and Allergic Diseases, Atatürk Chest Diseases and Chest Surgery Training and Research Hospital, Ankara, Turkey; [4]Pediatric Allergy and Asthma Unit, Hacettepe University School of Medicine, Ankara, Turkey; [5]Department of Pulmonary Diseases, Division of Clinical Immunology and Allergic Diseases, Ankara University Faculty of Medicine, Ankara, Turkey

Background: Magnesium sulphate ($MgSO_4$) is considered to be a safe, inexpensive, and effective medication for severe asthma exacerbations. We aimed to evaluate the attitude of Turkish physicians towards use of $MgSO_4$ for asthma exacerbations.

Method: A self-administered questionnaire was distributed through the Turkish National Society of Allergy and Clinical Immunology by e-mail and during the annual congress in 2013.

Results: Of the 456 respondents (mean age of 36.53 ± 9.11 yrs), 42.3% reported to use $MgSO_4$ in asthma exacerbations in their practice, and 48.7% agreed that $MgSO_4$ was included in asthma guidelines. The best known indication was that it was useful only in patients refractory to bronchodilators and systemic corticosteroids (67.7%). The two most common reasons to use $MgSO_4$ were to shorten hospital stay (94.7%), and prevent admission to intensive care unit (80.3%). The respondents mostly used $MgSO_4$ in the treatment of severe or life-threatening exacerbations. Thirty nine percent believed that $MgSO_4$ had no effect on discharge period, and 29% of them marked minor side effects. The use of $MgSO_4$ was most common among pediatric allergists (61%). The most common reason for not using $MgSO_4$ was inexperience (36.5%). Having an academic affiliation (OR = 3.17, $P < 0.001$), the number of asthmatic outpatients seen per month (OR = 1.820, $P = 0.007$), and more recent graduation from medical school (≤ 1991) (OR = 0.25, $P < 0.001$) were associated with the use of MgSO4 in the multivariate analysis.

Conclusion: Even though the effect of $MgSO_4$ in acute severe asthma is well known, only half of the physicians dealing with asthma patients have used it in their practice. Education and encouragement in this regard are necessary.

372
Acute exacerbations in subjects with asthma vs asthma-COPD overlap syndrome (ACOS)

Joon Young, C[1]; In Kyoung, K[1]; Hea Yon, L[1]; Hwa Young, L[1]; Chin Kook, R[1]; Ji Young, K[1]; Sook Young, L[1]; Young Kyoon, K[1]; Soon Suk, K[1]
[1]Division of Allergy and Pulmonology, The Catholic University of Korea School of Medicine, Internal Medicine, Seoul, Korea, Republic of

Background: The asthma-COPD overlap syndrome (ACOS) is composed of COPD patients with increased reversibility and/or of asthmatics with fixed or incompletely reversible airflow limitation. Its prevalence is estimated about 13–20% of subjects with COPD, but this syndrome is not yet well characterized. Previously we had published the report decribing the clinical characteristics of ACOS, this study was designed to evaluate the risk factors of acute exacerbation between asthma and ACOS.

Method: Of the 1898 patients over 40 years-old who were suspected diagnosis of asthma from Sep 2007 to Mar 2012 at Seoul St. Mary's hospital, a total of 256 patients were included, based on the criteria. (1) Physicians diagnosis with at least 3 months of chronic persistent respiratory symptoms and either (2) Positive response to bronchodilator: >200 ml FEV1 and >12% baseline or (3) Positive methacholine or mannitol provocation test. Among the patients, we defined the ACOS group with incompeletely reversible airflow obstruction (postbronchodilator FEV1/FVC < 70) at initial admission and continuing airflow obstruction after at least 3 months follow up. We retrospectively analyzed clinical features and risk of acute exacerbational events from first admission or clinic to recent follow up.

Results: Of the 256 patients, ninty seven patients were defined as ACOS group and 159 were asthma only group. ACOS group was older, contained more males and higher percentage of current or ex-smokers than asthma only group. ACOS group showed lower FEV1 (58 ± 1.2% vs 69.4 ± 1.2%, $P < 0.001$) and higher residual volume and functional residual capacity. During the mean 30.96 ± 20.3 months

© 2015 The Authors

follow up periods, total number of severe exacerbations which evoked emergency room visit or hospital admission was 33 (13.1%) and that of moderate exacerbations which were improved with oral steroid or antibiotics was 161 (64.1%). There was no difference in the incidence of acute exacerbations between ACOS and asthma group. In mutivariate analysis, female gender (OR 2.456, $P = 0.033$) and low FEV1 (%) (OR 0.976, $P = 0.020$) were significant factors associated with acute exacerbation.
Conclusion: Total number of moderate acute exacerbation was 5 times higher than severe exacerbation. But the numbers of events were not statistically different between ACOS and asthma only group. Female gender and low FEV1 might be clinical risk factors associated with acute exacerbation.

© 2015 The Authors

Poster Discussion Session PDS 13

New methods in molecular allergology

373

Recombinant FcεRIα of dog, cat and horse as novel diagnostic tools to detect IgE in these species

Einhorn, L[1,2]; Fazekas, J[1,2]; Muhr, M[1,2]; Schoos, A[1,2]; Panakova, L[3]; Herrmann, I[3]; Manzano-Szalai, K[1]; Oida, K[1,4]; Jensen-Jarolim, E[1,2]
[1]Comparative Medicine, Messerli Research Institute of the University of Veterinary Medicine, Vienna, Austria; [2]Institute of Pathophysiology and Allergy Medical University Vienna, Vienna, Austria; [3]Clinical Department of Small Animal Internal Medicine, University of Veterinary Medicine Vienna, Vienna, Austria; [4]Laboratory of Veterinary Molecular Pathology and Therapeutics, Tokyo University of Agriculture and Technology, Tokio, Japan

Background: Type-I allergy affects humans and their family pets in similar manner. IgE diagnosis in animals is today performed by using the human alpha chain of FcεRI receptor detecting canine, feline and equine IgE due to crossreactivity. The amino acid identity among the different alpha chains, however, ranges only between 54% and 56%. Therefore we hypothesized that introduction of species-specific alpha chains detection could improve veterinary allergy diagnosis. We present here the generation of recombinant soluble alpha-chains of the high affinity IgE receptors (FcεRIα) of dog, cat and horse as novel IgE detection tools.
Method: Combined with a custom pIRES SV40_Neo mammalian expression vector the Flag-tagged FcεRIα fusion proteins of canine, feline and equine species were expressed in CHO-DUKX B11 cells. The transfected 384 clones of each species were evaluated and selected with respect to their productivity and quality of the product, by Enzyme-Linked Immunosorbent Assay (ELISA) and immunoblots. Recombinant alpha chains were purified via anti-FLAG M2 affinity gel, again characterized by ELISA and Immunoblot.
Results: Purified alpha chains were tested for correct folding by CD-spectroscopy. Immunoblots and ELISA assays verified integrity and the affinity of the three alpha chains to serum IgE of each species. As further experiment we used solid phase assays for affinity evaluation and the successful detection of canine, equine and feline IgE with the species specific alpha chains.
Conclusion: Based on our data we propose that the dog-, cat- and horse-specific FcεRIα chains may improve the fidelity of IgE-diagnosis in allergic and atopic domestic animals.

374

INA (individual native In-vitro allergy diagnostics) cat-detector: a new and simple method to detect the distribution of cat allergens on the cats, their surroundings and in places where usually no cats are present

Wahl, R; Uhlig, J
ROXALL Medizin, Oststeinbek, Germany

Background: Nearly eight million cats are living in German households and nearly the same amount of people is sensitized to cat allergens (CA). So far, no simple test was available for the detection of CA. The objective was to use the newly developed INA-cat-detector to investigate the presence of CA at different places.
Method: INA-cat-detector is a combination of an INA-stick (with a special reaction field at its bottom) and specific IgE measurements (test-tube ELISA technique). The INA-stick was pulled through the fur of the cat, their walking and sleeping places and seats of public trains and busses to capture CA. Specific IgE measurement was performed by incubating the CA loaded INA-sticks with a high titer serum from a cat allergic patient (positive serum) and in parallel with a serum from a non-allergic patient (negative serum, to exclude a false positive reaction). The overall testing time was 2 h 25 min. For evaluation of the test results the colour reactions on the reaction fields obtained after incubation with the positive serum (dark purple) and negative serum (white/light purple) following the ELISA procedure were compared as yes/no outcome.
Results: CA could be detected over the whole cat body, in the saliva, in the surroundings of the cat and on the seats of public trains and busses. No CA could be found on the laminate floor at the cat owner's house. On this kind of material CA was easily wiped off.
Conclusion: The new INA-cat-detector ("Magic Stick") is easy and simple to handle and is effective in capturing CA from different surfaces. CA, mainly present in the saliva (major source of the major CA Fel d 1), was probably scattered over the whole cat body by licking their fur many times a day. CA could also be detected in places where usually no cats are present: on the seats of public trains and busses, most likely transported via the clothes of cat owners. These results indicate that cat allergic patients cannot avoid CA even when removing the cat from the house. CA seems to be ubiquitous. Therefore, one might consider allergen immunotherapy as a causal treatment for cat allergic patients.

375

Do different breeds of cats differ in their allergenicity? Application of the INA (individual native In-vitro allergy diagnostics) cat-detector on different breeds of cats

Wahl, R; Uhlig, J
ROXALL Medizin, Oststeinbek, Germany

Background: Cat allergen (CA) is said to be an ubiquitous allergen. To further investigate the distribution of CA in regard to different breeds we used our new INA-cat-detector to determine CA in samples collected from various sources of different breeds of cats.
Method: The INA-cat-detector is a combination of an INA-stick (with a special reaction field at its bottom) and specific IgE measurement (test-tube ELISA technique). To capture CA the stick was pulled through the fur and saliva of eight different breeds of cats: Don Sphynx (naked cat), Chartreux, Siam cat, European Shorthair, Persian cat, Persian cat Mix, Maine Coon, normal house cat. The captured CA was determined by specific IgE measurement using the test-tube ELISA technique in parallel with a high titer serum from a cat allergic patient (positive serum) and with a serum from a non-allergic patient (negative serum, to exclude a false positive reaction). The overall testing time was 2 h 25 min. For evaluation of the test results the colour reactions on the reaction fields obtained with positive serum (dark purple) and negative serum (white/light purple) following the ELISA procedure were compared as yes/no outcome.

© 2015 The Authors

Results: Using the INA-cat-detector CA could be detected in all samples collected from fur and saliva of the eight breeds of cats, even in samples from the belly of the naked cat.
Conclusion: The handling of the new INA-cat-detector is easy and simple as well as effective in capturing CA from fur and saliva. The reason why no difference has been found in the allergenicity between the breeds of cats and also on the body of the naked cat may be the fact that all cats are licking their fur many times a day and so distributing CA. Saliva is supposed to be the main source of major cat allergen Fel d 1. The INA-cat-detector shall be further used to investigate other breeds and whether washing the cats would reduce the allergen load on the fur, as mentioned in some publications.

376
INA (individual native In-vitro allergy diagnostics): a new and simple method to investigate dog allergen on different breeds of dogs

Wahl, R; Putensen, O
ROXALL Medizin, Oststeinbek, Germany

Background: It is controversially discussed whether different breeds of dogs show differences in their allergenicity and whether dogs with long hair are higher allergenic than dogs with short hair. Using the new INA-method the presence of dog allergens (DA) on different breeds including long and short haired dogs was investigated.
Method: The INA-stick is a plastic stick fitted with a special reaction field at the bottom of the stick. To capture DA the stick was pulled through the fur and saliva of nine different breeds of dogs: Boxer (long hair), Chihuahua (long hair), Yorkshire Terrier (long hair), Miniature Pinscher (short hair), American Pitbul Mix (short hair), Mixture Beagle/Terrier (short hair), Maltese (long hair), English Cocker Spaniel (medium long) and Mixture Bernes Mountain Dog/Leonberger (long hair). The captured DA was determined by specific IgE measurement using the test-tube ELISA technique in parallel with a high titer serum from a dog allergic patient (positive serum) and with a serum from a non-allergic patient (negative serum, to exclude a false positive reaction). For evaluation of the test results the colour reactions on the reaction fields obtained after incubation with the positive serum (dark purple) and negative serum (white/light purple) following the ELISA procedure were compared as yes/no outcome.
Results: DA could be detected with no significant difference in all nine breeds of the dogs when testing samples taken from saliva. Differences of positive reactions were detected in samples taken from the fur.
Conclusion: The handling of the new INA-dog-detector is easy and simple as well as effective in capturing DA from saliva and fur. No differences could be found in the allergenicity between the breeds of dogs (including long and short haired dogs) in the saliva samples, indicating that saliva is the main source of DA. Only in few samples taken from the fur a positive reaction could be determined, indicating less DA on the fur. In contrast to cats the dogs are seldom licking their fur, so the DA contained in the saliva is not permanently distributed over the fur. We recommend taking samples from saliva for investigating the allergenicity of dogs. This information is also important for the production of DA containing products used for diagnosis and immunotherapy in dog allergy to use the right raw material.

377
Evaluation of the Omega Allersys® allergy system for the detection of specific IgE

Tonutti, E[1]; Visentini, D[1]; Villalta, D[2]; Fontana, DE[3]
[1]Ospedale-Università di Udine, Laboratory of Immunopathology and Allergy, Udine, Italy; [2]Ospedale di Pordenone, Allergy and Immunology, Pordenone, Italy; [3]Ospedale-Università di Udine, Immunopathology and Allergy, Udine, Italy

Background: The Aim of our study was to evaluate the performance of a new method for the determination of specific IgE; the Allersys® assay (Omega Diagnostics, UK) is a two-step immunoassay method based on the principle of chemiluminescence. Biotinylated allergens are incubated with streptavidin coated magnetic micro-particles (solid phase), any specific IgE in the sample then binds to the allergen and is detected with a mouse monoclonal human anti-IgE antibody labeled with an acridinium ester derivative (conjugate). The generated signal, measured in RLU (Relative Light Units), is then read from a stored standard curve (standardized to the WHO 2nd IRP 75/502) to give the concentration of specific IgE present in the sample.

The ZENIT RA Analyzer (Menarini Diagnostics, Italy) automatically performs all the operations called for by the assay protocol: addition of the samples, calibrators, controls, magnetic particles, conjugate, and chemiluminescence activator solutions to the reaction vessel; magnetic separation and washing of the particles; measurement of the emitted light.

The results obtained with the Allersys® Allergy System were compared with the Phadia250 (Thermo Fisher IDD, Sweden).

Method: 179 sera from patients with a significant history for allergy were analyzed with both Allersys® Allergy System and Phadia 250; 18 sera were tested for IgE lactalbumin (f76), 24 sera for IgE Dermatophagoides farinae (d2), 19 sera for IgE rye grass (g5), 26 sera for IgE egg white (f1), 34 sera for IgE olive (t9), 9 sera for IgE blue grass (g8), 25 sera for IgE casein (f78) and 24 sera for IgE cod (f3). The results were expressed in kU_a/L; the cut-off used for the two methods was 0.15 kU_a/L.
Results: 121/179 samples resulted positive with Omega assay and 127/179 with Phadia 250. The overall agreement (pos/neg) was 93.3%. Of the 12 non-complying results 9 samples were ImmunoCAP pos/Allersys® neg; 10 out the 12 discordant samples had IgE specific concentration < 0.50 kU/L.

Even with the relatively small number of samples the correlation of quantitative results was excellent for 6 allergens: d2 ($R^2 = 0.8786$), g5 ($R^2 = 0.9239$), f1 ($R^2 = 0.8327$), t9 ($R^2 = 0.9511$), g8 ($R^2 = 0.9435$), f3 ($R^2 = 0.9205$); was good for f78 ($R^2 = 0.7236$) and f76 ($R^2 = 0.6307$).
Conclusion: The high analytical performance and on board storage capacity (up to 126 different allergens) make Allersys® suitable for specific IgE measurement in daily practice.

378
Evaluation of an automated microarray allergy test

Williams, P[1]; Önell, A[2]; Hui, V[2]; El Shanawany, T[1]
[1]Department of Immunology, University Hospital of Wales, Cardiff, United Kingdom; [2]Microtest Dx, London, United Kingdom

Background: Skin prick tests (SPT) and traditional allergen-specific IgE blood tests have been used for decades to seek to confirm a clinical diagnosis of allergy made from a patient's history. Microarray test platforms, enabling simultaneous measurement of many allergens with a small amount of serum sample are potentially very powerful tools in allergy diagnostics. The aim of this study was to evaluate the concordance between a new, automated microarray IgE test (Microtest Allergy System) and other allergy tests used in our service.
Method: Fifty (50) adult allergic patients referred to our clinic were included in the study. Patients were tested by four methods:

(1) SPT,
(2) ImmunoCAP,
(3) ISAC 112 and
(4) Microtest Allergy System.

© 2015 The Authors

SPT were performed for a panel of 11 common allergens. ImmunoCAP was requested for particular allergen extracts or recombinant allergens according to our routine practice. For ISAC and Microtest a predetermined allergen panel covering most common airborne and food allergens was tested. Test results from the different methods were analyzed and platform to platform comparisons were performed.

Results: The positive-negative % agreement between the four test methods is shown in the table below. Similar results were obtained when analyzing the agreement using IgE class scores or allergen-specific IgE concentration levels.

	SPT	Immuno CAP	MICRO TEST	ISAC
SPT	100	82	78	82
ImmunoCAP	82	100	88	80
MICROTEST	78	88	100	85
ISAC	82	80	85	100

[Positive-negative concordance between methods (%)]

Conclusion: In this study all four test methods showed similar results. All assay methods gave comparable results for high serum levels of specific IgE, and all methods showed similar deviations at low IgE levels (typically <2 kU/l). Data collection for additional 50 patients is ongoing.

The main advantages of the Microtest method are that it is simple and fully automated, can be run outside a laboratory (e.g. in a clinic) and requires only a small amount of serum sample (100 µl) to measure a patient's specific IgE profile.

The main difficulties in evaluating the performance or accuracy of a new allergy test are the lack of a gold standard to compare with, and differences in the allergen panel composition (extracts and/or components).

379
Evaluation of Microtest allergy system compared to three established diagnostic methods

Konradsen, J[1]; Nordlund, B[1]; Winkler, A[2]; Baldracchini, F[3]; Mazzoleni, G[3]; Önell, A[3]; Hedlin, G[1]; Grönlund, H[2]
[1]Woman's and Children's Health, Karolinska Institutet, Stockholm, Sweden; [2]Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; [3]Microtest Dx, London, United Kingdom

Background: Microarray platforms are potentially powerful diagnostic tools enabling simultaneous measurement of numerous allergens with a small amount of sample. The aim of this study was to evaluate the performance of a new, automated microarray platform, the Microtest allergy system.

Method: The Microtest system consists of an automated instrument, a reagent cartridge, software and a biochip. The test is fully automated and requires 100 µl sample for simultaneous measurement of 26 common airborn and food allergens, and includes both allergen components and extracts.

In order to evaluate the test system, 30 children (6 -18 years) were randomly selected from a cohort of 96 children with mild, moderate or severe persistent asthma. All patients were analysed against 9 allergens (egg, milk, cod, peanut, cat, dog, mite, timothy and birch) measured with four methods (Skin prick test (SPT), ImmunoCAP, Microtest and ISAC 112).

Results: Overall the four methods showed a good agreement with a Pearson correlation coefficient ranging between 0.76 - 0.86 (see Table below). Eight patients were non-sensitized and 22 patients showed individual IgE profiles with IgE antibody responses to a minimum of one and a maximum of seven triggering allergens. All patients were measured with four test methods against 9 allergens with only 5% missing values. This provided data to define a "reference" to compare with. The main triggering allergens and the overall IgE profile of each individual patient was always identified by Microtest and ImmunoCAP, and rarely missed for ISAC (dog undetected for 2 patients) or SPT (birch undetected for 2 patients). All four methods showed deviations at low IgE antibody levels (typically <2 kU/l).

Table 1: Correlation coefficients

	SPT	Immuno CAP	Micro Test	ISAC
SPT	1	0.76	0.78	0.80
ImmunoCAP	0.76	1	0.86	0.86
MicroTest	0.78	0.86	1	0.83
ISAC	0.80	0.86	0.83	1

Conclusion: Microtest show comparable test results to SPT, ImunoCAP and ISAC. The main triggering allergens and the overall IgE profile of an individual patient were never missed for any of the patients by Microtest. These preliminary results indicate that Microtest is a useful tool to efficiently characterize the IgE profile of asthmatic patients. However more data is needed for a more thorough evaluation. Data collection for additional patients is ongoing.

380
Skin response to recombinant birch pollen allergen (Bet v1a) in allergic patients during and out of the birch pollen season

Brzezińska-Pawłowska, O[1]; Lupinek, C[2]; Piechota-Polańczyk, A[1]; Kurowski, M[1]; Jabłońska, A[1]; Stolz, F[3]; Kopp, S[3]; Neubauer, A[3]; Kiss, R[2]; Valenta, R[2]; Kowalski, ML[1]
[1]Department of Immunology, Rheumatology and Allergy, Healthy Ageing Research Centre, Medical University of Lodz, Poland, Łódź, Poland; [2]Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria; [3]Biomay AG, Vienna, Austria

Background: Recombinant allergens allow specific and accurate diagnosis of allergy and may facilitate the diagnostic procedure in patients with complex allergy history. However, recombinant birch pollen allergen Bet v1 has never been tested before in a systematic SPT titration study in and outside the pollen season. The aim of our study was to assess skin reactivity to recombinant birch pollen allergen Bet v1a in allergic and healthy individuals, and to compare skin reactions during and after the birch pollen season in patients allergic to birch.

Material and Methods: We recruited 40 patients allergic to birch (each with positive SPT to birch allergen extract) and 10 non-allergic healthy controls. Seventeen patients were analyzed twice, during and 5–6 months after the birch pollen season. Patients and controls had skin prick tests with birch extract and increasing concentrations of recombinant allergen Bet v1a from 1:512 to 1:4 during the season and from1:2048 to 1:4 out of the season. All patients were out of antihistaminic drugs for at least a week.

Results: Recombinant Bet v 1a induced a positive ($\geq$3 mm) wheal response in 18 out of 21 patients tested during the season, and in 34 out of 36 patients teste out of the birch pollen season. The skin response was allergen dose dependent with some patients reacting even to 1:2048 dilution. During the birch pollen season all patients who reacted to Bet v 1a responded to a dilution of Bet v 1a >1:64 while out of the pollen season all Bet v 1a positive patients responded to lower allergen concentration (1:128). There was no skin reaction in any of the control subjects.

Conclusion: Recombinant birch allergen (Bet v1a) induces a specific wheal response in the skin of birch allergic patients, which seems to be modified by natural allergen exposure.

© 2015 The Authors

381
Identifying birch allergic patients: use of skin prick test and IgE classifications

Nelson, V; Sadoway, T; Pathmanapan, S; Patel, P; Salapatek, AM
Inflamax Research, Mississauga, Canada

Background: Birch pollen allergy is a significant problem in both North America and Europe, with birch trees being one of the highest pollen producing trees. Birch allergic patients are very likely to have oral allergy syndrome due to the cross reactivity of birch pollen with other food proteins. Properly identifying patients with birch allergy is crucial to ensuring that adequately allergic patients receive birch immunotherapy. It has been suggested that the majority of birch allergic patients show high sensitivity to primary birch allergen, bet v 1, however recent data suggests that this may not be the case.
Method: 183 patients, 18–65 years old, were screened for a history of birch allergy and a positive skin prick test (SPT) to birch allergen (wheal size >3 mm larger than negative control). Subjects meeting both conditions had Bet v 1 specific IgE levels quantified. IgE concentrations were then grouped into class levels (Class 0: <0.35KU/L; Class 1: 0.35–0.69KU/L; Class 2: 0.70–3.49KU/L; Class 3: 3.50–17.49KU/L; Class 4: 17.50–49.99KU/L; Class 5: 50.00–100.00KU/L; Class 6: >100.00KU/L), with Class 2 or higher indicating a positive Bet v 1 sensitivity.
Results: 67% of patients who screen positive for birch allergy based on a history and SPT had a specific IgE to birch pollen of Class 2 or higher and an average SPT wheal size of 14 mm larger than the negative control. 24% of patients who were SPT positive (average wheal size of 9 mm) had undetectable Bet v 1 specific IgE levels. There is a trend for average SPT wheal size to increase with IgE class levels; however the percent of patients in the higher class levels declines substantially, with only 3% of patients in Class 5 and 6.
Conclusion: A substantial number of patients who screen positive to birch allergy do not meet the criteria of Bet v 1-specific IgE Class 2 or higher, irrespective of SPT wheal size. More research should be done to examine Bet v -specific peptide phenotypes and patient symptom scores to help better understand the allergic response and assist in properly identifying birch allergic patients in North America.

382
Skin prick test and serum specific IgE level as predictors of nasal challenge response to *Dermatophagoides pteronyssinus* in children

Cho, E[1]; Baek, JH[2]; Jee, HM[2]; Shin, YH[3]; Koh, HY[1]; Han, MY[2]; Jung, Y-H[2]
[1]CHA University School of Medicine, Seongnam, Korea; [2]Department of Pediatrics, Bundang CHA Medical Center, CHA University School of Medicine, Seongnam, Korea; [3]Department of Pediatrics, Gangnam CHA Medical Center, CHA University School of Medicine, Seoul, Korea

Background: The ability of skin prick test (SPT) and measurement of serum specific IgE (sIgE) to *Dermatophagoides pteronyssinus* in predicting positive or negative nasal provocation test (NPT) results has not been determined in children. We sought to determine if skin prick test and/or serum IgE is diagnostic of allergic rhinitis in children.
Method: One hundred twenty children with rhinitis underwent NPT to *D. pteronyssinus* (a 5-step procedure; normal saline, 0.04, 0.4, 1, 2 and 4 ug/ml) following SPT and sIgE level measurement to *D. pteronyssinus*. Severity of each of the following symptoms of rhinitis (rhinorrhea, nasal obstruction, sneezing, nasal itch and eye symptom) was subjectively scored on a 0–10 scale upon each allergen dose, administration. SD index, degree of deviation from standard deviation of the difference between the default value and the value upon saline administration, was used to determine a positive reaction. An ROC curve was drawn for SPT and sIgE with NPT as the gold standard.
Results: Seventy-five of the 120 children tested positive to *D. pteronyssinus* on NPT. The negative predictive values for children with negative NPT results was 83.33% for SPT and 63.64% for sIgE., Positive predictive values for children with positive NPT result was higher than the negative predictive values: 98.67% for SPT and 93.59% for sIgE. Cutoff value for NPT-positive children was 3.5 mm ($P < 0.001$, area under curve 0.917) for SPT, and 3.48 IU/ml ($P < 0.001$, area under curve 0.908) for sIgE.
Conclusion: Although neither negative SPT result nor serum sIgE to *D pteronyssinus* predicted negative NPT results, SPT and sIgE to *D. pteronyssinus* were excellent predictors of positive NPT outcomes in children.

383
Clinical and immunological differences between house dust mite sensitized vs allergic patients

Zidarn, M[1]; Robic, M[1]; Krivec, A[1]; Silar, M[1]; Resch, Y[2]; Vrtala, S[2]; Kopac, P[1]; Kosnik, M[1]; Korosec, P[1]
[1]Allergy Department, University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia; [2]Center for Pathophysiology, Infectiology and Immunology Department of Pathophysiology and Allergy Research AKH, Vienna, Austria

Background: Sensitization to house dust mite does not always correlate with clinical allergy. With this study we evaluated clinical and immunological differences between patients with confirmed allergic airway disease and subjects with clinically irrelevant IgE sensitization to house dust mite.
Method: 36 subjects with positive skin prick test for *Dermatophagoides pteronyssinus* and/or *Dermatopagides farinae* were included into the study and divided in symptomatic and asymptomatic group on the basis of clinical history, juniper mini-RQLQ and nasal provocation test with four log concentration of house dust mite-allergen. In both groups the following immunological parameters were evaluated: CD 63 basophile response to *in vitro* stimulation using four log concentration of *D. pteronyssinus*-allergen extract, serum specific IgE to *D. pteronyssinus*, specific IgE to natural and recombinant house dust mite-allergens, total IgE and specific IgG to *D. pteronyssinus*. Thirteen control subjects with negative skin prick for *D. pteronyssinus* and *D. farinae* and negative house dust mite nasal provocation test were also included.
Results: 17 patients with allergic rhinitis and/or asthma were compared to 19 patients sensitized to house dust mite, who did not present with the history of perennial allergic rhinitis and had negative nasal provocation test, and 13 control subject. Symptomatic patients showed significantly higher basophil sensitivity compared to asymptomatic subjects, but there were no significant differences in specific IgE levels to *D. pteronyssinus*. For specific IgE to rDer p23 and rDer p7 house dust mite-allergens there was higher frequency of sensitization in symptomatic vs asymptomatic patients, but for other natural and recombinant allergens, frequency of sensitization was comparable.
Conclusion: The difference in basophile sensitivity to *D. pteronyssinus*-allergen extract and the difference in prevalence of specific IgE to rDer p7 and rDer p23 house dust mite-allergens prove useful for distinguishing clinical phenotype of allergic sensitization.

© 2015 The Authors

384
Validation of BAT testing with a wide spectrum of house dust mite recombinant allergens

Silar, M[1]; Resch, Y[2]; Zidarn, M[1]; Vrtala, S[2]; Kopac, P[1]; Kosnik, M[1]; Valenta, R[2]; Korosec, P[1]
[1]University Clinic for Respiratory and Allergic Diseases Golnik, Golnik, Slovenia; [2]Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Background: There is a lack of studies regarding the validation of recombinant allergens in BAT testing. The aim of this study was to establish the optimal dose-response curves for several potent house dust mite (HDM) allergens.
Method: Seven major recombinant house dust mite (HDM) allergens (Der p 1, Der p 2, Der p 5, Der p 7, Der p 10, Der p 21 and Der p 23) were evaluated for CD63 BAT testing in 30 clinically well-characterized HDM-allergic patients.
Results: The protocol was created in two consecutive steps. In the first step IgE reactivity to Der p 1, Der p 2, Der p 5, Der p 7, Der p 10, Der p 21 and Der p 23 was determined in sera of all patients. In the next step the patients were recall back and tested for IgE positive HDM recombinants in cellular BAT assay. We demonstrated the positive BAT response for all HDM allergens except for Der p 10. We had to use a large number of log recombinant concentrations to reach the whole dose response curve, with the maximum range from 10^{-35} up to 100 ng/ml. The lower concentration ranges were specific for every recombinant allergen. Surprisingly, the highest overall allergenic activity was showed for Der p 23.
Conclusion: BAT recombinant testing requires the two step protocol with inclusion of recombinant IgE measurement in the first step and with a BAT stimulation in the next step. A wide range of recombinant allergen concentrations is necessary to reaching the whole dose response curve.

385
Establishment of artificial human sera (ARTHUS) based on chimeras of FcgammaRI and human immunoglobulin domains

Offermann, N[1]; Plum, M[2]; Rathloff, K[2]; Sundermann, U[1]; Fooke, M[1]; Spillner, E[2]
[1]Dr. Fooke Laboratorien GmbH, R&D, Neuss, Germany; [2]Department of Engineering, Aarhus University, Aarhus, Denmark

Background: Human pool sera are the immunologist's first choice as controls for most diagnostic applications, but are strongly limited regarding availability, varying quality and high costs. The aim of this study was to circumvent these limitations by developing a versatile tool which allows the use of polyclonal antibodies in allergen-specific immunoassays.
Methods: The extracellular domains (ECD) of the human high affinity IgG receptor FcγRI (CD64) was fused to human immunoglobulin Fc regions of the epsilon isotype (IgE). Recombinant adapter molecules comprising the FcγRI ECD and human Ig Fc domains (CD64-Ig Fc) were expressed in human HEK-293 cells. Allergen-specific IgG antibodies were produced in rabbits by immunization with purified recombinant and native allergens as well as whole allergen extracts. Pre-incubation of polyclonal IgG with CD64-IgE Fc produced artificial allergen-specific reagents which were used in different types of allergen-specific immunoassays. A set of such artificial human sera was tested in three established assay Methods

ALLERG-O-LIQ (DFL), ALFA (DFL) and ImmunoCAP (Thermo Fisher). The deviation between the different immunoassays was calculated for each artificial serum sample.
Results: Artificial human sera show comparable results in three established diagnostic systems for the determination of specific IgE (sIgE). A reversed ELISA (ALLERG-O-LIQ), an Allergy Lateral Flow Assay (ALFA) and a direct ELISA (ImmunoCAP) show comparable results for both recombinant and native purified allergens as well as for allergen extracts. Agreement for each of more than 20 artificial human serum samples is within $\pm$ 1 RAST class.
Conclusion: Rabbit IgG complexed with the IgG-specific CD64-Ig Fc adapter molecule have the potential to provide a substitute for human reference sera with specificity for virtually any protein of interest. The variability of the Ig isotype used as a fusion partner makes the approach extremely versatile in different assay detection setups potentially overcoming limitations of current assay standardization protocols.

386
A fragmented detection antibody conjugate successfully eliminates falsely elevated tryptase levels due to heterophilic antibodies

Rydell, N; Magnusson, B; Ekström-Burell, A; Odelstad, L; Sjölander, A
Thermo Fisher Scientific, Uppsala, Sweden

Background: Heterophilic antibodies may bind to animal antibodies used in immunoassays, e.g. by crosslinking the capturing and detection antibodies. The presence of heterophilic antibodies may result in falsely elevated tryptase levels. Here, we evaluate whether removing the Fc region of the detection antibody will eliminate the binding of heterophilic antibodies and thus the risk of falsely elevated tryptase levels.
Method: The detection antibody in a sandwich immunoassay used for the detection of human tryptase in plasma or serum was subjected to enzymatic cleaving to produce a fragmented antibody. The fragmented antibody was subsequently conjugated with beta-galactosidase and used according to the same assay protocol as was used with the whole antibody conjugate. The whole antibody conjugate was compared with the fragmented conjugate using a panel of commercially available serum samples with known concentrations of heterophilic antibodies, human anti mouse antibodies (HAMA) or rheumatoid factor as well as in house samples with heterophilic antibodies. Different concentrations of heterophilic blocking antibodies and proteins were added to the two conjugates to enhance the protection against binding of heterophilic antibodies. The samples were also analyzed after pretreatment with commercially available heterophilic blocking tubes (HBT) to confirm the presence of heterophilic antibodies.
Results: Out of 17 tested serum samples, 15 proved positive for heterophilic antibodies when evaluated with HBT. All 15 samples (100%) showed elevated tryptase concentrations with the whole antibody conjugate without addition of blocking antibodies and proteins and 8 samples (53%) showed elevated tryptase concentrations with the whole antibody conjugate after addition of blocking antibodies and proteins. In contrast, none of the 15 samples showed elevated tryptase concentrations with the fragmented conjugate, neither with nor without addition of blocking antibodies and proteins.
Conclusion: The use of a fragmented detection antibody conjugate represents an efficient approach to eliminate falsely elevated tryptase levels due to heterophilic antibodies.

387
Heterologous production of food- and pollen-related allergen components for the detection of sensitization patterns

Sundermann, U; Britsch, M; Offermann, N; Fooke, M
Dr. Fooke Laboratorien GmbH, R&D, Neuss, Germany

Background: Food allergy affects not only children but also adults and prevalence is

© 2015 The Authors

worldwide increasing. Fruits, vegetables and nuts are important triggers and allergic reactions against these foods are often associated with pollen allergies. Allergen components belonging to the PR10 family like Bet v 1 are the main cause of such pollen-associated food allergies that may be responsible for the OAS. At the molecular level, this phenomenon is based on cross-reactions of specific IgE to Bet v 1 and homologous allergens in plant food. Further food allergies may be based on reactions against non-specific Lipid-Transfer-Proteins (nsLTP). These molecules are panallergens which might be linked to severe systemic reactions.

Methods: Codon optimized gene sequences, encoding for nsLTP from different food sources (e.g. peach) as well as members of the Bet v 1 family, were cloned via in-vivo-cloning into suitable *E. coli* expression vectors. The particular proteins linked to a polypeptide were expressed, purified by different chromatographic methods and analyzed by SDS-PAGE. These recombinant proteins were compared with commercially available and literature known ones. The reactivity of the allergen components were analyzed by Immunoblot and ELISA using characterized serum samples.

Serum samples known to be positive to pollen- and food-allergens were tested for specific IgE using commercially available direct (ImmunoCAP™; Thermo Scientific) and reversed (ALLERG-O-LIQ; DFL) ELISA systems. Additionally these serum samples were tested for specific IgE to the recombinant components described above. The reaction profile of each sample was compared to the structural homology of the different proteins.

Results: We produced four members of the two allergen families by heterologous expression. The yielded proteins show a reproducible purity $\geq$ 90%. All proteins are reactive and the observed results are comparable to commercially available allergen components.

The results for the serum samples show a good correlation between allergen extracts and corresponding components. But they differ in their IgE-reactivity against the different members of one allergen family. Although there is a significant structural homology between these proteins, an identical sensitization pattern cannot be observed for all cases.

Conclusion: Testing of allergen components in addition to the whole extract may help to find the responsible allergen and thus support further diagnosis.

© 2015 The Authors

Poster Discussion Session PDS 14

Atopic dermatitis and other skin conditions: mechanisms

388
Steroid fear, acceptability, usage frequency, quality of life and disease severity in childhood eczema

Hon, KLE; Tsang, YCK; Leung, TF
Chinese University of Hong Kong, Paediatrics, Hong Kong, Hong Kong, China

Background: Topical corticosteroids (CSs) are the mainstay of treatment for eczema but CS phobia and fears are prevalent and influence therapeutic efficacy. We evaluate if CS acceptability and fear affect patients' quality-of-life (QoL).
Method: Patients with eczema managed in the pediatric dermatology outpatient clinic of a university hospital were surveyed. Nottingham eczema severity score (NESS) for severity, Children's Dermatology Life Quality Index (CDLQI) for QoL, CS fear, acceptability and reported frequency of CS use were measured with quantified questions.
Results: CS fears were prevalent among parents and caregivers of patients with eczema. 58% of parents reported general acceptability of CS as being very good or good, and many applied CS to their child regularly every week. However, more than 40% of parents reported CS fear "always" or "often", 41% reported that they "always" or "often" apply CS only when eczema got worse, 57% would discuss CS fear with their doctors, 30% would request CS-sparing medications, and 14% "always" or "often" use traditional Chinese herbal medicine. Fears were predominantly interpersonal and less often iatrogenic in nature. Skin problems were the most concerned side effects of CS. CS acceptability, frequency of CS usage, CS fear and usage of alternative medications were independent domains in eczema management: CS fears correlated with CDLQI; CS usage frequency correlated with NESS and negatively with parental education; and CS acceptability correlated with parental education. Ordinal logistic regressions showed worse QoL was associated with more CS fear (Odds ratio: 1.092 [95% CI, 1.023 to 1.165], $P = 0.008$).
Conclusion: The extent of CS fears is independent of CS acceptability, but correlates with patients' QoL. Desensitization of parental CS fears should be integral part of eczema education and therapeutics in order to improve therapeutic efficacy and patients' QoL.

389
The perception of topical corticosteroid by caregivers of atopic dermatitis children

Murakami, Y; Amimoto, Y; Odajima, H
Fukuoka National Hospital, Pedatrics, Fukuoka City, Japan

Background: Although topical corticosteroids remain the mainstay of treatment for atopic dermatitis, poor adherence due to corticosteroid phobia and the subsequent therapeutic failures have been reported in some cases. We assessed factors relating to perception and degree of disinclination for topical corticosteroids in caregivers of atopic dermatitis children, and compared the proportion of those with the relevant factors over the 7 years (2007 vs 2014).
Method: Subjects were 198 children (76% of whom had AD) who visited allergic pediatric department in our hospital from November, 2007 to March, 2008 (mean age,3.9 years old), and 122 children (70% of whom had AD) who visited our hospital from May to July, 2014 (mean age, 3.0). We distributed a questionnaire to the 198 and 122 caregivers who agreed to response to our survey voluntarily. The questionnaire included inquiries relating to the degree of disinclination, side effect that they thought, and the reason for disinclination for or refusal of topical corticosteroid use. Further, we investigated the change of their disinclinations for topical corticosteroids in these seven years.
Results: Six percent, 70% and 19% of the subjects showed "absolute disinclination for topical corticosteroids", "mild disinclination" and "no disinclination", respectively in 2007. Reasons for such disinclination were fear of side effects (53%), unavoidable regular use of topical corticosteroid (13%) and bad image (6%), respectively. Caregivers of subjects thought pigmentation, skin atrophy, rebound and dependency as side effects of topical corticosteroid in 23, 12, 9 and 5%, respectively. On the other side, 5%, 63% and 29% of the subjects showed "absolute disinclination for topical corticosteroids", "mild disinclination" and "no disinclination", respectively in 2014. We observed no difference in proportion of caregivers reported disinclination between the two periods for children without AD ($P = 0.48$), while the disinclination was less frequently reported among caregivers of children with AD ($P = 0.06$) in 2014 than those in 2007.
Conclusion: The disinclination for topical corticosteroids of caregivers of the allergy children was still prevalent, but disinclination of caregivers with AD children might be decreasing over the 7-year period.

390
Methoxyluteolin inhibits human mast cells and reduces skin inflammation

Theoharides, T
Tufts University School of Medicine, Department of Integrative Physiology and Pathobiology, Boston, United States

Background: Over 40% of people have dry, sensitive skin that may also involve skin itching and inflammation in such conditions as atopic dermatitis (AD), chronic urticaria, multiple chemical sensitivity disorder, mast cell activation syndrome, mastocytosis and psoriasis (Ps). All these diseases involve mast cells (MCs), which act as "alarmins" to environmental triggers such as bacteria, heavy metals, mold,stress, toxins and viruses.
Method: We had shown that stress worsens this conditions by triggering skin inflammation through local secretion of corticotropin-releasing hormone (CRH), which stimulated MCs to release inflammatory and neurosensitizing molecules. We further showed that CRH works together with substance P (SP) secreted from peripheral nerves, and IL-33 secreted from epithelial cells to produce tumor necrosis factor (TNF), which causes further inflammation and skin damage. Activated MCs also secrete IL-6 and TGFb that induce maturation of Th-17 cell which are critical in the pathogenesis of auto-immune diseases. Both IL-33 and SP are upregulated in lesional AD and Ps skin. We show that SP and IL-33 synergistically stimulate MCs to produce and release IL-1β and TNF-α.

© 2015 The Authors

Unfortunately, there are no effective anti-allergic skin preparations. We had shown that the natural flavonoid luteolin inhibits MCs, keratinocytes and T cells. A pilot open-label study was conducted on patients with sensitive skin, AD, or Ps. We used human cultured MCs (LAD2) obtained from NIH.

Results: We have recently identified tetramethoxyluteolin (methlut), a flavone related to luteolin. This flavone is more potent than luteolin in inhibiting TNF release from human cultured MCs stimulated by SP and IL-33 together. Methlut also penetrates the skin better than luteolin. Here we report that methlut significantly inhibits IL-1β and TNF-α gene expression, production and release triggered by the combination of SP and IL-33. Since methlut has no color, unlike other flavonoids, it has been formulated in a skin lotion with significant anecdotal improvement in skin lesions. In particular, AD lesions in three female patients and Ps lesions in one female and one male patient almost disappeared after using the skin lotion twice daily for two weeks.

Conclusion: Tetramethoxyluteolin-based formulations could prove to be novel treatments for conditions involving skin inflammation.

Funded by NIH grants NS38326 and AR47652, as well as the National Psoriasis Foundation.

391
Dog exposure from birth decreases the risk of eczema in childhood

Thorsteinsdottir, S; Stokholm, J; Bisgaard, H
Copenhagen Prospective Studies on Asthma in Childhood, Faculty of Health and Medical Sciences, University of Copenhagen & Danish Pediatric Asthma Center, Gentofte Hospital, Copenhagen, Denmark

Background: Eczema is the most common chronic skin disease in childhood and preventive strategies are warranted. We have previously demonstrated that dog exposure in early life was associated with a decreased risk of eczema at age 3 years in a cohort of children born to mothers with asthma; odds ratio 0.44 [0.23–0.87], $P = 0.020$.[1] The aim of the present study was to replicate this finding in an unselected larger cohort.

Method: We enrolled 700 children in the Copenhagen Prospective Studies on Asthma in Childhood 2010 birth cohort. Childhood eczema was diagnosed prospectively in the research clinic based on the Hanifin-Rajka criteria during the first 3 years of life. Dog ownership was defined as dog in the home at birth obtained by personal interviews. Associations between dog ownership and eczema were analyzed by Cox' proportional hazards regression model.

Results: 24% ($N = 174$) of the children developed eczema during the first 3 years. 20% ($N = 135$) were exposed to dog in the home from birth. In this unselected cohort, we replicated the finding that perinatal dog exposure was associated with decreased risk of eczema: hazard ratio (HR) 0.55 [0.35–0.86], $P = 0.009$. The protective effect of dog was also found after stratifying for maternal asthma status; asthmatic mother: HR 0.32 [0.12–0.90], $P = 0.030$, non-asthmatic mother: HR 0.65 [0.39–1.09], $P = 0.099$.

Conclusion: Dog exposure from birth was associated with reduced risk of eczema in early life. The protective effect from dog exposure was especially large among children born to asthmatic mothers. The mechanisms by which dog protects against eczema remain unknown, but our study emphasizes the importance of the early environment as a trigger of disease trajectory.

References: 1. Bisgaard H, Halkjaer LB, Hinge R, Giwercman C, Palmer C, Silveira L, Strand M. Risk analyses of early childhood eczema. J Allergy Clin Immunol. 2009 Jun;123(6):1355–60.

392
Increased thymic stromal lymphopoietin and reduced filaggrin expression in the epidermis are associated with the development of atopic dermatitis in infants

Kim, J[1,2]; Kim, BE[3]; Lee, J-O[2]; Jun, H-Y[2]; Kim, H-M[2]; Choi, J[1,2]; Han, Y[2]; Leung, DYM[3]; Ahn, K[1,2]
[1]Samsung Medical Center, Seoul, Korea; [2]Environmental Health Center for Atopic Diseases, Seoul, Korea; [3]National Jewish Health, Denver, United States

Background: Biomarkers predicting the development of infantile atopic dermatitis (AD) are needed to determine the optimal time for effective prevention of AD.

Method: In a birth cohort study, we enrolled 388 pregnant women and serially followed 75 infants for 12 months in an AD risk group with family history of allergy and 12 infants in a control group. The presence of AD was observed, and tape stripping was done on the skin of the right arm at age of 2, 6 and 12 months. Epidermal barrier proteins and thymic stromal lymphopoietin (TSLP) were analyzed by mass spectrometry of epidermal tape strips.

Results: 42/75 (56%) and 2/12 (16.7%) infants developed AD in the AD risk group and the control group, respectively ($P = 0.011$). At the age of 2 months, filaggrin expression was lower in the AD risk group than the control group ($P = 0.027$). At 6 months, the infants in the AD risk group showed higher expression of TSLP and corneodesmosin than the control group ($P = 0.027$ and 0.049). When the infants in the AD risk group were divided into 2 subgroups according to TSLP level at 2 months, the high TSLP group was more likely to develop AD at 6 months ($P = 0.010$) and at 12 months ($P = 0.026$) than low TSLP group.

Conclusion: Our data suggest that increased TSLP and reduced filaggrin expression in the epidermis during the first 6 months of life are biomarkers for the development of AD in infants.

Funding support: This study was supported by Samsung Biomedical Research Institute grant (#GLI-B2-171-1).

393
Allergen immunotherapy in a murine model of atopic dermatitis using a monomeric allergoid adjuvanted with exopolysaccharide from Shigella sonnei

Babakhin, A[1]; Shershakova, N[1]; Kamishnikov, O[1]; Laskin, A[1]; Lvov, V[1]; Aparin, P[1]; Khaitov, M[1]; DuBuske, L[2,3]
[1]NRC-Institute of Immunology FMBA of Russia, Moscow, Russian Federation; [2]Immunology Research Institute of New England, Gardner, United States; [3]George Washington University, MFA, Washington, DC, United States

Background: This study assesses whether an exopolysaccharide (ePS) obtained from Shigella sonnei (S. sonnei) added to monomeric allergoid (succinilated ovalbumin - sOVA) can improve efficacy of allergen-specific immunotherapy (ASIT) in a murine model of experimental atopic dermatitis (EAtD).

Methods: To induce EAtD BALB/c mice were sensitized with OVA via epidermal applications (EDA) for 7 days with further repeat of EDA procedure for two times at 2 week interval. Between the 1st and 2nd EDA mice from:

a)Group 1 got 16 s.c. injections of PBS as a control of EAtD;
b)Group 2 got 16 s.c. injections of non-modified OVA at doses from 0.05 mg/kg to 50 mg/kg;
c)Group 3 got 7 s.c. injections of sOVA at doses from 6 mg/kg to 50 mg/kg;
d) Group 4 got 5 s.c. injections of sOVA at doses from 6 mg/kg to 50 mg/kg including 3 in a mixture with ePS at a dose of 5 mg/kg;
e)Group 5 negative control received just EDA with PBS. Anti-OVA IgE, IgG1, IgG2a antibodies in sera were detected by ELISA.

© 2015 The Authors

After 3rd the EDA IL-4, IL-5, IL-17 and IFN-γ were assessed by ELISA in supernatants after *in vitro* stimulation of spleen cells with OVA. Intensity of skin lesions was assessed by a visual evaluation score system with skin biopsies for histology review from the EDA sites.

Results: Anti-OVA IgE antibody levels in Groups 2 to 4 were decreased after experimental ASIT (EASIT) with lowest level of anti-OVA IgE seen in Group 4. Levels of anti-OVA IgG1 and IgG2a in all EASIT groups increased being maximal at EASIT conclusion. Lowest anti-OVA IgE/IgG2a ratios were in Groups 3 and 4. Decreased IL-4 occurred in Groups 2 and 3. IL-5 levels were similar in all EASIT groups. IL-17 levels were decreased in Groups 2 and 4 while IFN-γ levels were significantly increased in Groups 3 and 4. IL-4/IFN-γ ratio was low in Groups 3 and 4. Histologic examination of skin after the 3rd EDA demonstrated greatest improvement of allergic inflammation in Group 4. The intensity of allergic skin lesion inflammation in all groups receiving EASIT was less than that of Group 1.

Conclusion: ASIT using a monomeric allergoid adjuvanted by exopolysacchiride from S. sonnei improves both skin inflammation and inflammatory cytokine profiles in a murine model of atopic dermatitis.

394
Association of cord blood adiponectin, leptin, and C-reactive protein with atopic dermatitis in the first year of life

Logan, CA[1]; Koenig, W[2]; Reister, F[3]; Weiss, JM[4]; Rothenbacher, D[1]; Genuneit, J[1]
[1]Institut für Epidemiologie und Medizinische Biometrie, Ulm University, Ulm, Germany; [2]Department of Internal Medicine II - Cardiology, Ulm University Medical Center, Ulm, Germany; [3]Department of Gynecology and Obstetrics, Ulm University Medical Center, Ulm, Germany; [4]Department of Dermatology and Allergology, Ulm University Medical Center, Ulm, Germany

Background: A number of cross-sectional studies have reported associations between biomarkers of chronic inflammation and atopic dermatitis (AD), yet few reports of prospective studies have been published. Therefore, it remains unclear whether inflammatory processes play a role in AD development or if chronic inflammation is simply a byproduct of disease. To close this gap, we analyzed cord blood concentrations of adiponectin, leptin, and C-reactive protein (CRP) in relation to AD diagnosis in the first year of life.

Methods: In the Ulm SPATZ Health Study, 934 singleton newborns and their mothers were recruited during their hospital stay following delivery in the University Medical Center Ulm, Southern Germany, between 04/2012 and 05/2013. AD cases were defined as children reported to have been diagnosed with AD by both the parents and primary pediatrician at the 6-month or 1-year follow-up period. Inflammatory biomarkers were measured by ELISAs (n = 836). Logistic regression models were used to estimate relative risk of AD diagnosis based on an interquartile increase of each biomarker separately.

Results: Of 557 children included in the analysis, 34 (6.5%) were diagnosed with AD. Following adjustment for demographic, delivery-related, and anthropometric potential confounders, higher adiponectin was associated with decreased risk of AD diagnosis [Relative Risk 0.79, 95%CI (0.63; 0.97)] and higher CRP was marginally associated with increased risk [1.53 (0.97; 2.41)].

Conclusions: These results suggest inflammatory processes related to cord blood levels of adiponectin and possibly CRP may contribute to, or play a mediating role in, the development or progression of AD in the first year of life. Further research is warranted to determine if the present association extends to adiponectin, CRP, and possibly other related factors present in the intrauterine environment including fetal growth, infection, and pre-eclampsia.

© 2015 The Authors

Poster Discussion Session PDS 15

Food allergy: management

395

Can casein specific IgE levels predict oral desensitization results in children with persistent milk allergy

Karakoc, G[1]; Altintas, D[1]; Yılmaz, M[1]; Karagoz, D[2]; Kont, A[1]
[1]Cukurova University Faculty of Medicine, Adana, Turkey; [2]Baskent University Faculty of Medicine, Adana, Turkey

Background: Oral desensitization is a promising therapeutic approach in patients with persistent and severe cow's milk allergy (CMA). In this report we aimed to evaluate relationship between the oral desensitization results and serum casein spIgE levels in CMA.
Method: Oral desensitization was performed with semirush protocol in 20 children with persistent CMA aged 3–8 years. Clinical symptoms, tolerated milk amount, SPT results, spIgE levels, results of oral food challenges and levels of milk proteins were evaluated at the beginning and 6 months later.
Results: There were 17 boys and 3 girls with the mean age of 5.0 ± 1.41 years. Atopic dermatitis was diagnosed in 15 children (75%), asthma in 12(60%), GIS symptoms, rhinitis and anaphylaxis in 5.3 and 3; respectively(25%.15%,15%). Mean serum milk spIgE level was 124 ± 290 KuA/L(Median; 25.8)KuA/L, casein spIgE level; 32.25 ± 31.01 KuA/L, a-lactalbumin;16.69 ± 34.21(Median:4.62)KuA/L and ß-lactoglobulin;10.36 ± 20.74(Median; 2.38)KuA/L. Oral desensitization completed successfully in 17 children whereas failed in 3 patients because of anaphylactic reaction. Seventeen children could tolerate 30–200 ml undiluted milk after 6 months. We found statistically negative correlation between the casein spIgE levels and tolerated milk amounts (*r*:-0.380, *P*:0.043), however there were no significant correlation between the tolerated milk amounts and total milk spIgE levels(*r*: -0.271, *P*: 0.15), a-lactalbumin (*r*: -0.329, *P*: 0.096) and ß-lactoglobulin (*r*: -0.35, *P*: 0.079).
Conclusion: Casein is an important predicting component for the persistent and severe CMA. In this report we showed that casein spIgE levels can be used for predictor of oral desensitization success in children with CMA.

396

Oral immunotherapy for egg allergy with heat modified egg derivatives

Lazzarotto, F; Bonaguro, R; Toniolo, A; Celegato, N; Polloni, L; Muraro, A
Food Allergy Referral Centre, Department of Women and Child Health, University Hospital of Padua, Padova, Italy

Background: Dietary avoidance is the only current treatment for food allergy. Oral immunotherapy (OIT) is a promising approach to modify clinical reactivity. Being an experimental therapy, a heterogeneity of protocols have been proposed to date. We report our experience in pts affected by egg (E) allergy, who underwent an OIT protocol with E-containing baked products (BP).
Method: We recruited 31 patients (pts) who had reported severe allergic reactions (anaphylaxis) with egg, because of which they were on a restricted diet for E, and had been prescribed self-injectable epinephrine. Median age at recruitment was 6 yrs (range 1–17). All pts underwent an initial oral food challenge (OFC) with BP to define the threshold of tolerated protein dose. Up-dosing was scheduled at 4–6 weeks intervals, and performed at the hospital, using BP containing progressively higher doses of E protein. The newly tolerated dose was consumed daily, and adverse events were reported through a 24/7 line. A second OFC was scheduled after pts could tolerate 2 g of E protein in BP, approximately after 18–24 months after the first OFC. The OFC was negative if pts could tolerate both boiled and raw E, positive if pts reacted either with boiled or raw E. Specific IgE (sIgE) for E white, ovoalbumin and ovomucoid were tested at the initial and final OFC.
Results: During the protocol only 2 pts reported mild reactions. Final OFC was Negative in 13 pts (42%, N-OFC) who could add E to their diet without restrictions. Final OFC was Positive in 18 pts (50%, P-OFC), of which: 11 pts reacted with raw E, but tolerated boiled E; the remaining 7 pts reacted with boiled E, but were advised to assume BP *ad libitum*. An exploratory analysis of sIgE for E proteins showed baseline higher values in pts with final P-OFC compared to pts with N-OFC (table); in both groups sIgE decreased. Statistical significance is not allowed due to the low number of pts.

Baseline median sIgE(KU/L)	N-OFC (13)	P-OFC (18)
EGG WHITE	1.1	3.0
OVALBUMIN	0.2	2.1
OVOMUCOID	0.9	2.0

Conclusion: Our OIT protocol appears to be safe and effective. Pts who reacted at the final OFC did not show any anaphylactic reaction. Pts who did not tolerate raw E could introduce BP and cooked E; those pts who didn't tolerate even boiled E significantly improved their quality of life, thanks to the introduction of BP containing E.

397

Oral immunotherapy in severe peanut allergy in adults

Heliövaara, E[1,2]; Kukkonen, K[2]; Hakulinen, A[2]; Helin, T[2]; Voutilainen, H[2]; Pelkonen, A[2]; Mäkelä, M[2]; Kauppi, P[2]
[1]Department of Pulmonary Medicine, Heart and Lung Center, Helsinki University Central Hospital, Helsinki, Finland; [2]Skin and Allergy Hospital, Helsinki University Central Hospital, Helsinki, Finland

Background: Peanut allergy is common and can cause severe, life-threatening reactions. The current management of peanut allergy consists of strict allergen avoidance, education and provision of emergency medication. In children, oral immunotherapy has emerged as a potential treatment option for severe food allergies, including peanut allergy. In adults, however, studies on oral immunotherapy in severe food allergies are still lacking. In this study, we began oral immunotherapy in five patients with severe peanut allergy.
Method: Five Finnish adults (4 F, 1 M) with severe peanut allergy were enrolled in this study. The allergy diagnosis was verified with positive symptom history, allergen-specific IgE antibodies and an allergen challenge test. Patients were aged 18–41 years at the onset of oral immunotherapy. Immunotherapy was begun ingesting margarine containing peanut protein, starting from 0.1 mg peanut protein a day in the first week, with rising doses every one

© 2015 The Authors

or two weeks up to 50 mg a day in the 18th week. After that the patients ingested whole peanut, first one quarter a day in the 20th week, with rising doses up to several peanuts a day, and continued on a chosen maintenance dose of 1–3 peanuts a day.

Results: Two patients began oral immunotherapy in September 2013, and can now tolerate 1–3 peanuts a day as a maintenance dose. The three other patients have had oral immunotherapy for less than a year and have been able to ingest smaller amounts of peanut according to the immunotherapy plan. No severe allergic reactions or anaphylaxis have occurred during the oral immunotherapy. Results on long-term tolerance, possible allergen-specific IgE level changes, and the effect of oral immunotherapy on patients' quality of life are still pending.

Conclusion: This is the first study to show that oral immunotherapy in peanut allergy has leaded to increased allergen tolerance in our five adult patients. Larger patient series and longer follow-up time are needed to elucidate whether oral immunotherapy could have potential as a treatment option in severe peanut allergy in adults. In children it is known that the obtained tolerance often fades away if the oral immunotherapy is discontinued, and further studies will show if this is also true for adults.

398
Efficacy and safety of oral immunotherapy along with omalizumab in children with severe cow's milk allergy

Pajno, GB; Caminiti, L; Crisafulli, G; Arasi, S; Porcaro, F
Department of Pediatrics – Allergy Unit, University of Messina, Messina, Italy

Background: Given the rise in prevalence of food allergy in developed countries, oral immunotherapy(OIT), has given many patients hope for a widely available form of treatment. However some patients are included in the pattern of non-responders because of failure of active therapy.

Method: Since 2006 we have treated 53 children suffering from IgE mediated cow's milk allergy (CMA) with OIT. Among them four (7%) patients had severe or life-threatening events during up-dosing protocol with cow's milk (CM). Therefore, we used for these patients an anti -IgE monoclonal antibody (Omalizumab) as an adjuvant treatment with OIT, in order to enhance both the safety and efficacy of oral immunotherapy. The allergic children with severe food allergy caused by milk were 6, 9, 11 and 12 years old respectively. Median CM specific IgE levels 58 (+- 21) kU/L. Median total IgE 780 (+-125) kU/L. The dose of Omalizumab was of 0.016 mg/Kg/IgEU/mL (75-to 300 mg) every two weeks for 10 weeks. Afterwards, specific desensitization phase was started along with omalizumab.

Results: The patient n. 1 (male- 6y) after three months of treatment reached the dose of 32 ml of CM (1.0 g. of proteins); the patient n. 2 male (male-9y.) reached the dose of 64 ml of CM (2.0 g of proteins); the third patient(female-11y) reached 64 ml of milk(2.0 g of proteins).

The fourth (male 12y) achieved the dose of 128 ml (4.0 g of milk protein). No moderate to severe side effects were observed at this stage of desensitization. Currently the patients continue with the OIT with CM plus ongoing Omalizumab.

Conclusion: The treatment of severe food allergy with Omalizumab and oral desensitization combination therapy in significant IgE mediated CMA could result in an effective treatment in selected patients with severe food allergy non-responders to OIT.

399
Two year follow-up after rush oral immunotherapy for peanut-induced anaphylaxis

Nagakura, K[1]; Sato, S[2]; Yanagida, N[1]; Nishino, M[1]; Emura, S[1]; Asaumi, T[1]; Okada, Y[1]; Ogura, K[2]; Iikura, K[1]; Ebisawa, M[2]
[1]Pediatrics, Sagamihara National Hospital, Kanagawa, Japan; [2]Department of Allergy, Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, Kanagawa, Japan

Objective: The purpose of this study is to examine the efficacy and safety of rush oral immunotherapy (ROIT) to induce desensitization and tolerance for peanut.

Methods: We enrolled 24 peanut anaphylactic patients who underwent ROIT between 2011 and 2013. After ROIT in admission, they gradually increased ingestion of peanut powder up to 3 g/day at home. Thereafter they took maintenance dose daily. Premedication (antihistamine and leukotriene antagonist) was withdrawn, when they were able to consume 3 g of peanut without symptoms (desensitization). When no symptoms were seen for 3 months, they underwent an oral food challenge (OFC) after 2 weeks of peanut avoidance to confirm tentative tolerance acquisition. If the result of 3 g OFC was negative, they further received a 10 g OFC 1 year later.

Results: We analyzed 19 subjects (median age 8.8 y) whose treatment period exceeded 2 y after ROIT. Prior to ROIT, median threshold eliciting initial symptoms was 125 mg and anaphylaxis was 875 mg. Median peanut specific IgE was 39.2 kUa/L. All of the subjects reached desensitization by 8 months after ROIT. Eight (42%) were confirmed to have achieved tentative tolerance within 1 y, 16 subjects (68%) at 2 y and 3 subjects passed the 10 g OFC. Peanut specific IgE decreased gradually to 25.5 kUa/L after 1 y and 15.0 kUa/L after 2 y. Adverse reaction rate per ingestion during admission was 44% and with any home dose was 5%. Three subjects received adrenaline during ROIT and 2 subjects at home. After confirmed tentative tolerance acquisition, 4 subjects developed mild adverse reactions due to 3 g of peanut intake.

Conclusion: ROIT for peanut increased the threshold and induced desensitization. Even after developing tentative tolerance, careful long term observation is still necessary.

400
Study of reintroduction of tree nuts and peanuts (START-P)

Tibbott, R[1]; Foley, L[2]; King, Y[1]; Islam, S[2]; Deighton, J[2]; Ewan, P[2]; Clark, AT[1]
[1]Paediatric Allergy, Cambridge University Hospital Trust, Cambridge, United Kingdom; [2]Cambridge University Hospital Trust, Allergy, Cambridge, United Kingdom

Background: A recent study showed that following peanut oral immunotherapy (OIT), 90% of children could safely eat peanuts. Tree nut allergy often has a more severe phenotype and tree nut OIT has been less well studied. Children are often allergic to more than one nut type and multiple nut OIT has not been studied. Our objective was to assess the safety and feasibility of single tree nut OIT and multi nut OIT.

Method: Tree nut and peanut allergic children aged 7–15 years (with a positive nut specific IgE +/- relevant component resolved diagnostics) underwent double-blind, placebo controlled food challenges (DBPCFC) to confirm their allergies to one or more nuts (cashew, hazelnut, walnut, Brazil or peanut). Twenty three children underwent initial screening (clinical history, skin prick tests and nut specific IgE +/- component resolved diagnostics). Seventeen children underwent DBPCFC. Eleven were deemed eligible to commence either single or two nut OIT. (Participants were excluded if they passed the DBPCFC or if they had a severe reaction requiring intramuscular Adrenaline at the DBPCFC starting dose of 5 mg nut protein). OIT was administered by dose increments in 2 weekly intervals with a starting dose of 2 mg nut protein per nut up to a maximum of 800 mg nut protein per nut. Chil-

© 2015 The Authors

dren were reviewed following 6 weeks of maintenance therapy (and at 6 months).

Results: 3 children have successfully completed the immunotherapy programme to date (2 single cashew OIT, 1 single hazelnut OIT) with a further 8 children currently in the updosing phase. The mean length of time to reach the maintenance dose of OIT (800 mg) was 19.3 weeks. No dose reductions were required. Reactions during OIT were mild and included oral itching and nausea. 0/3 children required intramuscular Adrenaline.

Conclusion: Tree nut OIT has not been studied in detail. There are concerns that tree nut allergy has a more severe phenotype than peanut. Whilst this is a very small sample size, we have demonstrated that, to date, single tree nut OIT (including home adminsitration) is feasible and safe. The regime was well-tolerated. Results from our remaining 8 participants will provide further information on the feasibility and safety of multi nut OIT.

401
Reintroduction failure after negative food challenge in children

Amaral, L; Leão, L; Carolino, F; Coimbra, A; Placido, JL
Serviço de Imunoalergologia, Centro Hospitalar São João, Porto, Portugal

Background: A negative oral food challenge (OFC) should normally be followed by reintroduction of the food in the diet. However, reintroduction fails in a subset of children.

Aim:

1) To analyse the frequency and the reasons for not reintroducing the implicated food in the diet after negative OFC in children.
2) To determine the proportion of food avoidance in patients who did not undergo OFC.

Method: A retrospective study of children with an OFC scheduled between January 2012 and December 2014 was performed. The patients' clinical records were reviewed and we inquired the caregivers about current food consumption status (CFCS).

Results: Data of 101 patients (60.4% male; median age [minimum-maximum] 5 [1–17] years); 60.4% atopic (44.6% allergic rhinitis; 37.6% asthma and 25.7% atopic eczema) was collected.

A total of 178 OFC were scheduled and 150 were performed with a negative outcome in 103; the majority with cow's milk (32%), hen's egg (23.4%) and fish (22.3%). Data on the CFCS was obtained in 92.7% of the total 178 OFC scheduled. The mean time (± std) between the OFC and the assessment of CFCS was 14.01 ± 7.3 months.

Food avoidance was maintained in 26.7% of the 103 negative OFC. Refusal of the food was the main reason (41.4%), followed by the caregivers' fear of reaction (34.5%). Further comparison of children with or without food avoidance revealed no significant differences with respect to gender ($P = 0.527$), age ($P = 0.067$), suspected food group ($P = 0.2$), clinical manifestation of the index reaction ($P = 0.257$) or the presence of allergic comorbidities ($P = 0.952$).

In the 28 OFC scheduled that were not performed, avoidance of the suspected food was maintained in 63.2% in contrast to the 38.6% when an OFC was performed, independent of the result; $P < 0.001$.

Conclusion: Children with suspected food allergy often present with a long-lasting elimination diet. This study reveals that despite a negative challenge outcome and advice to re-introduce the food in the diet, more than one fourth (26.7%) of the children were still avoiding the implicated food. Almost two thirds (63.2%) of the children who did not undergo OFC, maintained avoidance of the suspected food. These data highlight the importance of performing an OFC in preventing unnecessary restrictive diets as well as to alert the need in reassessment of food consumption after negative OFC.

402
Continuing food-avoidance after negative food challenges in adults with suspected food allergy

Carolino, F; Amaral, L; Leão, L; Pereira, AM; Coimbra, A; Plácido, JL
Centro Hospitalar São João, E.P.E., Porto, Portugal

Background: Oral food challenge (OFC) is the gold standard for the diagnosis of food allergy (FA) and, when negative, it should be followed by reintroduction of the food in the diet.

Aims:: 1) To determine the frequency of food reintroduction after negative OFC in adults with suspected FA;

2) to ascertain the reasons for continuing avoidance in cases of negative OFC.

Methods: Adult patients referred to our Department for suspected FA, and with an OFC scheduled between January 2012 and December 2014, were selected. Data were collected by revision of the medical records and through a short structured phone or paper questionnaire on current food consumption status (CFCS).

Results: A total of 122 patients (68.9% females; median age 35 years [interquartile range 25–44]) were included; 74.6% were atopic and 33.6% had asthma. Of the 169 OFC scheduled, 139 (82.2%) were performed and, of these, 120 (86.3%) were negative. The main food groups tested were seafood (32.4%), fresh fruit (23.0%) and fish (18.7%). Data on CFCS was obtained in 114 of the performed OFC. The mean time (± standard deviation) between the OFC and CFCS assessment was 19.1 ± 9.0 months.

Food eviction was maintained in 24.2% of the 99 negative OFC with assessed CFCS. The main reason was fear of reaction (68.4%); 15.8% reported recurrent symptoms after new ingestion. Continuing avoidance after negative OFC had no significant association with gender ($P = 0.25$), age ($P = 0.98$), suspected food group ($P = 0.34$), clinical manifestations at the index reaction ($P = 0.13$) or the presence of allergic comorbidities ($P = 0.79$).

CFCS was verified in 23 of the 30 scheduled OFC that were not performed and avoidance of the suspected food was maintained in 87.0%, in contrast to 31.6% of those who performed OFC, independently of the result ($P < 0.001$).

Conclusion: This study reveals that food eviction was maintained in nearly one-quarter of the suspected food allergies even after a negative challenge, mostly due to fear of having a reaction. In almost 90% of cases, patients who did not perform OFC maintained avoidance of the suspected food. These data highlight the importance of performing OFC in the prevention of unnecessary restrictive diets and alert to the need of reassessing food consumption after negative OFC. A subset of patients continued to react in real-life settings after negative OFC.

403
Impact of food allergies on the allergic person's travel decision, trip organization and stay

Dano, D[1,2]; Michel, M[3]; Astier, C[1]; Couratier, P[4]; Steenbeek, N[5]; Sarr, PY[2]; Bonnefoy, M[3]; Boulangé, M[1]; Kanny, G[1,6]
[1]Université de Lorraine, Vandoeuvre les Nancy, France; [2]Genclis SAS, Vandoeuvre les Nancy, France; [3]ORSAS Lorrane, Vandoeuvre les Nancy, France; [4]AFPRAL, Paris, France; [5]OASIS, Belgium, Belgium; [6]University Hospital Nancy, Vandoeuvre les Nancy, France

Background: Management of food allergy (FA) relies on the establishment of a targeted and personalized eviction diet. During a stay abroad, people with FAs may have difficulties in applying/following eviction measures due to the language barrier as well as to the various laws in countries outside the European Union (EU). The aim of this study is to evaluate the impact of FA in the travel decision, trip organization and stay of an allergic person.

© 2015 The Authors

Method: The survey was conducted through a questionnaire available online from October 1st to December 15th 2014 on the website of the Regional Observatory of Health and Social Affairs in Lorraine, the French and Belgian Associations for the Prevention of allergies and was disseminated through social networks. The survey included 45 questions of whether FA impacts the travels abroad, induces specific behaviors and whether allergic reactions occurred during the stay.

Results: One hundred and two allergic persons (62 women / 40 men) aged 20.25 ± 14.6 years completed the questionnaire. Allergies were due to: nuts (52%), peanuts (43%), milk (31%), soybean (22%), eggs (19%), sesame (15%), and other allergens (46%). Of these 102 patients, 89 (87%) carry an emergency kit. Seven people don't travel for personal reasons, 14 (15%) don't travel due to their FA, 81 (79%) have traveled abroad. Translation of the allergen in the language of the country visited is unknown by 18 (18%) persons, 48 (47%) don't know the legislation regarding mandatory allergen labeling in the EU and 72 (70%) don't know that of the country visited outside EU, 37 (36%) carry their food during a stay abroad. Allergic reactions while traveling are reported by 24 people (23%).

Conclusion: This survey demonstrates the significant impact of FA on the allergic person's travel decision, trip organization and stay. FA forced 15% of allergic people not to travel abroad and 36% to transport their food. A quarter of respondents had an allergic reaction abroad. Attention is drawn to the lack of knowledge concerning allergen legislation. This study highlights the need to raise patient awareness to control the risk of allergies, especially mandatory allergen labeling and the need for harmonization of regulations internationally.

404

Health-related quality of life in children with objectively-diagnosed allergies to staple foods assessed using a disease-specific questionnaire

Protudjer, JLP[1,2]; Östblom, E[2,3,4]; Jansson, S-A[2,5]; Middelveld, R[1,2]; Dahlen, S-E[1,2]; Ahlstedt, S[2]; Health Economics of Food Allergy (FoodHE)
[1]Karolinska Institutet, Institute of Environmental Medicine, Stockholm, Sweden; [2]Karolinska Institutet, Centre for Allergy Research, Stockholm, Sweden; [3]Södersjukhuset, Sachs' Children and Youth Hospital, Stockholm, Sweden; [4]Södersjukhuset, Department of Clinical Research and Education, Stockholm, Sweden; [5]Umeå University, Department of Public Health and Clinical Medicine, Umeå, Sweden

Background: The health-related quality of life (HRQL) of children with food allergy is incompletely understood. Others have considered the impact of allergy to various foods on children's HRQL using both generic and disease-specific questionnaires. However, the impact of objectively-diagnosed allergies to staple foods on HRQL amongst children has not been investigated, despite the fact that these foods are difficult to avoid in a typical western diet. To this end, we aimed

(1) to investigate various food allergy-related exposures associated with HRQL amongst staple food allergic children, and
(2) to contextualize poorer food allergy-associated HRQL vs controls.

Method: Swedish children (0–12 years) with objectively-diagnosed allergy to the staple foods cow's milk, hen's egg and/or wheat ("cases"; $n = 85$). Cases were identified from medical records and recruited in 2010–2012 by a paediatric nurse specialising in allergy. Inclusion criteria were a convincing history of allergy to at least one staple food ascertained either by a positive food challenge with evident symptoms, or by high levels of food specific Immunoglobulin E antibodies associated with 95% probability of a positive double-blind placebo controlled food challenge. Age- and sex-matched controls ($n = 94$) were recruited via advertisements from the same geographical area as cases. We administered a food allergy-specific parent-completed questionnaire originally developed by EuroPrevall (to cases only), and a generic HRQL questionnaire (EQ 5D; to cases and controls).

Results: The mean age for cases and controls were 6 years and 7 years, respectively. No statistically significant demographic differences were found between cases and controls. Hen's egg was the most common offending staple food, affecting 76% of cases. Approximately 7% of cases were allergic to all three staple foods. Parent-reported respiratory and cardiovascular symptoms were associated with poorer HRQL. Elements of disease severity (previous anaphylaxis ($P < 0.001$; epinephrine autoinjector prescription ($P < 0.003$)) were negatively associated with HRQL. Cases had poorer HRQL measured by EQ-5D compared to controls ($P < 0.01$).

Conclusion: Use of a disease-specific questionnaire revealed that disease severity in children with objectively-diagnosed allergy to the staple foods cow's milk, hen's egg and/or wheat is associated with poorer health-related quality of life. Cases have poorer health-related quality of life than controls.

405

Thresholds reactivity and clinical evaluation (TRACE) study: investigation of the effect of extrinsic factors on peanut allergic reactions

Dua, S[1]; Ruiz-Garcia, M[2]; Bond, S[3]; Boyle, R[2]; Durham, S[2]; Ewan, P[1]; Felgate, L[3]; Foley, L[4]; Hardy, S[5]; Islam, S[4]; King, Y[4]; Mills, C[6]; Pasea, L[3]; Skypala, I[7]; Turner, P[2]; Wilson, E[2]; Clark, A[3]
[1]Department of Allergy, Cambridge University, Cambridge, United Kingdom; [2]Imperial College London, London, United Kingdom; [3]Cambridge University, Cambridge, United Kingdom; [4]Cambridge University Hospital Trust, Cambridge, United Kingdom; [5]Food Standards Agency, London, United Kingdom; [6]University of Manchester, Manchester, United Kingdom; [7]Imperial College (NHLI) and Royal Brompton Hospital, London, United Kingdom

Background: Determining individual and population thresholds of reaction is critical for assessing the risk posed by food allergens. Peanut is widely used in food manufacturing and is known to cause severe and fatal allergic reactions. Both the threshold and severity of an allergic reaction are known to vary between individuals in a population. However within individual variation can also occur and may in part be explained by extrinsic factors.

The TRACE study aims to measure the impact of two such extrinsic factors: sleep deprivation and exercise on the threshold and severity of responses to peanut in a representative sample of the UK peanut allergic population.

Method: This is a multicentre randomised crossover study in progress, with 45 of 100 peanut allergic adults, aged 18–45, recruited. After screening, the participants undergo a DBPCFC to determine their threshold to peanut at baseline. They then undergo three further challenges in a random order: one with no intervention (repeat baseline) and two with extrinsic factors (exercise and sleep deprivation). Incrementally increasing doses of peanut flour (3 μg to 1 g) in a dessert matrix are delivered at fixed time intervals and the eliciting dose of reaction is recorded. Thresholds of reaction are judged on objective symptoms.

Results: Initial pilot work was performed to assess the safety and practicability of the challenge protocols. Methods were piloted on 26 participants (16 peanut allergic and 10 non peanut allergic). On the basis of pilot data the PRACTALL criteria to score food challenges have been adapted. Exercise piloting allowed finalisation of the challenge protocol: participants are given peanut doses interspersed with exercise bouts at 85% of their maximum exercise capacity. For the sleep deprivation arm, participants are allowed to sleep for a maximum of 2 h on the night before the challenge. The Psychomotor Vigilance Task is used to objectively assess tiredness.

© 2015 The Authors

Initial data have guided the development of a new severity score to grade food allergic reactions. New participant and investigator visual analogue scales to assess symptoms during challenges have been developed giving insight into food allergic reactions.

Conclusion: This study will provide the first UK eliciting dose data. In addition, novel information on the impact of extrinsic factors on food allergic reactions will be provided. These data will be used to inform food policy makers and will have public health significance.

406
Specific peanut allergen levels in foods show marked variability and depend on the food

Filep, S; Block, D; King, E; Chapman, MD
INDOOR Biotechnologies, Inc., Charlottesville, United States

Background: Immunoassays are commonly used in the food industry to assess allergen contamination during food processing. Although 13 peanut allergens are listed in the WHO/IUIS nomenclature, the levels of these allergens in foods have not been extensively studied. We compared levels of Ara h 1, Ara h 2 and Ara h 6 in foods, flours and oils from peanut and other nuts.

Method: Ara h 1, Ara h 2 and Ara h 6 were measured by ELISA in over 60 peanut and tree nut food products from local stores or by purchasing these products online. The products included several varieties of peanut butter, as well as peanut and tree nut flours, powders, and refined or unrefined oils.

Results: Roasted peanut butters contained much more Ara h 1 than other peanut allergens and typically had 2–4 fold more Ara h 1 than Ara h 2 or Ara h 6. The reverse was true for peanut flours which showed markedly higher levels of Ara h 2 and Ara h 6, up to 20 fold higher than Ara h 1. In general, there was no cross-reactivity in any of the ELISA and peanut allergens were below detection in tree nut butter. Among peanut oils, the allergens were only detected in roasted unfiltered oil and not in refined oils.

Conclusion: Specific major allergen levels in peanut foods show great variation and composition. Exposure to high levels of Ara h 2 in peanut flour products may explain in part why Ara h 2 is a dominant peanut allergen. The variability of the allergen levels also suggests that specific allergen measurements have advantages for allergen monitoring in the food industry, as compared to generic assays for total peanut, which may not adequately measure such variability.

407
The BASALIT multicentre trial: ELISA quantification for Gly m 4 threshold data and consistency control of challenge meal batches

Holzhauser, T[1]; Treudler, R[2]; Franke, A[3]; Schmiedeknecht, A[3]; Randow, S[1]; Becker, W-M[4]; Lidholm, J[5]; Vieths, S[1]; Simon, JC[2]; for the BASALIT Study Group
[1]Division of Allergology, Paul-Ehrlich-Institut, Langen, Germany; [2]Universität Leipzig, Klinik für Dermatologie, Venerologie und Allergologie, Leipzig, Germany; [3]Universität Leipzig, Zentrum für Klinische Studien, Leipzig, Germany; [4]Division of Clinical & Molecular Allergology, Research Center Borstel, Borstel, Germany; [5]ThermoFisher Scientific, Uppsala, Sweden

Background: The BASALIT trial (EudraCT 2009-011737-27) aims to determine efficacy of rBet v 1 SCIT on threshold levels (LOAEL) after soy food challenge in patients with birch-associated and Gly m 4-mediated soy allergy [1]. For this purpose, the development of standardised provocation meals with consistently stable allergen levels was needed. We present data on the determination of Gly m 4 levels for the verification of batch-to-batch consistency of challenge meals.

Method: Soy meal included soy protein isolate (SPI, 88% total soy protein) at nine dose levels (0.00044 g–15 g total soy protein) [1]. An inhibition (i) ELISA based on polyclonal rabbit anti-rGly m 4 antiserum was developed, validated and quality controlled. Over a period of 56 months, 24 individual batches of active and placebo meals were prepared. At weeks 0, 6 and 12, each of the individual batches of placebo (level 9), active soy meals (levels 4 and 9) and SPI were quantified for Gly m 4 using iELISA.

Results: Gly m 4 was non-detectable below the detection limit in any placebo batch. With ≤20% mean coefficient of variation (CV), Gly m 4 levels were equivalent between 24 active batches and within a storage and testing period of 12 weeks for each batch.

Conclusion: A novel Gly m 4 specific iELISA was successfully developed and it proved batch-to-batch consistency of challenge meals over a 4-year period. Gly m 4 levels were low in the active SPI material but comparable to published levels in soybean. Based on quantified Gly m 4 levels in SPI, LOAEL of Gly m 4 in active soy challenge meal can be calculated once LOAEL data on SPI in challenge meal are available.

Ref.: [1] Treudler R et al., EAACI 2014 (Abstract 101).

© 2015 The Authors

Poster Discussion Session PDS 16

Novel Immune Mechanisms

408

Up-regulation of periostin production in tears of allergic ocular disease patients

Okada, N[1]; Fujishima, H[2]; Fukagawa, K[3]; Izuhara, K[4]; Ohta, S[5]; Ono, J[6]; Matsuda, A[1]; Saito, H[1]; Matsumoto, K[1]
[1]Department of Allergy and Immunology, National Research Institute for Child Health & Development, Tokyo, Japan; [2]Department of Ophthalmology, Tsurumi University School of Dental Medicine, Yokohama, Japan; [3]Ryogoku Eye Clinic, Tokyo, Japan; [4]Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, Saga, Japan; [5]Department of Laboratory Medicine, Saga Medical School, Saga, Japan; [6]Shino-Test Corporation, Sagamihara, Japan

Background: Atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC) are severe, chronically relapsing, ocular inflammatory diseases that threaten vision. These diseases are characterized by T helper-cell 2 (Th2 cells)-dominant conjunctival inflammation, with proliferative changes in the conjunctiva that often cause corneal damage. However, the mechanism increasing the severity of these diseases remains unknown. Furthermore, discrimination of each allergic ocular disease and prediction of corneal damage are sometimes difficult. We investigated whether periostin, a novel mediator and a biomarker in allergic inflammation, is involved in the pathogenesis of allergic ocular diseases and whether it can serve as a biomarker for these diseases.
Method: Patients with allergic ocular diseases (AKC, VKC or seasonal allergic conjunctivitis (SAC)) and age-matched control subjects were enrolled, and tear and serum samples were collected. Tear periostin levels were measured by ELISA and compared with tear IL-13 and serum periostin levels. We also investigated the expression of periostin in conjunctival tissues by immunohistochemistry.
Results: Tears from allergic ocular disease patients contained significantly higher periostin levels compared to tears from control subjects. Especially, tear periostin levels in AKC patients were associated with serious comorbidities such as giant papilla formation and corneal damage. Although tear periostin levels were positively associated with tear IL-13 and serum periostin, tear periostin levels appeared to enable the most accurate discrimination of allergic ocular diseases. Moreover, intense periostin immunochemical staining was observed in the stromal tissues of giant papillae from AKC patients.
Conclusion: Periostin produced in conjunctival tissues, presumably induced by IL-13, may contribute to the pathogenesis of allergic ocular diseases. Furthermore, tear periostin levels seem to be potentially useful as a biomarker for distinguishing allergic ocular diseases from other conditions and diagnosing allergic ocular disease involving corneal damage.

409

Effects of ONO-4053, a DP1 (prostaglandin D_2 receptor) antagonist, on antigen-induced nasal congestion and sneezing

Yamaguchi, S; Okada, Y; Matsunaga, Y; Nishikawa, R; Takeda, H; Nambu, F
Ono Pharmaceutical Co., Ltd., Osaka, Japan

Background: As prostaglandin D_2 (PGD_2), a chemical mediator released in large amounts from mast cells, is known to be involved in a number of allergic responses, blockage of PGD_2 action might be beneficial for the treatment of nasal allergies. ONO-4053 is an orally active DP1 (PGD_2 receptor) antagonist in phase 2 clinical development for the treatment of allergic rhinitis. The purpose of this study was to evaluate the effects of ONO-4053 on antigen-induced nasal congestion and sneezing in animal models.
Method:

1 Naturally sensitized cynomolgus monkeys were anesthetized, intubated and mechanically ventilated. Nasal volume, as indicator of nasal congestion, was measured by acoustic rhinometry before and after antigen nasal challenge. ONO-4053, montelukast or cetirizine was intravenously administered the monkeys before antigen nasal challenge.
2 Mice were intraperitoneally sensitized to ovalbumin (OVA) on day 1 and boosted by subcutaneous injection of OVA on day 5. From day 18 to day 24, the animals were challenged by intranasal application of OVA once a day. On day 25, ONO-4053 or chlorpheniramine maleate was orally administered the mice followed 1 h later by antigen-challenged into the nostril. The numbers of sneezes and nasal rubbing were counted for each animal.

Results:

1 Antigen challenge induced nasal congestion in cynomolgus monkeys. While montelukast and cetirizine partially inhibited antigen-induced decrease in nasal volume, ONO-4053 inhibition of this parameter was complete, resulting in a superior efficacy on nasal congestion.
2 In the mice, antigen challenge induced sneezing and nasal rubbing. Both ONO-4053 and chlorpheniramine maleate significantly decreased the frequency of sneezing and nasal rubbing with comparable efficacy.

Conclusion: ONO-4053 strongly inhibits antigen-induced nasal congestion, sneezing and nasal rubbing in animal models. It is therefore believed that ONO-4053 would be clinically useful for the treatment of allergic rhinitis.

410

Modulation of the IgE response and cytokine production in BALB/c mice by synthetic peptides mimicking B and T-cell epitopes from the major birch pollen allergen Bet v 1

Purgina, D; Andreev, S; Bashkatova, E; Babakhin, A; Khaitov, M
NRC Institute of Immunology FMBA, Moscow, Russian Federation

Background: Synthetic peptides mimicking epitopes (mimotopes) of natural allergens are promising candidates for a safe allergen-specific immunotherapy. The purpose of study was to prepare mimotopes mimicking B and T epitopes of the major birch pollen allergen Bet v 1, evaluate their allergenicity and the possibility of their use to suppress the allergic response in experimental model of allergic hypersensitivity.
Method: Synthetic peptides (sequences 42–57, 59–71, 104–116, 120–132, 141–156 with added Cys residue at N-terminal) of Bet v 1were synthesized by Fmoc chemistry. To enhance their immunogenicity for generation of peptide-specific antibodies some of these peptides were conjugated with carrier protein KLH (keyhole limpet hemocyanin)

© 2015 The Authors

via maleimide spacer. All peptides were tested in ELISA for binding with serum IgE- and IgG-antibodies generated to birch pollen extract (T3), while anti-peptide sera were assessed for binding with the T3. BALB/c female mice were divide into 4 groups (8 mice each) where 2 groups were sensitized by *i.p.* injections of 30 μg of T3 mixed with 1 mg/mouse $Al(OH)_3$ and 2 other groups were control (sensitization/challenge: PBS/PBS and PBS/T3). The protocol of treatment incuded five s.c. dorsal injection with 25 μg of peptide 141–156. Cytokine production by splenic cells stimulated with T3 was tested by ELISA.

Results: It has been shown that none of the peptides adsorbed on a micro titer plate did bind to IgE antibodies against to T3, while peptide 120–132 demonstrated a significant reactivity towards the T3/IgG antibodies. However, we observed some binding of peptide 141–156 to T3-specific IgE antibodies in solution. Therapeutic immunization with peptide 141–156 prior a challenge with T3 reduced the level of T3-specific IgE. Peptide injections resulted in significant IFNγ production compared with the control. Changes in the levels of IgE, IgG, IL-4, IL-5 and IFNγ indicated reduction in reactivity to the T3 antigen.

Conclusion: Subcutaneous administration of peptide 141–156 of major allergen Bet v 1 resulted in a reorientation of Th2-type immune response towards Th1-type immune response.

411
Mast cells-nerve interactions in allergic rhinitis

Le, DD[1]; Schmit, D[1]; Heck, S[1]; Bals, R[2]; Schick, B[3]; Al-Kadah, B[3]; Dinh, QT[1,2]
[1]Department of Experimental Pneumology and Allergology, Saarland University Hospital, Homburg/Saar, Germany; [2]Department of Internal Medicine V, Saarland University Hospital, Homburg/Saar, Germany; [3]Department of Otorhinolaryngology, Saarland University Hospital, Homburg/Saar, Germany

Background: Neurogenic inflammation constitutes an important pathway in allergic diseases. Mast cells (MCs) are well recognized as key players in allergic rhinitis. It was shown that MCs represent an important link between the nervous system and the immune system. The interactions between MCs and nerve in allergic rhinitis remain to be poorly understood. In this study we investigated the distribution and the proportion of tryptase and chymase expressing mast cell (MC_{tc}) and the interaction of MC with nerve fibers in human nasal mucosa biopsy samples.

Method: Nasal biopsy specimens were taken from 8 patients with perennial allergic rhinitis and from 6 patients without any allergic diseases. Immunohistochemistry was performed to identify mast cells by their tryptase- and chymase-expression and nerve fibers by PGP9.5.

Results: A significant increase in mast cells numbers was found in epithelium only (Cells/mm²: Rhinitis 121.5 ± 35.18 $n = 8$ vs control 18.45 ± 9.327 $n = 5$, $P = 0.0459$). Very few MC_{tc} were found in epithelium with no difference in both patient groups (MC_{tc}/MCs: Rhinitis 4.754 ± 3.074% $n = 8$ vs control 19.82 ± 9.735% $n = 5$, $P = 0.1026$). The proportion of MC_{tc} decreased significantly in submucosa of the rhinitis group (MC_{tc}/MCs: Rhinitis 25.64 ± 6.369% $n = 8$ vs control 49.75 ± 8.169% $n = 6$, $P = 0.0357$). In the epithelium only a few MCs were detected to have contact with nerve fibers, and there is no significant change in both patient groups (Rhinitis 5.156 ± 2.869% $n = 7$ vs control 1.176 ± 1.176% $n = 5$, $P = 0.2921$). The proportions of MCs having contact with nerve were found to be increased significantly in submucosa of rhinitis patients (Rhinitis 37.73 ± 3.296% $n = 8$ vs control18.08 ± 1.771 $n = 6$, $P = 0.0005$). The MC_{tc} having contact with nerve were found in submucosa only, their proportion is increased significantly in the rhinitis group (Rhinitis 45.58 ± 2.721% $n = 8$ vs control 18.24 ± 2.541% $n = 6$, $P < 0.0001$).

Conclusion: Allergic rhinitis induces an increase in the amount of MCs in epithelium and reduces the proportion of MC_{tc} in submucosa. The increased morphologic association of MCs with nerve may indicate an important role of neuroimmune interaction in allergic rhinitis.

412
Effects of ONO-4053, a DP1 (prostaglandin D_2 receptor) antagonist, on prostaglandin D_2-induced nasal allergic reactions

Okada, Y; Yamaguchi, S; Matsunaga, Y; Nambu, F
Ono Pharmaceutical Co., Ltd., Osaka, Japan

Background: As prostaglandin D_2 (PGD_2), a chemical mediator released in large amounts by mast cells, is known to be involved in a number of allergic responses, blockage of PGD_2 action might be beneficial for the treatment of allergic diseases. ONO-4053 is an orally active DP1 (PGD_2 receptor) antagonist in phase 2 clinical development for the treatment of allergic rhinitis. The purpose of this study was to evaluate the effect of ONO-4053 on PGD_2-induced nasal reactions in dogs.

Method: Dogs were anesthetized, intubated, and mechanically ventilated. Nasal volume (NV), as indicator of nasal congestion, was determined before and after intranasal application of PGD_2 using acoustic rhinometry. In addition, the amount of nasal secretion was measured after application of PGD_2. ONO-4053 was intravenously or orally administered prior to PGD_2-treatment.

Results: Nasal application of PGD_2 decreased NV and increased the amount of nasal secretion. These allergic responses continued for up to 60 min after PGD_2 application. ONO-4053, given intravenously 30 min prior to PGD_2 application, inhibited PGD_2-induced responses. In addition, ONO-4053, given orally 4 h before PGD_2 application, completely inhibited PGD_2-induced decrease in NV.

Conclusion: These findings indicate that PGD_2-induced nasal allergic reactions are predominantly mediated via the DP1 receptor and that ONO-4053 could be effective for the treatment of such reactions, including allergic rhinitis.

413
Increase in CC10 production from human nasal epithelial cells by histamine H_1 receptor antagonists, levocetirizine

Tokita, E[1]; Asano, K[2]; Furuta, A[1]; Kobayashi, H[1]
[1]Otorhinolaryngology, Showa University School of Medicine, Tokyo, Japan; [2]Physiology, Showa University School of Nursing and Rehabilitation Sciences, Yokohama, Japan

Background: CC10 is well known to be an immuno-suppressive protein secreted from airway epithelial cells after inflammatory stimulation and plays important roles in the development of allergic disorders. Although the third generation histamine H_1 receptor antagonist, levocetirizine (LCT) is developed and used for the treatment of allergic disorders with remarkable success, the influence of the agent on CC10 production is not fully understood. In the present study, we examined the influence of a histamine H_1 receptor antagonist, cetirizine (CT) and LCT on CC10 production *in vitro* and *in vivo*.

Method: Nasal epithelial cells were stimulated with 20 ng/ml TNF-α in the presence of various concentrations of the agents for 24 h. CC10 levels in culture supernatants were examined by ELISA. We also examined the influence of the agents on CC10 mRNA expression and mRNA translation by RT-PCR and wheat germ cell-free protein synthesis technique, respectively. In the second set of experiments, pollinosis patients against Japanese cedar pollen were treated orally with 5 mg LCT once a day for 4 weeks during Japanese pollen season (January to April, 2014). CC10 levels in nasal secretions were also examined by ELISA.

Results: The addition of LCT into epithelial cell cultures caused increase in the abil-

© 2015 The Authors

ity of cells to produce CC10 in response to TNF-α stimulation as well as its mother drug, CT. The minimum concentrations that caused significant increase was 0.05 μM for LCT, which is lower levels than that induced by its mother drug, CT (0.1 μM). Although treatment of cells with LCT caused inhibition of CC10 mRNA expression, which was increased by TNF-α stimulation, the agent increased the translation of CC10 mRNA to produce specific proteins. Oral administration of LCT also increased CC10 levels in nasal secretions from pollinosis patients along with attenuation of clinical symptoms.

Conclusion: The ability of histamine H1 receptor antagonists, LCT to enhance CC10 production may account, at least in part, for the clinical efficacy of the agent on allergic disorders, including allergic rhinitis.

414

Phlp 5-specific antibody responses and their impact on antigen uptake and cell polarization in CD1c+ dendritic cells from non-allergic humans living in different environments

Isakovic, A[1]; Fichtinger, G[1]; Hillebrand, C[1]; Thalhamer, J[1]; Danzer, M[2]; Gabriel, C[2]; Weiss, R[1]
[1]University of Salzburg, Molecular Biology, Salzburg, Austria; [2]Red Cross Blood Transfusion Service, Linz, Austria

Background: In our present project we study primary $CD1c^+$ dendritic cells from non-allergic individuals as these have been hotly debated in the maintenance of allergic – and probably also non-allergic – immune responses via interaction with antigen-IgG immune complexes. Dendritic cells can be activated or inhibited by immune complexes binding to functionally different FcμR which link humoral and cellular immune responses. The balance of activating and inhibitory FcμR therefore might play a decisive role in the regulation of a non-allergic immune response against the major grass pollen allergen Phl p 5. However, it is still unknown how the ratio between signals from the activating FcμR and the inhibitory FcμRIIb determines the immunological outcome. Thus, we measured the amount of Phl p 5-specific antibodies in non-allergic donors living in different environments (farm vs. urban environment) and investigated their influence on antigen uptake and polarization of $CD1c^+$ dendritic cells.

Method: IgG1, IgG4 and IgE antibody titers were measured by ELISA. $CD1c^+CD19^-CD20^-$ dendritic cells from non-allergic donors were purified using a BD FACSAria III cell sorter and antigen uptake in the absence and presence of autologous IgG was assessed by flow cytometry. Afterwards, polarization of antigen loaded DCs was determined based on secreted cytokines and gene expression profiles, both measured by Luminex system.

Results: We found that a high percentage of non-allergic individuals display considerable amounts of Phl p 5 specific IgG1 and IgG4 with significant differences depending on the living conditions. Furthermore, IgG1, which is associated with TH1 immune response is 100-fold elevated compared to TH2 associated IgG4 in farmers and towns people. Furthermore, we could show that allergen uptake by $CD1c^+$ dendritic cells is highly dependent on the presence of allergen specific IgG. Moreover, we established and measured gene expression of the Fc receptors and genes involved in activation/polarization as well as cytokine and chemokine secretion after IgG mediated antigen uptake.

Conclusion: We found that donors living in farming vs. urban environment differ in seroconversion indicating that the environment and the amount of antigen exposure might have an influence on the non-allergic immune response. The established methods will allow us to analyze the impact of allergen immune complexes on polarization and T cell priming capacity of CD1c+ dendritic cells.

415

Immunophenotype of human olfactory mucosa-derived mesenchymal stem cells

Hancharou, AY[1]; Antonevich, NH[1]; Chekan, VL[2]; DuBuske, LM[3,4]
[1]Republican Scientific and Practical Center for Epidemiology and Microbiology, Minsk, Belarus; [2]Belarusian Medical Academy of Postgraduate Education, Minsk, Belarus; [3]Immunology Research Institute of New England, Gardner, MA, United States; [4]George Washington University, MFA, Washington, DC, United States

Background: The olfactory mucosa from nasal cavity is an easily available source of resident multipotent cells. Human olfactory mucosa-derived mesenchymal stem cells (hOM-MSCs) are similar to bone marrow and adipose tissue MSCs. This study assesses the immunophenotypic properties of hOM-MSCs.

Method: Nasal mucosa samples were taken from 16 patients with non-inflammatory diseases of nasal cavity. Explant culture method was used to obtain hOM-MSCs cells. Cells were assayed for 30 markers using flow cytometric analysis (CD11b, CD11c, CD15, CD31, CD33, CD34, CD40, CD45, CD54, CD71, CD73, CD80, CD86, CD90, CD105, CD106, CD117, CD123, CD 133/2, CD273, CD274, HLA-ABC, HLA-DR, nestin, vimentin, β-III-tubulin, p75NTR, GFAP, MAP-2, O4).

Results: The analyzed cell cultures satisfied the minimal criteria used to define human MSC according to Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy. HOM-MSCs expressed mesenchymal markers CD105, CD73 and CD90, but were negative for lymphocyte common antigen CD45 and the specific endothelial marker CD31. Hemapoetic stem cell proteins CD177 and CD133/2 were not observed on hOM-MSCs surface, but cells expressed low levels of CD34. Among markers inherent to neuroglial-linege, hOE-MSC expressed nestin, vimemtin, β-III-tubulin, p75NTR and GFAP. Markers of mature neurons (MAP-2) and oligodendrocytes (O4) were absent. hOM-MSCs were negative for erythroid (CD71) and myeloid receptors CD15, CD11c and CD33, while weak expression of CD11b was seen. Cells expressed co-inhibitory molecules CD273 and CD274, co-stimulatory molecule CD40, but were negative for CD80 and CD86 markers. Moreover, hOM-MSCs were positive for cell adhesion molecules CD54 and CD106. Cells expressed HLA class I (HLA-ABC) molecules, but the expression of HLA-DR was absent.

Conclusion: The hOM-MSCs belong to the MSC-superfamily based on the pattern of antigen expression. Some neuronal stem/progenitor markers were observed in analyzed cells suggesting that hOM-MSCs may represent a distinct tissue-specific population of stem cells. The presence of co-inhibitory molecules may explain the immunosuppressive effect of MSC on human T-cells.

416

Dynamic changes of B-cell subpopulations after renal allograft transplantation

Svachova, V[1]; Sekerkova, A[1]; Cecdrlova, E[1]; Brabcova, I[2]; Hruba, P[2]; Striz, I[1]; Viklicky, O[3]
[1]Department of Clinical and Transplant Immunology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Institute for Clinical and Experimental Medicine, Transplant Laboratory, Prague, Czech Republic; [3]Department of Nephrology, Transplant Center, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

B lymphocytes play an important role in immune responses affecting the outcome of kidney allograft transplantation. Each subpopulation of these cells is involved in both alloimmune response and regulation. The long lived plasma cells derived from bone marrow (PCs) are the main source of donor specific antibodies (DSA) involved in the pathogenesis acute or chronic rejection. Memory cells have an indispensable role in early antibody mediated rejection in sensitized recipients. Many donor reactive

© 2015 The Authors

naive B cells are formed too, to but due to immunosuprressed T lymphocytes these cells are rarely differentiated into alloreactive PCs or memory B cells secreting DSA. The role of B regulatory cells is connected with long-term graft tolerance based on production of IL-10 and other cytokines suppressing immune response. The phenotype of B regulatory cells is overlapped with populations of transient and mature naive B cells.

The aim of our study was to characterize the proportions of B lymphocyte subpopulations in kidney allograft recipients. B cell subsets were defined by multicolor flow cytometry in peripheral blood according to lineage specific markers (CD45, CD19, CD27, IgG, IgM, CD38, CD24, CD21) and their dynamic changes were observed in time intervals before transplantation, on day 7, and 3 months after the surgery in 76 kidney transplant recipients. We compared also the effect of induction therapy by rabbit antithymocyte globulin (rATG) and basiliximab on redistribution of B cells. Although the absolute $CD19^+$ B cell count was not affected, we demonstrated an increase of both the non-switched ($CD27^+IgM^+$) and the switched-memory ($CD27^+IgM^-$) B cells in early phase after transplantation and after rATG induction. Plasmablasts ($CD24^-CD27^{high}CD38^{high}$) and transient ($CD24^{high}CD38^{high}$) B lymphocytes remained significantly decreased for 3 months after Tx in both regimes of induction therapy. In contrast, the absolute number of mature naive ($CD24^+CD27^-IgD^+IgM^+$) B cells was not affected by immunosuppression.

The monitoring of dynamic changes in population of B cells after the transplantation may be helpful for understanding the mechanism of early antibody-mediated responses and follow-up study contribute to diagnosis of possible complications of the outcome including kidney graft rejection.

417
The role of IL-33/ST2 axis in enhancing inflammation, tissue damage and structural changes in acute inflammation

Stankovic, M[1]; Pantic, I[2]; Puskas, N[3]; Zaletel, I[3]; De Luka, SR[1]; Milutinovic-Smiljanic, S[4]; Trbovich, AM[1]
[1]Department of Pathophysiology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia; [2]Faculty of Medicine, University of Belgrade, Institute of Medical Physiology, Belgrade, Serbia; [3]Faculty of Medicine, University of Belgrade, Institute of Histology, Belgrade, Serbia; [4]General and Oral Histology and Embryology, Faculty of Dentistry, University of Belgrade, Belgrade, Serbia

Background: IL-33 is a new member of IL-1 cytokine family. From 2005, when IL-33 was discovered, a lot of research has been done about IL-33 and its receptor, ST2. However, the role of IL-33/ST2 axis in acute inflammation is still not completely understood. The aim of this study was to examine the effects of IL-33/ST2 axis on intensity of inflammation, tissue damage and structural changes in acute inflammation.

Method: Wild-type and ST2 knock-out BALB/c mice were divided into four groups: WT-C (wild-type control group), KO-C (ST2 knock-out control group), WT-I (wild-type inflammation group), and KO-I (ST2 knock-out inflammation group). An acute inflammation was induced by injection of turpentine oil into the right and left hind limb muscles, while control animals received intramuscular injection of saline. After 12 h animals were anesthetized, treated muscles and b*lood from the heart* were collected, and creatine kinase activity in serum and volume density of inflammatory infiltrate in muscle, were determined. Fractal and grey level co-occurrence matrix (GLCM) analysis of treated muscle was performed. Namely, fractal dimension as a measure of complexity, angular second moment as a measure of uniformity, and GLCM correlation were determined.

Results: Creatine kinase activity in serum increased in both inflammatory groups, WT-I and KO-I, but it was significantly higher in WT-I than in KO-I. In addition, volume density of inflammatory infiltrate was significantly higher in WT-I than in KO-I. Fractal dimension was significantly lower in WT-I and KO-I, when compared to WT-C and KO-C, respectively. GLCM correlation and angular second moment were significantly lower in WT-I when compared to WT-C as well as to KO-I, while there was no significant difference between KO-C and KO-I.

Conclusion: Our results indicated that IL-33/ST2 axis had important role in enhancing inflammation, tissue damage and structural changes in acute inflammation.

418
Sensitivity of basophils to recombinant birch pollen allergen (Bet v 1a) during and out of pollen season in allergic patients

Piechota-Polanczyk, A[1]; Lupinek, C[2]; Brzezinska-Pawłowska, O[1]; Kurowski, M[1]; Jabłońska, A[1]; Stolz, F[3]; Kopp, S[3]; Neubauer, A[3]; Kiss, R[2]; Valenta, R[2]; Kowalski, ML[1]
[1]Department of Immunology, Rheumatology and Allergy, Healthy Aging Research Centre, Medical University of Lodz, Lodz, Poland; [2]Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria; [3]Biomay AG, Vienna, Austria

Background: The measurement of changes in CD63 or CD203c expression on basophiles after *in vitro* exposure to an allergen (Basophil Activation test; BAT) has been used in both diagnostic and research settings. However, the capacity of recombinant allergens to activate basophils in allergic patients with respect to the environmental allergen exposure during birch pollen season has not been tested in detail. We aimed at comparing basophil reactivity with recombinant birch allergen (Bet v 1a) in allergic patients during and after birch pollen season.

Method: The study comprised 40 patients allergic to birch and 10 non-allergic controls. Sixteen patients were analyzed twice, during and 5–6 months after the birch pollen season. Allergy to birch pollen was confirmed by SPT with birch pollen extract. Whole blood was stimulated with increasing concentrations of Bet v 1a (from 0.001 ng/ml to 10,000 ng/ml) or anti-FcεRI, and analyzed by flow cytometery. Basophils were identified as CD45posHLADRnegCD123-pos cells and changes in CD63 and CD203c expression were used to assess cell activation. BAT test was considered positive when the percentage of CD63 positive cells was higher than 30% or if the stimulation index (SI) for CD203c expression was higher than 2, for at least one of all Bet v 1a concentrations tested.

Results: When assessed by CD63 expression BAT was positive in 9/16 (56%) patients during birch pollen season and in 7/35 (20%) after the pollen season. BAT assessed by CD203c expression was positive in 9/16 (56%) patients during pollen season and in 5/31 (16%) out of the pollen season. The basophil reactivity to Bet v 1a was weaker 5 months after the birch pollen season for each of the examined Bet v 1a concentrations. Statistically significant differences in the percent CD63 expression during and out of season were observed for 0.01 ng/ml ($P = 0.005$) and 0.1 ng/ml of Bet v 1a ($P = 0.03$). For CD203c expression significant seasonal changes were noticed for 0.1 ng/ml ($P = 0.02$) and 1 ng/ml of Bet v 1a ($P = 0.003$). No basophil activation was observed in healthy controls.

Conclusion: The study documented, that recombinant Bet v 1a specifically activates basophils of allergic patients, but the response varies depending on environmental allergen exposure.

© 2015 The Authors

419
Alteration of Treg and Th17 cytokine levels in patient with recurrent spontaneous abortion, before and after lymphocyte immunotherapy

Roomandeh, N[1,2]; Saremi, A[2]; Salehian, P[2]; Naderi, M[2]; Yonesi, B[2]; Zare, A[2]; Arasteh, J[3]; Mirmohammadkhani, M[4]; Kokhaei, P[1,5]; Pak, F[1]
[1]Department of Immunology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran; [2]Sarem Cell Research Center, Sarem Women's Hospital, Tehran, Iran; [3]Department of Biology, Faculty of Basic Sciences, Islamic Azad University Central Tehran Branch, Tehran, Iran; [4]Research Center for Social Determinants of Health, Semnan University of Medical Sciences, Tehran, Iran; [5]Immune and Gene Therapy Lab, Cancer Centre Karolinska, Karolinska University Hospital, Stockholm, Sweden

Background: Immunotherapy with paternal mononuclear cells is used as a treatment for recurrent spontaneous abortion (RSA). Th17 and Treg cells have been described as two subsets with opposing actions in autoimmunity and maintenance of maternofetal tolerance. Among immunological causes, an imbalance between Th17and Treg cells have been investigated in women with RSA, but the mechanism of the action has not been fully elucidated. The objective of the present study is to evaluate the variation in Treg and Th17 associated cytokine levels before and after immunotherapy.
Method: 40 women with at least three RSA, were included in this study as an experimental group. Paternal immunotherapy, were performed for each patient. The levels of IL-17, IL-21, IL-22, IL-10 and TGF-β were measured in serum and peripheral blood mononuclear cells (PBMCs) culture supernatant of patients, before and after treatment by paternal mononuclear cells using ELISA method.
Results: Serum concentration of IL-17 and IL-21, were significantly decreased in RSA patients after immunotherapy ($P < 0.03$), while no differences were observed in serum levels of IL-22 after treatment. Also, TGF-β serum levels as a Treg cytokine, were significantly increased ($P < 0.01$) but IL-10 level showed no major changes. Supernatant of cultured PBMC from RSA patients showed a significant decrease in levels of IL-17 and IL-22 ($P < 0.03$, $P < 0.005$ respectively), but levels of IL-21, TGF-β and IL-10 showed no significant change after the treatment.
Conclusion: These results suggest that lymphocyte immunotherapy for RSA patients may induces a dominant state of Treg cells and we found that alteration of Th17 and regulatory T cell related cytokines would be beneficial for pregnancy.

420
Production of recombinant rat monoclonal antibodies and design of a biologically-relevant model for the preclinical evaluation of IgE antibodies for cancer therapy

Josephs, DH[1,2]; Saul, L[1,2]; Bax, H[1,2]; Dodev, T[3]; Karagiannis, P[1]; Selkirk, C[4]; Lentfer, H[4]; Beavil, AJ[3]; Jones, P[4]; Gould, HJ[3]; Blower, PJ[5]; Spicer, JF[2]; Karagiannis, SN[1]
[1]King's College London, St John's Institute of Dermatology, London, United Kingdom; [2]Division of Cancer Studies, King's College London, Research Oncology, London, United Kingdom; [3]Randall Division of Cell and Molecular Biophysics, King's College London, London, United Kingdom; [4]Cancer Research UK, Centre for Drug Development, South Mimms, United Kingdom; [5]Division of Imaging Sciences, King's College London, London, United Kingdom

Background: Monoclonal antibody cancer therapeutics are historically of the IgG1 antibody class. However, other classes, namely IgE, may demonstrate superior tissue bioavailability and higher receptor affinity, which may result in improved efficacy (AllergoOncology). To investigate this hypothesis, MOv18 IgE was engineered against the human ovarian carcinoma antigen, folate receptor alpha (FRα).

The lack of cross-reactivity of human IgE with murine FcεRs precludes preclinical evaluations of therapeutic IgE antibodies in immunocompetent murine models. Due to genetic similarities between humans and primates, and similarities in the FcεRI structure and distribution between humans and rats, 1) primate effector cells and 2) a fully immunocompetent rat model with surrogate rat antibodies, were investigated for the preclinical evaluations of MOv18 IgE immunotherapy.
Method: Recognition of human IgE by effector cells from cynomolgus monkey and man was compared *ex vivo*. Surrogate rat MOv18 IgE and IgG2b chimeric antibodies were cloned from the human equivalents and purification was performed. *In vitro* antibody characterisation and functional assessments were then performed.
Results: Human IgE bound with different characteristics to human and cynomolgus monkey IgE effector cells. Molecular characteristic, functional and effector equivalence of surrogate rat MOv18 IgE and IgG2b with human counterparts was confirmed by demonstrating:

1 expected size and purity
2 binding of the rat antibodies to their target, human FRα, and to rat FcεRI and FcγRI receptors respectively,
3 triggering of mast cell degranulation upon cross-linking of rat MOv18 IgE and
4 triggering of ADCC and ADCP of FRα-expressing tumour cells.

Conclusion: Primate models may not be useful for preclinical evaluations of human IgEs. Instead, the production of recombinant rat IgE to develop a biologically-relevant surrogate rat tumour model demonstrated similar characteristics and functional profiles for human and rat IgE. This model will enable *in vivo* preclinical efficacy and toxicity studies.

© 2015 The Authors

Poster Discussion Session PDS 17

Pediatric asthma

421

How body mass index may influence the diagnosis of airway diseases by impulse oscillometry

Cavaleiro Rufo, J[1]; Madureira, J[1]; Oliveira Fernandes, E[1]; Martins, C[2,3]; Moreira, A[2,3]
[1]INEGI – Institute of Science and Innovation on Mechanical Engineering and Industrial Management, Porto, Portugal; [2]Immunology Department, Faculty of Medicine of the University of Porto, Porto, Portugal; [3]Allergy Department, Centro Hospitalar São João E.P.E., Porto, Portugal

Background: Lung function assessment is a very important phase in the diagnosis and monitoring of airway diseases. The impulse oscillometry system (IOS) is a versatile non-invasive tool that may be used for lung function evaluation requiring minimal effort from the patient, thus being particularly useful in assessing young children that are unable to correctly perform spirometry. Transient IOS reference values for children aged 3 to 18 years old have already been published in previous studies and are known to be influenced by age and height. Since obesity and dyspnoea are often associated, the aim of this pilot study was to investigate how the body mass index (BMI) in children may influence the IOS parameters. Moreover, the study also focused on how BMI affected the variation of IOS parameters after administration of a bronchodilator.
Method: IOS with bronchodilation test (400 μg Salbutamol) was performed in 141 children (64 girls), aged between 3 and 18 years old (9.1 ± 4.2 years), using a MS-IOS MasterScreen (Jaeger, Cardinal Health, Germany), according to international recommendations. BMI was calculated and children were regarded as "normal weight" (61%), "overweight" (22%) and "obese" (17%), according to the BMI percentiles.
Results: There were no statistically significant correlations between BMI percentiles and the measured IOS parameters. However, there were statistically significant differences between the three groups of children when considering the variations of impedance (ΔZ) and reactance (ΔX) at 5 Hz after administration of the bronchodilator ($P = 0.022$ and $P = 0.003$, respectively). These variations were significantly higher in the obese group (ΔZ5 = -2.4 [95% CI -3.5: -1.3]; ΔX5 = 1.0 [95% CI 0.5: 1.5]) when compared to overweight (ΔZ5 = -1.5 [95% CI -2.1: -0.9]; ΔX5 = 0.3 [95% CI -0.1: 0.7]) and normal weight (ΔZ5 = -1.5 [95% CI -1.7: -1.2]; ΔX5 = 0.4 [95% CI 0.2: 0.6]) groups.
Conclusion: There appears to be no associations between BMI percentiles and IOS parameters. Nevertheless, a subgroup analysis showed that BMI percentiles appear to influence ΔZ5 and ΔX5 after the administration of 400 μg of salbutamol, suggesting that different responses to the bronchodilator may be associated with the BMI. The highest ΔZ5 and ΔX5 were observed in obese children.

422

Longitudinal study of lung function growth in Chinese children with asthma

Tang, MF[1]; Sy, HY[1]; Tam, WWS[2]; Chan, WC[1]; Wong, GWK[1]; Leung, TF[1]
[1]Department of Paediatrics, Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China; [2]National University of Singapore, Alice Lee Centre for Nursing Studies, Yong Loo Lin School of Medicine, Singapore

Background: Asthma is caused by complex interactions between many predisposition genes and early-life and environmental factors. The Childhood Asthma Management Program reported that a proportion of American asthmatic children had decreased lung function with time. Prevention of asthma exacerbations is an important goal in the management of these patients. On the other hand, there is limited longitudinal data on lung function growth of asthmatic children. This study characterised changes in spirometric indices among Chinese asthmatic children in Hong Kong and explored genetic and environmental determinants on lung function growth.
Method: Of 186 Chinese asthmatic children aged 6–12 years were recruited from paediatric allergy clinic of our university-affiliated teaching hospital. These patients were prospectively followed by the same paediatrician for 5 years. Disease control by Asthma Control Test and pre-bronchodilator spirometry were recorded at baseline and then monitored at least annually. Spirometric indices were compared with local references. Generalised estimating equation was used to analyse longitudinal changes in these lung function outcomes.
Results: The mean (SD) age of patients at baseline was 9.7 (1.9) years, and 117 (63%) of them were male. Twenty-nine percent had passive smoking, 54% ever received inhaled corticosteroid (ICS) treatment during follow-up, and 57% had good asthma control. Adjusting for age and presence of upper respiratory infection within 2 weeks before visits, we found significant decline in FVC of 1% per year, and significant increase in FEV_1/FVC and $FEF_{25–75}$ of 1.5% and 3.7% per year respectively. Male patients had 4.8% higher FEV_1 ($P = 0.012$) and 6.9% higher FEV_1/FVC ($P = 0.025$) than females in any single year. However, there was no significant gender disparity in longitudinal changes for FEV_1 and FEV_1/FVC ($P = 0.718$ and 0.487 respectively). Patients treated with ICS had 4.0% lower FEV_1 and 3.1% lower FEV_1/FVC than those without ICS, but the former group had less rapid FVC decline over time. Among asthmatic children, we could not find any relationship between lung function growth and passive smoking in household as well as the presence of eczema or atopy.
Conclusion: Chinese schoolchildren with asthma have significant annual decrease in FVC and increase in FEV_1/FVC and $FEF_{25–75}$. Boys with asthma had higher FEV_1 than females. Asthmatics who received ICS treatment have lower FEV_1 and FEV_1/FVC but less rapid FVC decline.

423

Reference values of oscillometric indices assessing small airway function in Finnish preschool children

Knihtilä, H[1]; Kotaniemi-Syrjänen, A[1]; Mäkelä, MJ[1]; Kalliola, S[1]; Pelkonen, AS[1]; Malmberg, LP[1]
[1]University of Helsinki, Helsinki University Hospital, Skin and Allergy Hospital, Allergology, Helsinki, Finland

Background: Impulse oscillometry (IOS) has a potential advantage of reflecting small and large airway function separately, and the measurement is suitable also for preschool children. Although small airways may play a significant role in the development and control of asthma, no reference values suitable for Finnish children exist with regard to IOS parameters that are suggested to reflect small airway function; the difference between respiratory resistance at 5 and 20 Hz (Rrs5–20) and area under the reactance curve (AX).
Method: Healthy children were recruited from seven kindergardens in Espoo, Finland, and screened with a standardized

© 2015 The Authors

health questionnaire, skin prick tests and clinical examination to exclude asthma and other allergic diseases. Technically accepted triplicate IOS measurements from 106 children (53 boys) aged between 2.1 to 7.0 years were used to create reference equations for Rrs5–20 and AX. Age, standing and sitting height, weight and body surface area were introduced as independent variables in the regression equations, and the equations were also evaluated separately by gender.

Results: Both Rrs5–20 and AX showed positive correlation with all tested anthropometric predictors, and Rrs5–20 showed minimal variability with gender. However, standing height was the best independent variable for reference equations of both Rrs5–20 (adjusted r^2=0.151) and AX (adjusted r^2=0.463). Results were not significantly altered by including other tested factors in the equations.

Conclusion: The present study introduces reference equations for oscillometric parameters Rrs5–20 and AX in Finnish children. Further studies are required to determine the clinical significance of these height-adjusted oscillometric reference values.

424
Early-onset atopic dermatitis phenotypes at risk of asthma in the ORCA cohort

Amat, F[1,2]; Saint Pierre, P[3]; Foueré, S[4]; Bourrat, E[4]; Nemni, A[1]; Boutmy-Deslandes, E[5]; Sarahoui, F[1]; Pansé, I[6]; Bagot, M[7]; Just, J[1,2]; Couderc, R[8]
[1]AP-HP – Hôpital d'Enfants Armand Trousseau, Paris, France; [2]Sorbonne Universités, UPMC Univ Paris 06, INSERM, UMR_S 1126, Institut Pierre Louis d' Epidémiologie et de Santé Publique, Equipe EPAR, Paris, France; [3]Sorbonne Universités, UPMC Univ Paris 06, Laboratoire de Statistiques Théoriques et Appliquées, Paris, France; [4]Hôpital Saint-Louis, Dermatology Department, Paris, France; [5]Hôpital Saint-Louis, Service de Biostatistiques et Informatique Médicale, INSERM, U 717, Université Paris 7, Paris, France; [6]Hopital Saint Louis, Paris, France; [7]Hôpital Saint-Louis, Paris, France; [8]AP-HP – Hôpital d'Enfants Armand Trousseau, Laboratoire de Biologie Moléculaire, PARIS, France

Background: Atopic dermatitis (AD) is known to predate asthma and other atopic disorders, described under the term "atopic march". However, this classical sequence is not always present and only a few studies have addressed children at risk of developing asthma. The objective of the study was to define early-onset AD phenotypes leading to asthma.

Method: We performed a cluster analysis with 9 variables of 214 infants with early-onset AD, prospectively enrolled in the ORCA cohort and followed each year on the occurrence of asthma until the age of 6.

Results: We identified 3 clusters: – cluster 1 (n = 94) with low to no sensitization to food (27.7%) or aeroallergens (10.6%) and moderate AD severity (SCORAD 25.29 ± 14.6) called "mild AD with low sensitization"; – cluster 2 (n = 84) characterized by a higher AD severity (SCORAD 32.66 ± 16.6) and frequent sensitization to food allergens (98.9%) or aeroallergens (26.2%), most likely multiple (96.4% for food allergens), called "severe AD with multiple sensitizations" – cluster 3 (n = 36) with parental history, moderate AD severity (SCORAD 24.46 ± 15.7), moderate rate of sensitization to food (38.9%), exclusively unique, with no sensitization to aeroallergens, called "moderate AD with familial history of asthma". Percentages of children suffering from asthma at the age of 6 were higher in clusters 2 and 3 (36.1% and 33.3% respectively versus 14.9% in cluster 1, $P < 0.01$).

Conclusion: Risk of developing asthma during childhood in infants with early-onset AD is higher in phenotype with multiple sensitizations or in phenotype with familial history of asthma.

425
Adaptation and validation of the pediatric asthma quality of life questionnaire in Macedonian children with asthma

Cvejoska-Cholakovska, V[1]; Vlaski, E[1]; Velik Stefanovska, V[2]
[1]University Children Hospital, Pulmonology and Allergology, Skopje, Macedonia; [2]Institute of Epidemiology and Biostatistics, Skopje, Macedonia

Background: In order to better the control of asthma in the last decades, despite the use of clinical and pulmonary functional assessment, a lot of emphasis has been put on the assessment of the quality of life (QL). The aim of the study was to validate the Macedonian version of the Pediatric Asthma Quality of Life Questionnaire (PAQLQ).

Method: The study included 64 children with asthma from the ages ranging from 7 to 17 from a Macedonian nationality, treated in an ambulant or hospital facility within the University Children's Hospital in Skopje in a time period of 2 years. The children were assessed 3 times during a period of 3 months. To assess the control of the condition we used a Clinical Severity Score (CSS), and for the assessment of the quality of life (QL) we used the Macedonian version of the PAQLQ from Elizabeth Juniper which is contained of 23 questions organized into three domains: symptoms, activities, and emotions. The results were analyzed with the appropriate statistical tests.

Results: There has been an increase in the average values of the scores of the PAQLQ, which means better control of the asthma by the end of the 3 months. There was not a statistically significant difference ($P > 0.05$) in the change of the QL and the age of the children with asthma in all the three domains and the overall score. Better scores on the PAQLQ were achieved by the children with persistent asthma, as well as the ones with better CSS. A significant correlation was determine between the assessed clinical parameters (asthma diary, FEV1, PEF и CSS) and the domain of activities; symptoms and overall score ($P < 0.05$); and in the domain of emotions only in correlation to CSS ($R = -0.31$). There was a significant improvement of all the scores of PAQLQ after the beginning of the anti-inflammatory therapy with ICS. According to CSS, at the end of the study all of the children were classified as stable (good QL), in contrast to the beginning of the study which was characterized with 78% of stability.

Conclusion: The Macedonian version of the PAQLQ shows exceptional validity, reliability, excellent responsiveness in the QL of the children with asthma. It can be used for the assessing of the condition; has good measurement properties for all children between 7 and 17; successfully assesses the effects of the anti-inflammatory therapy and achieves complete control of the condition. It is simple and easy for use, and gives a new dimension to monitoring children with asthma.

426
Low levels of vitamin D found to associate with increased airway responsiveness in young children with blood eosinophilia

Määttä, A[1]; Kotaniemi-Syrjänen, A[1]; Malmström, K[1]; Malmberg, LP[1]; Sundvall, J[2]; Pelkonen, AS[1]; Mäkelä, MJ[1]
[1]Skin and Allergy Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; [2]Department of Health, Genomics and Biomarkers Unit, National Institute for Health and Welfare, Helsinki, Finland

Background: In addition to bone metabolism, vitamin D has been found to play a central role in normal maturation of the immune system. Suboptimal serum vitamin D levels have been suggested to be associated with biased T cell responses such as those seen in allergies and asthma. However, the role of vitamin D in the development of airway responsiveness is not clear.

Method: Eighty children aged 6–27 months suffering from recurrent and/or prolonged lower respiratory tract symptoms were physically examined and measurement of maximal expiratory flows with whole body plethysmography, rapid thoracoabdominal compression, methacholine challenge test and skin prick tests were per-

© 2015 The Authors

formed. Serum vitamin D, IgE levels and blood eosinophils were determined.

Results: Serum vitamin D levels were deficient (< 25 nmol/l) in none of the patients, insufficient (25–50 nmol/l) in 21 (26%), suboptimal (51–-74 nmol) in 43 (54%), and optimal (≥75 nmol) in 16 (20%) children. Of the patients, 35 (42%) had atopic eczema, 21 (26%) were skin prick test positive, 21 (26%) had blood eosinophilia (blood eosinophils > 4% of the leukocytes) and 61 (76%) had increased airway responsiveness to methacholine (PD40VmaxFRC < 0.9 mg).

Vitamin D insufficiency was not associated with any of the atopic manifestations (atopic eczema, skin prick test reactivity) or high levels of IgE. However, seven (54%) of the 13 children with blood eosinophilia and increased airway responsiveness to methacholine had insufficient levels of vitamin D, whereas 13 (23%) of those 56 with either eosinophilia or increased airway responsiveness, and one (9%) of those 11 with neither eosinophilia nor airway responsiveness had vitamin D ≤ 50 nmol/l ($P = 0.045$). Among those children with increased airway responsiveness defined as PD40VmaxFRC < 0.3 mg, the association with insufficient amount of vitamin D was even stronger ($P = 0.007$).

Conclusion: Insufficient vitamin D levels were more common in those young children with troublesome lung symptoms and airway responsiveness linked to eosinophilia. The results highlight the need for controlled, prospective interventional studies for preventing asthma by increased supplementation with vitamin D during early childhood.

427

Exhaled nitric oxide and bronchial hyperresponsiveness between asthmatic children with and without allergic rhinitis sensitized to the same house dust mite

Yoon, J[1]; Park, AL[2]; Park, JS[1]; Lee, E[1]; Yang, SI[1]; Hong, SJ[1]; Yoo, J[1]
[1]Department of Pediatrics, Childhood Asthma Atopy Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [2]Childhood Asthma Atopy Center, Asan Medical Institute, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Background: Children with asthma frequently have allergic rhinitis (AR) as co-morbidity. Allergic rhinitis increased exhaled nitric oxide (eNO) and bronchial hyperresponsiveness (BHR) in children with asthma. The purpose of this study is to compare lung function, eNO and BHR between asthmatic children with and without AR, and investigate whether the difference between two groups are associated with atopic degree.

Method: We recruited 69 asthmatic children with AR, 19 asthmatic children without AR, 38 children with AR, and 43 non-atopic controls. We measured forced expiratory volume in 1 s (FEV1) and forced expiratory flow at 25–75% of the FVC (FEF 25–75%), dose response slope (DRS) of methacholine and adenosine 5'-monophosphate (AMP), and eNO, and performed skin prick tests with common aeroallergens.

Results: Asthmatic children with AR had higher eNO levels than those without AR ($P = 0.035$). However, there was no difference in lung function, BHR to methacholine and AMP between asthmatic children with and without AR. After adjustment of age and serum total IgE, there was no significant difference in the levels of eNO between asthmatic children with and without AR. In total asthmatics, the levels of eNO were significantly correlated with serum total IgE levels ($r = 0.478$, $P < 0.001$).

Conclusion: AR increased exhaled nitric oxide (eNO), but did not BHR in asthmatic children. Moreover, the levels of eNO were associated with serum total IgE levels. It suggests that airway inflammation increased by AR as co-morbidity may not lead to BHR and be dependent on atopic degree in children with asthma.

428

Bronchodilator response in asthmatic children and adolescents with normal FEV1 values

Cruz, C[1]; Didenko, I[1]; Tomaz, E[1]; Ferreira, F[1]; Reis, R[1]; Ferrão, A[1]; Campos, L[1]; D'Elia, C[2]; Inácio, F[1]
[1]Hospital de São Bernardo, Serviço de Imunoalergologia, Setúbal, Portugal; [2]Hospital de São Bernardo, Serviço de Pediatria, Setúbal, Portugal

Background: Spirometry measurements before and after short-acting bronchodilator administration are recommended in the diagnosis and evaluation of asthma control. The bronchodilator response (BDR) is used to determine if reversible limitation of airflow exists. Several studies recommend the assessment of the BDR no matter what the values of the basal spirometry. The purpose of the study was to determine the relation between the spirometric variables and the BDR in a population of paediatric patients with asthma.

Method: We retrospectively analysed all spirometry data from asthmatic patients with ages comprised between 6 and 17 years old, referred to our pulmonary function laboratory from 2011 up to and including March 2012. From this database, the following subset was selected: patients with spirometry measurements before and after short-acting bronchodilator and with a pre-bronchodilator FEV1 ≥ 80%. For patients who had multiple studies in the laboratory, only the first test was used for analysis. Pearson correlation was used to select the variable with better correlation to bronchodilation. Then a model to predict bronchodilation greater than 12% was built, by logistic regression, taking the selected variable as a predictor.

Results: We obtained a sample of 215 asthmatic patients with an average age of 10.2 ± 2.8 years old, 64% male, BMI of 19.9 ± 4.6 kg/m^2. The correlation between the BDR and the spirometric parameters were tested, and we found that FEF 25–75 had the better correlation to bronchodilation – R −0.5380885, $P < 0.0001$. Cut-off FEF 25–75 values, with errors below 30%, may be chosen from 60% to 70%, according to the desired probability of bronchodilation (55–33%).

Conclusion: We found that FEF25–75 was sensitive and specific to predict bronchodilator responsiveness. The FEF25–75 is considered a measurement of small airway patency, but the existing guidelines do not suggest that its assessment plays a significant role in the measurement of airflow obstruction. This finding concurs with previous reports that found the BDR predicts an increase in FEV1 after inhaled corticosteroid treatment and is associated with several indicators of poor asthma control. The cut-off value obtained is consistent with the results of other studies.

429

Assessing corticosteroid side-effects by measuring the long term cortisol levels in scalp hair of asthmatic children, the Netherlands, 2014

Smit, MP[1]; van Leer, EHG[1]; Noppe, G[2]; de Rijke, Y[3]; Hoekx, JCM[1]; van den Akker, ELT[4]
[1]Groene Hart Hospital, Paediatrics, Gouda, the Netherlands; [2]Erasmus MC, Paediatrics and Internal Medicine, Rotterdam, the Netherlands; [3]Erasmus MC, Clinical Chemistry, Rotterdam, the Netherlands; [4]Erasmus MC, Paediatrics, Rotterdam, the Netherlands

Background: Inhalation corticosteroids (ICS) as treatment for asthma can interfere with the hypothalamic-pituitary-adrenal axis and could lead to hypocortisolism. The frequency of this systemic side effect and the correlation to ICS usage is still unclear. Given that the cortisol excretion is pulsatile and determined by acute stress and circadian rhythms, the usefulness of tests based on momentary measures, such as saliva, serum, or urine are therefore limited. Cortisol levels measured in scalp hair provide a marker for long-term cortisol exposure and seems promising for diagnosing hypercortisolism. The aim of this study is to determine the long-term hair cortisol

© 2015 The Authors

concentrations (HCC) in children with asthma and corticosteroids usage compared to healthy controls.

Method: A case-control study was conducted at the Groene Hart Hospital, Gouda, 2014. All asthmatic children (4–18 years of age) using ICS for more than 3 months and visiting the general hospital were eligible as cases. Healthy controls were voluntarily enrolled children (4–18 years of age) from schools or from healthy siblings attending the pediatric outpatient clinic at Erasmus MC, in Rotterdam. Anthropometric characteristics and hair samples from the posterior vertex were obtained from cases and controls. HCC in three cm scalp hair was analyzed by Liquid chromotography-tandem mass spectrometry.

Results: Eighty cases and 258 controls were enrolled for analysis. Median age of cases 10.8 (4.1–17.6) and controls 11.5 (4.3–18) were comparable ($P = 0.56$). For both cases and controls, the male/female ratio was 0.5. The use of Budesonide equivalent by cases was 17.26 μg/kg (95%CI 6.03–54.55) per day. Mean HCC-levels did not differ between cases and controls ($P = 0.94$) even after adjusting for age, gender, height-SDS, and BMI-SDS ($P = 0.81$). No correlation was found between the Budesonide doses and HCC levels.

Conclusion: This study suggests that the average adrenal function in children with asthma and ICS is similar to their healthy controls. In our study the hair cortisol concentration is not influenced by ICS doses.

430
The impact of moderate to high dose inhaled corticosteroids on hypothalamic-pituitary-adrenal-axis suppression

Cavkaytar, O[1]; Vuralli, D[2]; Arik Yilmaz, E[1]; Buyuktiryaki, B[1]; Soyer, O[1]; Sahiner, UM[1]; Kandemir, N[2]; Sekerel, BE[1]
[1]Department of Pediatric Allergy, Hacettepe University Medical Faculty, Ankara, Turkey; [2]Department of Pediatric Endocrinology, Hacettepe University Medical Faculty, Ankara, Turkey

Background: The possible risk of systemic side effects due to use of inhaled corticosteroids (ICS) is a an issue of concern. Our aim was to find out the rate of hypothalamus-pituitary-adrenal-axis-suppression (HPA-AS) in children&adolescents taking ICSs regularly and to describe the factors that have an impact on HPA-AS.

Method: HPA-axis status investigated 0–18 year old patients on moderate & high dose ICS[>176 μg and >264 μg daily fluticasone propionate (FP HFA) for patients 0–11 years and ≥12 years, respect ively] were included. Various types and doses of ICS were converted to FP HFA equivalent according to NAEPP&GINA guidelines, daily dose, daily dose/body mass index (BMI), total dose and total dose/BMI were calculated. Participants with a baseline (8am) serum cortisol< 15 μg/dl underwent a low-dose-ACTH-stimuation test (LDAT) for diagnosis of HPA-AS.

Results: Ninety-one patients (58.2% male, median age 6.8 years) were included. Majority (74.7%) had been treated with FP, while budesonid (17.6%) and ciclesonide (7.7%) were also used. Sixty (75.9%) participants underwent LDAT, seven (7.7% of the whole population) were diagnosed with HPA-AS. Participants with HPA-AS didn't differ from those with a normal HPA-axis in terms of gender, current age, age at asthma diagnosis, follow-up period, the frequency of hospitalization in previous year, taking of any additional controller treatment, different types of devices used for taking ICS, FEV_1 or BMI levels. Ciclesonide was more frequently used by the participants with HPA-AS (42.9%) compared to patients with a normal HPA-axis (4.8%) ($P = 0.009$). Daily dose, daily dose/BMI, total dose and total dose/BMI of ICS taken at moderate & high doses were significantly higher in participants with HPA-AS compared to those with a normal HPA-axis ($P < 0.05$). Use of ICS at moderate to high doses for at least 7 months discriminated participants with HPA-AS from those with a normal HPA-axis ($P = 0.036$). Within duration, type and dosage of ICS, only, use of ICS at a dose of ≥22 μg/kg/m^2 FP was found to increase the risk for HPA-AS (OR: 7.22.95%CI: 1.23–42.26, $P = 0.028$). With a ROC-curve analysis, a cut-off value of 291 μg/day FP was determined (AUC = 0.840, $P = 0.003$) for predicting HPA-AS. Sensitivity, specificity, NPV and PPV of this cut-off value were 100%, 51.9%, 100% and 15.6% respectively.

Conclusion: The prevalence of HPA-AS was 7.7% in a group of children taking ICS not only in high but also in moderate doses. Dose alone was found to be an actual risk factor for HPA-AS.

431
High sensitivity C-reactive protein can reflect small airway inflammation & obstruction in childhood asthma

Ko, AR; Kim, YH; Sol, IS; Park, YA; Lee, KE; Hong, JY; Kim, MN; Kim, YS; Oh, MS; Yoon, SH; Kim, MJ; Kim, KW; Sohn, MH; Kim, K-E
Department of Pediatrics, Severance Hospital, Institute of Allergy, Brain Korea 21 PLUS Project for Medical Science, Seoul, Korea

Background: High-sensitivity assays for C-reactive protein (hs-CRP) enabled the identification of C-reactive protein at levels that were previously undetectable using routine assays, and can reflect even low-grade inflammation.

Relations between asthma and hs-CRP has been raised in several publications; Pellizzaro AM and Heuertz RM showed in 2010 that individuals with asthma or shortness of breath have significantly higher serum hs-CRP levels than the control group, and Derax TE et al. published an article in 2012 showing that serum hs-CRP levels are significantly higher in patients with steroid-naïve asthma than in ICS and healthy control group.

Therefore, we conducted a study to see if there are any significant relations between asthma severity and hs-CRP, or, spirometry or impulse oscillometry (IOS) parameters and hs-CRP.

Method: A retrospective study was conducted for 276 asthmatic children who visited pulmonology and allergy department outpatient clinic of Severance children's hospital from January 2012 to April 2014, and whose serum C-reactive protein levels and pulmonary function tests were available. Patients who had had acute infection signs within past 7 days before blood test were excluded. Asthma severity was classified according to National heart, lung, and blood institute (NHLBI) guideline. Patients were divided into two groups according to their serum hs-CRP levels; patients with hs-CRP levels higher than 3.0 mg/l as hs-CRP positive group, and patients with hs-CRP levels equal to or lower than 3.0 mg/l as hs-CRP negative group.

Results: There was no significant relationship between asthma severity according to NHLBI guideline and hs-CRP.

Among the spirometry parameters, there were significant relevance between forced expiratory volume in 1 s (FEV_1, maximum midexpiratory flow (MMEF, FEF25–75%), and hs-CRP.

Among the IOS parameters, there were significant relevance between reactance area (AX), difference between R5 (resistance at 5 Hz) and R20 (resistance at 20 Hz) (R5–R20), R5, reactance at 5Hx (X5), and hs-CRP.

Spirometry and IOS parameters versus hs-CRP had more relevance in patients with higher serum neutrophil levels.

Conclusion: Hs-CRP appears to reflect small airway obstruction in childhood asthma, and it seems to be more prominent in neutrophil dominant asthma phenotype.

© 2015 The Authors

Poster Discussion Session PDS 18

Clinical management of rhinitis

432
Hyaluronic acid and seawater effectively relieves nasal symptoms and reduces inflammation associated with non-allergic rhinitis

Pascolini, L[1]; Buonomo, A[1]; Colagiovanni, A[2]; Pecora, V[1]; Rizzi, A[1]; Aruanno, A[1]; Ricci, AG[1]; Di Rienzo, A[1]; Mezzacappa, S[1]; Centrone, M[1]; Nucera, E[1]; Schiavino, D[1]
[1]Allergy Department, Policlinico A. Gemelli, Università Cattolica del Sacro Cuore, Rome, Italy; [2]Presidio Ospedaliero A. Cardarelli, Campobasso, Italy

Background: Non-allergic rhinitis (NAR) is a prevalent condition, affecting ≈5 million people in Italy (prevalence ≈8.6%) and can be difficult to treat. It is not IgE-mediated and is defined in terms of inflammatory cell infiltrate as NAR eosinophils (NARES), NAR mast cells (NARMA), NAR neutrophils (NARNE) and NAR eosinophils and mast cells (NARESMA). Ialumar (Rottapharm Spa) is an isotonic nasal spray consisting of hyaluronic acid and seawater (HS) recommended for patients with rhinitis to cleanse, hydrate and regenerate nasal mucosa. The aim of this study was to assess the anti-inflammatory properties of HS and its effectiveness for the relief of nasal symptoms in patients with NAR.
Method: NAR patients (n = 16; mean age: 35.3 yrs [SD 11.4]) were randomized into a placebo (PLA)-controlled, parallel group trial to HS (n = 8, 1 spray/nostril3 to 4 times per day) or PLA (n = 8; no treatment). NAR patients had a negative skin prick test to a common aeroallergen and were IgE negative. The severity of nasal symptoms was assessed using a visual analogue scale (VAS) ranging from 0 mm (not at all bothersome) to 100 mm (extremely bothersome) on Days 0 and 60. Epithelial cells were obtained from patients on Day 0 and Day 60 by nasal scraping of the middle one-third of the inferior turbinate with Rhinoprobes. Samples were fixed by air drying, stained with Wright-Giemsa stain and examined under an optical microscope.
Results: HS provided effective nasal symptom relief for NAR patients, reducing the VAS from 56.3 mm (SD 17.7) at baseline to 20.0 mm (SD 7.6) at Day 60. No reduction in VAS score was observed for PLA patients. The table shows the cytological profile at baseline and the effect following 60 days of treatment with HS or PLA. HS significantly ($P < 0.01$) reduced the number of goblet cells in those patients with mucous mucipara and eradicated mast cells in those with NARMA.
Conclusion: HS effectively relieved nasal symptoms associated with NAR and reduced the number of goblet cells and mast cells in these patients. A larger sample size is needed to confirm the anti-inflammatory and nasal epithelium repairing properties of HS, and to correlate these findings with effectiveness.

433
Quality of life in patients with rhinitis: randomized clinical trial comparing bilastine *versus* loratadine

Santos, K; Locks, RB; da Silva, J
University of Southern Santa Catarina – Unisul, Postgraduate Program in Health Sciences, Palhoça, Brazil

Background: Allergic rhinitis is a disease of the upper airways, with high prevalence and morbidity leading to a significant impact on quality of life (QoL) in adults and in children. Estimating the QoL is particularly important when one wishes to evaluate the efficacy of antihistamines, because they are the most commonly used drugs in the control of the most prevalent symptoms of allergic diseases such as allergic rhinitis. The lack of studies, the clinical trial type, comparing the efficacy of bilastine and loratadine on the QoL of patients with rhinitis was a major reason for this research. The aim of study was determine whether there are differences in QoL of patients with allergic rhinitis treated with 20 mg of bilastine compared to those treated with 10 mg loratadine.
Method: We conducted a randomized, double-blind clinical trial with 73 patients, aged between 18 and 63 years, with 36 patients in the loratadine 10 mg group and 37 in the 20 mg group bilastine. All patients were followed in otolaryngology clinic of Criciuma-SC and were recruited from May 2013 to September 2014. The QoL assessment tool chosen for the study was the Quality of Life Questionnaire Modified in Rhinoconjunctivitis (RQLQm), previously translated and validated into Portuguese. The RQLQm was applied at baseline and after 10 days of treatment.
Results: Participants were 49 women (67.1%), and 27 allocated in the group of loratadine and 22 bilastine group. The 24 men (32.9%) who participated in the study were divided into: 9 in the group of loratadine and 15 in bilastine group. QoL of the participants was considered poor, according to the high scores RQLQm before the start of treatment. The use of bilastine 20 mg or 10 mg of loratadine significantly reduced RQLQm score after 10 days of treatment ($P < 0.001$). Antihistamines promoted an efficacy of 40% improvement in the assessment of severity, scores of nasal symptoms, symptoms, practical problems and emotions and an efficiency of 46.4% in the improvement of obstruction, rhinorrhea, sneezing and nasal itching. When comparing the two drug treatments, there was no statistically significant difference in QoL of patients for the studied variables.
Conclusion: QoL of patients with rhinitis improved significantly after 10 days of use of antihistamines evaluated and the effectiveness of both bilastine and loratadine was equivalent. Furthermore, they showed an acceptable safety profile.

434
Apolipoprotein A-IV is a new target molecule for allergic rhinitis

Fujieda, S[1]; Ninomiya, T[1]; Imoto, Y[1]; Kato, Y[1]; Yamaguchi, S[2]; Nanbu, F[2]; Makino, Y[3]; Noguchi, E[3]
[1]University of Fukui, Otorhinolaryngology, Yoshida-gun, Japan; [2]Ono Pharmaceutical Co., Ltd., Minase Research Institute, Research Headquarters, Osaka, Japan; [3]University of Tsukuba, Medical Genetics, Tsukuba, Japan

Background: Sublingual allergen-specific immunotherapy (SLIT) is the available treatment that can alter the natural course of allergic rhinitis. We performed proteomic in the patients treated with SLIT. Apolipoprotein A-IV (ApoAe-IV) was significantly increased in SLIT-treated patients but not in placebo-treated patients. The serum level of ApoA-IV correlated with the clinical symptom-medication scores and quality of scores in the case of SLIT-treated patients. In this study, we investigated the function of ApoA-IV in the allergic rhinitis.
Method: Histamine release from the basophils *in vitro* was examined after the addition of recombinant ApoA-IV. We made ApoA-IV knock out mice (ApoA-IV$^{-/-}$) and administrated antigen to ApoA-IV$^{-/-}$.

© 2015 The Authors

Recombinant ApoA-IV was administrated to monkey with allergic rhinitis caused by Japanese cedar pollen.
Results: The addition of recombinant ApoA-IV reduced histamine release from human basophils in the presence of antigen. Frequency of sneezing and nasal itching was decreased after antigen challenge in ApoA-IV$^{-/-}$, compared to wild type. However, cytokine (Th1 and Th2) and IgE production, and eosinophil infiltration in the nasal mucosa in ApoA-IV$^{-/-}$ were same level to those to those in wild type. The administration of recombinant ApoA-IV increased nasal flow in allergic monkey, but the recovery rate was smaller than topical corticosteroid treatment.
Conclusion: Although biological ability of ApoA-IV is not strong, ApoA-IV works to increase nasal flow and decrease histamine release in SLIT-treated patients.

435
Usability of nasal filters in managing seasonal allergic rhinitis: an observational, open-label in-season study

Kenney, P[1,2]; Hilberg, O[3]; Sigsgaard, T[1]
[1]Department of Public Health, Aarhus University, Aarhus C, Denmark; [2]Rhinix ApS, Aarhus N, Denmark; [3]Department of Respiratory Diseases and Allergology, Aarhus University Hospital, Aarhus C, Denmark

Background: A recently published small out-of-season study reported promising results for the use of nasal filters in preventing symptoms of seasonal allergic rhinitis. However, in-season studies on efficacy and usability (particularly concerning convenience, comfort and treatment costs) in larger populations are needed to verify this.
Objective: To evaluate the usability of nasal filters (Rhinix, Rhinix ApS, Aarhus, Denmark) in a large population during a regular pollen season.
Method: An open-label, in-season, at-home study (NCT02108379) conducted throughout Denmark during the peak of the main grass pollen season in 2014 included 1073 participants with seasonal allergic rhinitis, with or without asthma. Participants received Rhinix for a two-week use period. Identical online questionnaires were answered after each week of use. End points included ratings on satisfaction, usage and interest in continued use, stratified by allergy and asthma severity.
Results: The response rates for the online questionnaires were 95% in both weeks. 854 and 735 participants used the filters in week 1 and 2, respectively. In both weeks, 339 (40%/46%) said they would continue use of the filters after study end, 308 and 222 (36% and 30%), respectively, said they might. In both weeks, satisfaction with the filters and interest in continued use were correlated (week 1, OR: 10.32; 95% CI: 8.19–13.01 and week 2, OR: 14.39; 95% CI: 10.80–19.18). Satisfaction with the filters increased with increased total nasal symptom scores (severity from 0 to 12) (week 1, OR: 1.06; 95% CI: 1.01–1.12 and week 2, OR: 1.07; 95% CI: 1.01–1.13). For those with asthma ($n = 410$), increased asthma severity (mild-moderate-severe) correlated with higher satisfaction of the filters (week 1, OR: 1.79; 95% CI: 1.31–2.44 and week 2, OR: 1.45; 95% CI: 1.05–2.01).
Conclusion: A significant share of participants found the filters useful for the everyday management of their seasonal allergy. The filters appear sufficiently convenient and comfortable for everyday use. Participants with moderate-to-severe allergy or asthma seemed to benefit more with the filters.

436
The effect of Korean Red Ginseng (Ginsenosides) on allergic inflammation induced using EOL-1 cell line in nasal polyp epithelium

Jung, JH; Kang, IG; Kim, ST
Gachon University, Gil Medical Center, Incheon, Korea

Background: Korean red ginseng (KRG) is reported to have anti-allergic properties, including beneficial effects on asthma and atopic dermatitis. However, its effect on allergic rhinitis has not been studied extensively. The purpose of this study was to examine the effect of Korea red ginseing on the allergic inflammation induced using EOL-1 cell line in nasal polyp epithelium.
Method: After 7 days incubation of EOL-1 cell, we collected the culture supernatant (Eosinophil obtain conditioned media, EoCM). Nasal epithelial cells were obtained from CRS patients with polyps and cultured with EoCM. IgE, Interleukin-4 (IL-4) and Interleukin-13 (IL-13) were measured to determine the activation of epithelial cells. We treated activated nasal epithelial cell with KRG.
Results: Nasal epithelial cells stimulated with EoCM produced larger amount of IgE, IL-4 and IL-13 than negative controls ($P < 0.05$). After treated with ginsenoside IgE and IL-4 were decreased ($P < 0.05$).
Conclusion: KRG may suppress allergic inflammation on nasal epithelial cell. KRG might be a useful treatment modality in allergic rhinitis patients. However, we need to better understand the long-term effects of KRG with further studies.

437
Pharmacists can help to detect undiagnosed or poorly-controlled allergy

Salerni, G[1]; Alfieri, M[1]; De Carlo, G[2]; Finkwagner, A[2]; De Simone, M[1]
[1]FederAsma e Allergie Onlus Federazione Italiana Pazienti, Prato, Italy; [2]European Federation of Allergy and Airways Diseases (EFA), Brussels, Belgium

Background: Many patients with respiratory allergy do not recognise their condition and fail to consult a physician. Others tend to use proven or unproven means to relieve their symptoms (ARIA Guidelines for Pharmacists). A study conducted in Austria on customers of pharmacies of the Vienna area (Austrian "Allergy Risk Check" programme, 2013) showed that around 50% of patients with high-moderate risk of allergy had never been tested for allergy. As trusted healthcare professionals in the community, pharmacists can play a major role in early identification of the condition and guide patients to a correct diagnosis.
Aim: The Allergy Risk Check 2014 – Genoa, Italy aimed at evaluating the role of pharmacists in the early identification of customers with respiratory allergy by using the validated *Allergy Screening Test ASF Questionnaire* (Fischer, Allergologie, JG 29, 2006).
Method: Of 299 pharmacies in Genoa proposed the questionnaire, in Italian, to customers with symptoms of respiratory allergy or asking for OTC treatment for allergic symptoms. Each pharmacy received 50 copies of the questionnaire, 1 poster to promote the Allergy Risk initiative, 100 leaflets on allergy in plain language including a pollen calendar, and printed instructions on how to conduct the survey and information on the initiative.
Results: Of 1663 questionnaires were collected. The symptoms reported were sneezing (49%), itchy nose (49%), itchy eyes (44%), runny nose (41%) and blocked nose (37%). The symptoms usually appeared in Spring (64%). 71% of participants were at a moderate-severe risk of allergy and 78% of the total was never tested for allergy, 70% of the never tested participants "clearly felt unwell". Notably, 94% of patients previously tested for allergy claimed a poor control of symptoms.
Conclusion: These results confirm those previously reported by the Austrian "Allergy Risk Check" programme. Pharmacists are often the first healthcare professionals seeing a person at risk of respiratory allergy. They may play a role in symptom recognition and early detection of allergy. They may motivate at-risk subjects to consult a physician to receive an early and correct diagnosis. In addition, they may help to detect cases of poorly controlled allergy.

© 2015 The Authors

438
Allergic rhinitis: clinical symptoms, skin prick test or ARIA guidelines?

Kit Liang, G[1]; Eugene, WHC[1]; Abu Bakar, MZ[1]
[1]Otorhinolaryngology, University of Malaya Medical Centre, Kuala Lumpur, Malaysia

Background: Rhinitis symptoms are commonly seen in allergic rhinitis (AR), non-allergic rhinitis (NAR) and infective rhinitis. However, the diagnosis of allergic rhinitis can be very difficult at the first presentation to the clinicians without relevant investigations like IgE or skin prick test (SPT). Thus, a proper guideline is needed to screen and manage these patients, especially with chronic presentation of rhinitis symptoms.
Objective: To review the management of allergic rhinitis in our setting in regards to the ARIA guidelines (2010) and its usefulness.
Method: Retrospective study of 126 patients who underwent skin prick test in 2014 in University of Malaya Medical Centre, Kuala Lumpur, Malaysia. Twenty-two non-rhinitis cases were excluded. The demographic and clinical profiles of patients with AR and NAR were also studied and analysed.
Results: A total of 104 out of 126 cases (82%) referred for skin prick test presented with rhinitis symptoms, of which 67.3% was diagnosed with allergic rhinitis. Majority of both AR and NAR patients were females, Malay ethnicity, and aged between 20 and 50 years old. The commonest presenting symptoms for both AR and NAR patients were runny nose (77.1% and 73.5% respectively), nasal obstruction (64.3% and 70.6% respectively) and sneezing (54.3% and 55.9% respectively). However, there were no statistically significant associations between these symptoms and SPT results (all $X^2 < X^2_{critical}$ for 1df, $P > 0.05$). In AR patients, the commonest allergens were found to be *D. Pteronyssinus* (51.4%), *BlomiaTropicalis* (47.1%) and *D. Farinae* (40%). Most patients with AR have 3 or more positive allergens (55.7%). The commonest association in both AR and NAR was bronchial asthma (18.6% and 8.8%). There were no statistically significant association between presence of asthma and SPT results and between number of allergens and presence of asthma (all $X^2 < X^2_{critical}$ for 1df, $P > 0.05$). The commonest treatment for both AR and NAR were intranasal steroid (84.3% and 91.2% respectively) and oral antihistamine (82.9% and 82.4% respectively).
Conclusion: Diagnosis of allergic rhinitis using clinical symptoms is difficult as shown in this study. Therefore, the application of ARIA guidelines in clinical setting would aid the early diagnosis and provide an easier guide for management of rhinitis patients.

439
Strong correlation between the result of skin prick test and ImmunoCAP in house dust mites allergy

Kim, JY; Ahn, J-C; Kim, D-Y; Rhee, C-S; Lee, CH; Han, DH
Department of Otorhinolaryngology, Seoul National University Hospital, Seoul, Korea

Background: Serum specific immunoglobulin E (sIgE) test has been used as a substitutionary tool of the skin prick test (SPT) in allergic rhinitis.
Objective: This study investigated their correlation in house dust mite (HDM) allergens in rhinitis patients.
Method: This study retrospectively reviewed medical records of patients with rhinitis symptoms who underwent both SPT and ImmunoCAP of two HDMs, Dermatophagoides pteronyssinus (Dp) and farinae (Df) from January 2011 through December 2013. Positve sensitization of the allergy tests was defined by an allergen to histamine ratio in wheal diameter (A/H ratio) ≥ 1 in SPT and sIgE ≥ 0.35 U/mL in ImmunoCAP. Discordant group 1 (DG1) and 2 (DG2) of the allergy tests were defined by positive SPT with negative ImmunoCAP and vice versa, respectively.
Results: Total 5158 patients were enrolled; 3269 male and 1889 female patients with the average age 30.3 years old. The positive rates of Dp and Df were 42.7% and 56.2% in SPT and 47.4% and 52.8% in ImmunoCAP. We found strong linear correlation between the A/H ratio and sIgE after logarithmic transformation ($R = 0.881$, $P < 0.001$ for Dp; $R = 0.865$, $P < 0.001$ for Df). Male and aging were risk factors of DG2 in both Dp (male OR 1.624, 95% CI 1.214–2.173; aging 1.014, 1.008–1.021) and Df (1.932, 1.270–2.934; 1.046, 1.037–1.056).
Conclusion: The remarkably strong correlation of both tests was verified in HDMs. However, ImmunoCAP could provide more accurate information of allergy in male and older patients than SPT.

440
Cut-off value of ImmunoCAP in house dust mites and animal danders based on skin prick test

Hong, SD; Chung, S-K; Kim, HY; Dhong, H-J
Otorhinolaryngology – Head and Neck Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Background: The most common tests for allergen sensitization are skin-prick testing (SPT) and *in vitro* tests to detect allergen-specific IgE. Among the *in vitro* tests, ImmunoCAP (CAP) testing has been known to most accurate. However, cut-off value of CAP testing has been applied uniformly as 0.1, 0.35, 0.7 and so on without regard to each antigen and characteristics of patient. The purpose of this study is (1) to determine the antigen specific cut-off value of CAP testing in house dust mites and animal danders and (2) to analyze the difference of cut-off value according to age and gender.
Method: Of 1829 Patients with more than one rhinitis symptom who underwent both SPT and CAP testing were retrospectively evaluated between March 2008 and May 2012. Male was 1227 (67.1%) and female was 602 (32.9%)/Mean age was 33.06 years (7–79). Gold standard for allergen sensitization was defined as more than SPT 3 + (wheal size more than histamine control). Youden index method was used for calculating the cut-off value of CAP testing.
Results: Cut-off value of Dermatophagoides pteronyssinus (Dp), Dermatophagoides farinae (Df), Cat fur and dog fur was 0.7, 0.84, 0.12, and 0.46 kU/l, respectively (*Youden index method*). The cut-off value was changed according to age in Dp and Df, but not in cat and dog fur. When categorizing the age group according to under twenty, twenties, thirties, forties, and over fifty, cut-off value for Dp and Df had tendency becoming smaller with age ($P < 0.05$; Cochran-Armitage trend test). There was no significant difference in cut-off value according to gender ($P < 0.05$; Fisher's exact test).
Conclusion: The cut-off value of the CAP testing is different for each antigen and is changed according to the age. Physicians should select the proper cut-off value for the appropriate criteria according to the antigen and age of subjects rather than the uniform one.

442
Introducing the Ghent score, a new nasal polyp scoring system

Pauwels, B[1]; Jonstam, K[2]; Derycke, L[1]; Bachert, C[1]
[1]University Hospital Gent, Gent, Belgium; [2]Karolinska University Hospital, Stockholm, Sweden

Background: The Davos nasal polyp score is a scoring system used to estimate polyp size based on nasal endoscopy. It is often used as primary outcome in clinical trials with new therapeutics for chronic rhinosinusitis with nasal polyps (CRSwNP). However, this scoring system never was validated and does not seem to reflect real polyp size in some patients. Our objective is to introduce the Ghent score, a new

© 2015 The Authors

scoring system that would better reflect real polyp size and might be a better indicator for drug response in clinical trials.

Method: Endoscopy videos of patients enrolled in a clinical study with a new drug for CRSwNP were scored according to the Davos score and the Ghent score by two independent investigators. The scoring system was validated by calculation of the inter-observer agreement. CT based Lund-Mackay scores and a new method to calculate sinonasal volume were used to estimate real polyp size and correlated with both the Davos and the Ghent score. Efficacy of the two scoring systems to prove drug response was demonstrated by comparison of the number of responders to treatment (reduction in nasal polyp score of 2 or more points) according to both scores.

Results: Volumetric measurements of the nose and sinuses showed a significant increase in sinonasal volume after treatment. This increase correlated significantly with the reduction in Ghent polyp score. When comparing both scoring systems more responders could be identified with the Ghent scoring system.

Conclusion: Compared to the Davos scoring system the Ghent scoring system is a more accurate way to demonstrate real polyp size. Therefore it can be proposed as a new outcome parameter in clinical trials to demonstrate drug response.

443
Sensitization to Anisakis simplex and nasal polyposis

D'Amelio, CM[1]; Corral, M[2]; Goikoetxea, MJ[1]; Ferrer, M[1]; Vega, O[1]; Bernad, A[1]; Gastaminza, G[1]
[1]Allergy and Clinical Immunology, Clinica Universidad de Navarra, Pamplona, Spain; [2]Universidad de Navarra, Pamplona, Spain

Background: We have detected a high frequency of positive skin prick test to Anisakis simplex in patients affected with nasal polyposis (NP). Nasal polyposis is a severe disorder that can have great impact in the quality of life of affected patients. Its physiopathology remains still unknown, although eosinophilic infiltration and Th2 immune response have been described. We sought to determine the prevalence of sensitization to Anisakis sp. in patients with NP and patients without NP.

Method: A retrospective case-control study was assessed in 640 patients who underwent detailed clinical history and skin prick test (SPT) with a panel of allergens, including *Anisakis sp*., between 01/2008 and 12/2013 in our hospital. 320 cases (patients with NP) and 320 controls (subjects without diagnosis of NP) were enrolled. Patients were paired by estimated fish consumption by geographic zone of residence in Spain (EFC), age group and gender. All patients were grouped regarding their EFC, in low (L) (< 10 Kg per person/year), medium (M) (10–14 Kg) or high (H) (>14 Kg) consumers, age group and gender.

Results: Global prevalence of positive SPT to *Anisakis sp*. in our sample was 93/640 (15%). Fifty-five patients (17%) with NP showed positive SPT to Anisakis sp., compared to 38 (11.8%) controls ($P < 0.05$). Compared to histamine wheal diameter, 33 (60%) of NP-patients with positive SPT to *Anisakis sp*. showed inferior wheal size, while 32 (84%) of positive-SPT controls showed a greater size. Higher prevalence of positive SPT to *Anisakis sp*. was observed in H-consumers (29%), compared to M-consumers (11%) and L-consumers (8%), ($P < 0.05$). Patients aged 65 or more, showed higher frequency of positive SPT, 10 (31%) *versus* the 18–25 year-old group (11%), ($P < 0.05$). Male patients showed higher prevalence of sensitization to *Anisakis sp*. compared to female patients (17% *versus* 12%, respectively) without statistical significance.

Conclusion: The prevalence of sensitization to *Anisakis sp*. in our patients is high. We have found statistically significant relation among sensitization to *Anisakis sp*. and NP, which prevalence increases with age and higher fish consumption. Further studies are needed to investigate the subjacent physiopathology of this finding.

© 2015 The Authors

Poster Discussion Session PDS 19

Mechanisms of AIT

444

The number of FceRI receptors on basophils decreases during subcutaneous immunotherapy

Schmid, JM[1]; Dahl, R[2]; Hoffmann, HJ[1]
[1]Department of Respiratory Medicine and Allergy, Aarhus University Hospital, Aarhus C, Denmark; [2]Allergy Centre, Odense University Hospital, Odense, Denmark

Background: Allergen specific immunotherapy is the only disease modifying treatment of allergic diseases. It induces complex cellular and humoral changes leading to an inhibition of type-1 allergic reactions.
Method: Twenty four young grass pollen allergic adults suffering from seasonal rhino-conjunctivitis were randomized to receive standard subcutaneous immunotherapy ($n = 18$) or to an open control group ($n = 6$). The number of FcεRI receptors on basophils was measured by quantitative analysis of indirect immunofluorescence by flow cytometry. Grass binding to basophils was measured by staining with grass-conjugated fluorescent dye and analyzed by flow cytometry. Basophil sensitivity was determined by the EC50, the logarithm of the allergen concentration leading to half-maximum basophil activation.
Results: The number of FcεRI receptors decreased from a median 105196 (95% CI: 81283–132038) to 30903 (95% CI: 11482–85114)/basophil in the treatment group ($n = 0.016$), while it remained constant in the control group (pre: 102424; post: 103753). We found only minor changes in maximal grass binding on the basophils. The allergen concentration leading to half-maximum grass binding decreased slightly during SCIT, whereas the EC50 increased significantly by 2.48 (1.38–3.50, $n = 16$, $P < 0.0001$) in a full blood basophil activation test and by 2.11 (1.51–2.70, $n = 16$, $P < 0.0001$) in washed basophils reconstituted with plasma from the present visit after 3 years of SCIT. Basophil sensitivity did not change significantly in the control group.
Conclusion: Surface density of the FcεRI receptors on basophil granulocytes decreased significantly during 3 years of subcutaneous immunotherapy. This might contribute to the development of allergen tolerance during immunotherapy and may contribute to decrease in basophil sensitivity.

445

Allergen immunotherapy decreases LPS-induced NF-κB activation in neutrophils from allergic patients

López, C[1]; Bellido, V[1]; Ventura, I[1]; Vega, A[1]; Chamorro, C[1]; Aroca, R[1]; Pineda, R[2]; Palacios, R[2]; Monteseirín, J[1,3]
[1]Hospital Universitario Virgen Macarena, UGC-IC Alergología, Sevilla, Spain; [2]Laboratorios Diater, Madrid, Spain; [3]Departamento de Medicina, Facultad de Medicina, Universidad de Sevilla, Sevilla, Spain

Background: Allergen-specific immunotherapy (IT) is widely used to treat allergic diseases. The molecular mechanisms have not been clarified yet completely. The present work was undertaken to analyze the effect of IT in the activation of NF-κB.
Method: Neutrophils from 15 pollen-allergic IT-treated patients, 10 untreated pollen allergic patients, and 10 healthy donors were *in vitro* stimulated with LPS. NF-κB activation (p65/p52) was measured in their nuclear extracts by enzyme-linked immunosorbent assay (ELISA). IκBα phosphorylation, NF-κB-repressing factor (NRF) activation, and thromboxane A_2 (TXA_2) and Interleukin-8 (IL-8) release were measured by ELISA.
Results: TXA_2 release were significantly lower in neutrophils from IT-treated patients than from untreated patients. There was a positive correlation between the score of symptoms and NF-jB activation in human neutrophils. IT significantly decreased NF-κB activation levels in neutrophils compared with neutrophils from untreated patients. IκBα phosphorylation and NRF activation levels were, respectively, significantly lower and higher in neutrophils from IT-treated patients than from untreated patients. IL-8 and TXA_2 release were significantly lower in neutrophils from IT-treated patients than from untreated patients.
Conclusion: IT positive effects are at least in part mediated by the negative regulation of NF-κB activation in human neutrophils. These observations represent a novel view of neutrophils as possible cell target to treat IgE-dependent diseases through NF-κB downmodulation.

446

Immunotherapy reduces allergen-mediated CD66b expression and myeloperoxidase levels on human neutrophils from allergic patients

Bellido Linares, V; Lopez, C; Aroca, R; Chamorro, C; Vega, A; Ventura, I; Monteseirin, J
Hospital Universitario Virgen Macarena, UGC Intercentros de Alergologia, Sevilla, Spain

Background: CD 66b is a member of carcinoembryonic antigen family which mediates the adhesion between neutrophils and to endothelial cells. It is well know that it is a cell marker of activation but its functions are unknown. Allergen-specific immunotherapy is widely used to treat allergic diseases but the mechanism underlying this therapy is not completely understood.
Method: The present work was undertaken to analyze the *in vitro* effect of allergens and immunotherapy on cell-surface CD66b expression of neutrophils from patients with allergic asthma and rhinitis and the *in vivo* effect of immunotherapy on cell surface CD66b expression of neutrophils from nasal lavage fluid during the spring season.

Myeloperoxidase expression and activity was also analyzed in nasal lavage as a general marker of neutrophil activation.
Results: CD66b surface expression is up regulated *in vitro* in response to allergens and significantly reduced by immunotherapy ($P < 0.001$). Myeloperoxidase activity in nasal lavage fluid was also significantly reduced by immunotherapy, as were neutrophils cell-surface expression of CD66b and myeloperoxidase ($P < 0.001$).

CD66b expression was higher in neutrophils from nasal lavage fluid than in those from peripheral blood, and the number of CD66 + MPO+ cells is reduced in nasal lavage fluid.
Conclusion: These results presented here provide evidence of possible involvement of the CD66b molecule in the allergic asthma and rhinitis and reveal that immunotherapy has a positive effect on this molecule. Thus, immunotherapy positive effects might be mediated, at least in part, by the negative regulation of CD66b and myeloperoxidase activity in human neutrophils.

© 2015 The Authors

447
Substance P in nasal lavage fluid of mugwort pollen allergic rhinitis patients after 3 years course of specific immunotherapy

Gawlik, R[1]; Jawor, B[1]; Barbara, R[1]
[1]Medical University of Silesia, Katowice, Poland

Background: Neural mechanisms is proved to play a role in the mechanism of allergic rhinitis symptoms. The purpose of our study was to examine the impact of specific allergen immunotherapy on concentration of substance P in nasal lavage fluid of patients with allergic rhinitis sensitive to mugwort.
Method: Twenty four patients with seasonal allergic rhinitis, mean age 34.20 ± 8.1 years were included into trial. Fifteen sensitive to mugwort (skin prick test and serum antigen specific IgE (Pharmacia CAP system) patients were desensitized with hypoallergic extract of mugwort (Allergovit, Allergopharma) for 3 years. The control group consisted of 9 polyallergic rhinitis patients whose symptoms were controlled only by pharmacotherapy. Nasal allergen provocation tests with using *mugwort extract* followed by collection of lavage fluid were repeatedly before the pollen season. All patients recorded clinical symptoms in the diary. The substance P (SP) nasal lavage fluid level were determined by EIA methods # (Assay Designs Inc., USA) in all patients.
Results: There were similar levels of SP in both groups at baseline prior to therapy (SIT group: 56.1 ± 15.5 vs controls 54.3 ± 10.1 pg/ml). SP concentration in NLF after mugwort challenge in 1 year were lower in SIT group (48.1 ± 13.8 pg/ml versus 53.6 ± 6.8 pg/l; $P < 0.05$) with a further, but not statistically significant decrease in SP levels after 2 and 3 year of SIT (40.9 ± 17.6 pg/ml vs 51.2 ± 9.2 pg/ml). Significant reduction of symptoms and medications use was observed in hyposensitized patients.
Conclusion: Normal 0 21 false false false MicrosoftInternetExplorer4 Substance P seems to play a role in the pathophysiology of nasal allergic responses to mugwort pollen in allergic rhinitis patients. Decreased level of SP as a result of SIT suggests that some of the symptomatic benefits of SIT may be related to the reduced release of substance P into nasal secretions.

448
Variability of serum tryptase during venom immunotherapy

Tziotou, M[1]; Syrigou, E[2]; Zande, M[2]; Sinaniotis, A[2]
[1]National & Kapodistrian University of Athens, 2nd Pediatric Clinic, Allergy Unit, Athens, Greece; [2]Department of Allergy, Sotiria General Hospital, Athens, Greece

Background: Tryptase is the most abundant protein in mast cells and its levels reflect the burden of mast cell load and activation. High levels of baseline tryptase are a risk factor for severe anaphylaxis. Little is known about the interindividual variability of tryptase in healthy controls and in patients receiving immunotherapy. Our purpose was to evaluate the variability in tryptase levels in patients receiving venom immuonotherapy (VIT).
Method: We retrospectively reviewed the medical records of eight patients undergoing VIT, whose serum tryptase levels were measured before and 1 year after the initiation of treatment. Four of these patients were further evaluated with a controlled sting challenge.
Results: We included 8 patients, 5 with serum tryptase levels before VIT > 11.4 ng/mL (group A) and 3 with serum tryptase levels before VIT < 11.4 ng/mL (group B). A significant reduction of baseline tryptase levels was observed in all patients in group A undergoing VIT. Moreover, two patients in group A underwent a sting challenge that was negative, while the sting challenge in two patients from the group B was positive.

	Tryptase (ng/mL) before VIT.	Tryptase (ng/mL) after VIT.	Sting challenge
Group A	24.2	4.0	Negative
	13.6	4.5	Negative
	17.4	3.1	Not Done
	16.7	2.5	Not Done
	18.0	1.8	Not Done
Group B	7.2	2.7	Positive
	3.6	5.2	Positive
	4.0	4.0	Not Done

[Tryptase variability during VIT].

Conclusion: Successful venom immunotherapy confirmed by negative controlled sting challenges is associated with a significant decrease in tryptase levels in patients with serum tryptase levels before VIT > 11.4 ng/mL. More studies are needed in order to confirm these observations regarding the potential use of tryptase measurement in the monitoring of venom immunotherapy.

449
Expression of microRNA in initial phase of venom immunotherapy

Specjalski, K[1]; Jassem, E[1]
[1]Department of Allergology, Medical University of Gdansk, Gdansk, Poland

Background: MicroRNAs are small non-coding molecules regulating functioning of immune system. They are also involved in allergic inflammation. Recent studies have demonstrated different microRNA profiles in some allergic conditions e.g. asthma, allergic rhinitis atopic dermatitis and eosinophilic esophagitis. The crucial processes influenced by microRNAs are eosinophils development, T cells differentiation and activation, development and activation of mast cells. Although our knowledge about impact of microRNAs on allergic inflammation has considerably expanded over the last years, multiple areas warrant future investigation. The aim of the study is to describe profile of microRNAs expression in patients undergoing initial phase of specific immunotherapy with wasp venom.
Method: Seven adult patients with a history of severe systemic reaction after stinging by wasp (grade III or IV in Mueller's classification) and sensitization to wasp venom confirmed by skin tests and sIgE were included. Initial phase of wasp venom immunotherapy (VIT) was performed according to ultra-rush protocol. Cumulative dose of 101.1 μg of wasp venom was administered over 210 min. Venous blood was collected before VIT and 24 h after completing initial phase. RNA was isolated and reversely transcripted in order to obtain single-stranded cDNA. In the next step cDNA was amplified with the use of sequence-specific primers and probe on microRNA array. Finally, the amount of 740 microRNAs was compared between pre- and post-VIT samples with the 2-fold difference as a significant.
Results: After completing initial phase of VIT proile of microRNA was changed with lower expression of miR-21- in 3 patients, miR-106 – in 4 patients, miR146 – 6 patients, miR-181- 5 patients, miR-485 3p – 5 patients. No significant changes were found in expression of in miR-125b, miR-126, miR-223.
Conclusion: Profile of microRNA expression was changed after initial phase of VIT with lower expression of microRNAs involved in allergic inflammation.

© 2015 The Authors

450
Oral and subcutaneous immunotherapy in a peanut allergy mouse model

Wagenaar, L[1]; Vonk, MM[2,3]; van Roest, M[1]; Kruijssen, LJW[1]; Knippels, LMJ[2,3]; van Esch, BCAM[2,3]; Pieters, RHH[1]; Smit, JJ[1]
[1]Utrecht University, Institute for Risk Assessment Sciences, Utrecht, the Netherlands; [2]Utrecht University, Institute for Pharmaceutical Sciences, Utrecht, the Netherlands; [3]Department of Immunology, Nutricia Research, Utrecht, the Netherlands

Background: Although the prevalence of food allergy is increasing, no curative treatment is available yet. Human studies have shown encouraging suppression of food allergy by either subcutaneous or oral allergen-specific immunotherapy (IT). However, this therapy is still hampered by serious side effects and a lack of long-term tolerance. Improving the efficacy and safety of oral and subcutaneous immunotherapy (OIT and SCIT) is therefore necessary before widespread application. The NUTRALL consortium aims at developing knowledge-based immunotherapy protocols with improved efficacy and safety using dietary adjuvants.
Method: In the present study, a C3H/HeOuJ mouse model for intragastric induced peanut allergy was used to study the efficacy, mechanism of action and the dose responsiveness of OIT and SCIT. We looked at clinical efficacy (acute allergic skin responses, anaphylactic shock symptoms and body temperature), antibody and cytokine production and number of various immune cells after IT of sensitized mice. After sensitization, mice were treated with three different doses of peanut via the intragastric (5 times/week, OIT) or subcutaneous route (3 times/week, SCIT) for three consecutive weeks. Hereafter, mice were exposed to peanut via an intradermal, intragastric and intraperitoneal challenge.
Results: Both SCIT and OIT were able to lower the acute allergic skin response, anaphylactic symptoms and mast cell degranulation after peanut exposure. In addition, serum levels of IgG1 and IgG2a, next to serum levels of IgE, were raised after both IT regimes. Furthermore, OIT prevented the increase of IgE after allergen challenge. The production of cytokines IL-5 and IL-10 was elevated after SCIT and allergen challenge, but not after OIT. IL-13 and IFN-γ production did not differ between the groups. Despite the observed changes in clinical parameters and antibody levels, the number of regulatory T cells, Th1 cells and Th2 cells in the spleen were not changed. Activated CD8-positive T cells were elevated after both SCIT and OIT, while activated CD4-positive T cells were only elevated in the SCIT groups.
Conclusion: These data show that in a mouse model for peanut allergy, both SCIT and OIT protect against allergic and anaphylactic responses. Furthermore, we show that IT induced IgG1 and IgG2a and OIT prevented the increase of IgE after challenge. In future experiments, we will investigate whether dietary adjuvants improve the efficacy and/or safety of OIT and SCIT.

451
Comparing oral and subcutaneous antigen-specific immunotherapy in a cow's milk allergy mouse model

Vonk, MM[1,2]; Wagenaar, L[3]; Diks, MAP[1]; Hofman, GA[1]; Dingjan, GM[1]; Smit, JJ[3]; Pieters, RHH[3]; Willemsen, LEM[1]; Garssen, J[1,2]; Knippels, LMJ[1,2]; van Esch, BCAM[1,2]
[1]Utrecht University, Utrecht Institute for Pharmaceutical Sciences, Pharmacology, Utrecht, the Netherlands; [2]Nutricia Research, Immunology, Utrecht, the Netherlands; [3]Utrecht University, Institute for Risk Assessment Sciences, Immunotoxicology, Utrecht, the Netherlands

Background: Allergen specific immunotherapy is a promising therapeutic approach for cow's milk allergy (CMA), but needs to be optimized since both oral immunotherapy (OIT) and subcutaneous immunotherapy (SCIT) coincide with a high incidence of allergic side effects in humans. The NUTRALL consortium aims at developing knowledge-based immunotherapy protocols with improved efficacy and safety using dietary adjuvants. This study was designed to compare the efficacy of OIT and SCIT and to determine the dose of allergen needed to suppress the allergic response.
Method: C3H/HeOuJ mice were sensitized intragastrically (i.g.) to whey and subsequently mice were treated orally (0.1, 1, 10 and 100 mg, 5 times/week) or subcutaneously (2.5, 10 and 25 μg, 3 times/week) with whey for three consecutive weeks. The acute allergic skin response, anaphylactic shock symptoms and changes in body temperature were measured upon intradermal (i.d.) challenge, and mast cell degranulation was measured upon i.g. challenge. Whey-specific antibodies were measured in serum at different time points before, during and after IT. Activated Th2, Th1 and regulatory T cells (Treg) derived from spleen and mesenteric lymph nodes were assessed using FACS analysis and *ex vivo* cytokine production was measured after re-stimulation with whey.
Results: Both OIT and SCIT decreased the acute allergic skin response, anaphylactic shock symptoms, the drop in body temperature and mast cell degranulation upon challenge. A rise in IgE was observed during immunotherapy in the OIT and SCIT mice. However, OIT and SCIT mice seemed to be protected against the increase in whey-specific IgE caused by systemic challenge. Whey-specific IgG1 and IgG2a increased over the course of treatment and challenges. Both OIT and SCIT reduced the percentage of activated Th2 cells and increased the percentage activated Th1 cells in the spleen compared to non-treated allergic mice. In addition, Treg cells were increased in the MLN of OIT mice. A reduction of Th2 cytokines IL-5 and IL-13 was observed after *ex vivo* stimulation of spleen and MLN cells with whey in the OIT groups.
Conclusion: Both OIT and SCIT effectively suppressed the allergic response after challenge with an observed change in T cell subsets, prevented the increase in whey-specific IgE after challenge and induced whey-specific IgG1 and IgG2a. Future experiments should investigate whether dietary adjuvants might improve OIT and SCIT in terms of efficacy and/or safety.

452
Larger homing receptor expression on Tregs suggests increased efficacy of epicutaneous compared to oral or sublingual immunotherapy for the treatment of food allergy

Dioszeghy, V[1]; Mondoulet, L[1]; Pelletier, B[1]; Puteaux, E[1]; Ligouis, M[1]; Dhelft, V[1]; Plaquet, C[1]; Dupont, C[2]; Benhamou, P-H[1]
[1]DBV-Technologies, Bagneux, France; [2]Université Paris Descartes - Hôpital Necker, Paris, France

Background: Allergen specific immunotherapy is an attractive strategy to actively treat food allergy. In the 3 different routes investigated, oral (OIT), sublingual (SLIT) and epicutaneous (EPIT), regulatory T cells (Tregs) play a pivotal role. Their sites of induction may influence their homing properties and organ targeting. The aim of the study was to compare the expression of homing receptors on Tregs after EPIT, OIT or SLIT.
Method: BALB/c mice were orally sensitized to peanut and then treated by EPIT, OIT, SLIT or not treated (Sham). After 8 weeks of treatment with 100 μg of peanut protein extract, the proportion of Tregs and their expression of homing receptors (CCR9, CLA, CCR4, CCR8, CCR6, CXCR3 and CCR3) was analyzed by flow cytometry in spleen and in related lymph nodes (inguinal (iLN) or mesenteric (mLN)).
Results: Whatever the method, spleen Foxp3 + Tregs increased with immunotherapy ($P < 0.001$ compared to Sham), more significantly with EPIT ($P < 0.01$ compared to OIT and SLIT). In spleen, whereas Tregs CCR4 expression increased with the 3 methods ($P < 0.05$ compared to sham),

© 2015 The Authors

only EPIT-induced Tregs significantly increased expression of CCR8, a well-defined Th2 homing receptor. Analysis of skin (CLA) and gut (CCR9) homing receptors revealed a significant induction of CLA+CCR9 + Tregs by EPIT ($P < 0.001$ compared to Sham, OIT and SLIT) and CLA-CCR9 + Tregs by OIT ($P < 0.01$ compared to Sham, EPIT and SLIT) whereas SLIT-induced Tregs increased neither CLA nor CCR9 expression. In iLN, Tregs level increased only after EPIT with induction of CLA+CCR9- and CLA+CCR9 + Tregs. In mLN, only EPIT and OIT induced higher level of Tregs with increased expression of CCR9 ($P < 0.001$ compared to Sham and SLIT).

Conclusion: EPIT induced more Tregs than OIT or SLIT. The increase in gut homing receptor CCR9 and in Th2 homing receptor CCR8 in these Tregs strongly suggests a wider range of action for EPIT over SLIT and OIT during food allergy.

453
Mechanistic characteristics of peanut allergic children undergoing oral food challenge and oral immunotherapy

Bajzik, V[1]; Farrington, M[2]; Marchesini, G[1]; Suttner, J[3]; Kwok, WW[1]; Wambre, E[1]
[1]Benaroya Research Institute, Seattle, United States; [2]Virginia Mason Medical Center, Seattle, United States; [3]Allergen Research Corporation, San Mateo, United States

Background: Peanut OIT can dramatically improve the quality of life for allergic children but the molecular and immunological changes that occur during treatment are poorly understood.

Method: Whole blood samples were collected pre- and post-oral food challenge to peanut at baseline and at 6 months from peanut allergic patients participating in a double-blind, placebo-controlled, randomized trial of peanut Oral Immunotherapy (POIT). Basophil activation test (BAT) using CD203c expression was performed after stimulation with different concentration of peanut extract. Peanut-reactive CD4 + T cell response were monitored using the CD154-based assay following stimulation with pool of Ara h 1, 2, 3, 6, 8 and Ara h 9 peptides. Sorted peanut-reactive CD4 + T cells were then run on the Fluidigm 96.96 dynamic array chip to assess changes in gene expression.

Results: Positive reactions to the BAT and presence of peanut-reactive effector TH2 cells lacking CD27 expression reflect the status of sensitization to peanut, proved by Oral food challenge. No significant change in Basophil allergen threshold sensitivity was observed post POIT. Oral food challenge to peanut drastically increase the frequencies of peanut-reactive CD4 + T cells before POIT but at a lower extent in the active group post treatment. Changes in the frequency, phenotype and molecular signature of peanut reactive T cells are predictive of early clinical responses induced by POIT. Our results also confirmed that allergen-specific TH2 cells exhibit an 'exhausted' phenotype and are preferentially targeted by allergen immunotherapy.

Conclusion: These results reveal novel immunological and transcriptional signatures as surrogate markers of successful immunotherapy.

454
Epicutaneous immunotherapy leads to sustainable GATA-3 hypermethylation and Foxp3 hypomethylation in peanut sensitized mice

Mondoulet, L[1]; Tost, J[2]; Puteaux, E[1]; Ligouis, M[1]; Dhelft, V[1]; Plaquet, C[1]; Dupont, C[3]; Benhamou, P-H[1]
[1]DBV Technologies, Bagneux, France; [2]CNG-CEA, Evry, France; [3]Université Paris Descartes – Hôpital Necker, Paris, France

Background: Early treatment by EPIT is able to induce epigenetic modifications of the DNA expression of Th2 without influencing the expression of Th1. The purpose of this study was to investigate the kinetics of these epigenetic modifications underlying the therapeutic effect of EPIT and its persistence.

Method: BALB/c mice were orally sensitized to peanut and then treated by EPIT ($n = 56$) or sham ($n = 56$), with 8 naive mice serving as controls. Mice were sacrificed during EPIT, at 1, 2, 4, 6 and 8 weeks; and 8 weeks after the end of EPIT. DNA methylation was analysed in spleen and blood by restrictive enzyme digestion and quantitative PCR as well as in sorted CD4, CD8 and CD19 cells from spleen and blood by pyrosequencing.

Results: In the whole spleen of peanut-sensitized mice, EPIT increased methylation in GATA-3 CpG islands, significantly versus Sham at the 4th, 6th and 8th weeks of treatment and 8 weeks after the end of EPIT ($P < 0.05$). In the whole blood, methylation increased in the CpG islands of GATA-3 only from the 8th week of EPIT ($P < 0.01$, vs Sham) and was also sustained 8 weeks after the end of EPIT. In spleen and blood CD4 cells, a significant hypermethylation of CpG island of GATA-3 occurred at the 4th week of EPIT and persisted after the end of EPIT. A significant hypomethylation of Foxp3 CpG islands was concomitantly obtained in spleen and blood CD4 T cells, persisting after the end of EPIT. No modification was observed for Tbet and RORg transcription factors whatever the cells or the organs.

Conclusion: The prolonged and continuous skin exposure of allergen through EPIT leads to sustained epigenetic modifications of the DNA expression of Th2 (down regulation) and Treg (up regulation) transcription factors.

455
Virosomes show faster uptake dynamics and a stronger activating potential than liposomes in an *in vitro* model of pulmonary dendritic cells

Blom, RAM[1]; Amacker, M[2]; Moser, C[3]; Blank, F[1]; van Garnier, C[1]
[1]Berne University Hospital, Respiratory Medicine, Berne, Switzerland; [2]Mymetics SA, Epalinges, Switzerland; [3]Swiss Federal Institute of Intellectual Property, Berne, Switzerland

Background: The respiratory tract represents an attractive target organ for inhaled bio-mimetic antigen carriers for immunemodulation. It is easily accessible and has a dense network of dendritic cells (DCs) which is embedded in a vast surface area. However, immune effects and interaction with antigen presenting cells remain poorly understood at present, therefore we investigate the interaction of biomedical nanoparticles with key resident antigen presenting cells in order to use them as novel therapeutic agents.

Method: Virosomes and liposomes were surface-labelled with Atto647 for fluorescent detection and ovalbumin (OVA) as a model antigen. Virosomes additionally contained hemagglutinin (HA) from the influenza virus A/Brisbane/59/2007 (H1N1). Both virosomes and liposomes were characterized for particle size, purity and HA and OVA content.

Murine bone marrow derived and *in vitro* differentiated DCs (BMDCs) were exposed to virosomes or liposomes for 18 h. Particle uptake and BMDC phenotype was determined by flow cytometry (FACS) and laser scanning microscopy (LSM). Cells were tested for viability following exposure to particles by FACS.

Results: All particles showed a homogenous size-distribution and consistent HA and OVA concentration. Particles were successfully taken up by BMDCs with virosomes showing a more rapid dynamic (plateau reached at 0.5 h) whereas liposomes were taken up slower (plateau reached at 2 h). Virosomes showed a trend for stronger DC activation than liposomes as determined by co-stimulatory and phenotypic markers without affecting cell viability.

Conclusion: Reproducible, uniform virosomes and liposomes were successfully designed and thoroughly characterized for optimal interaction with BMDCs. Both

© 2015 The Authors

virosomes and liposomes were successfully taken up by DCs showing different dynamics and up-regulation of phenotypic and co-stimulatory markers. In-depth *in vivo* investigation of the potential of biomimetic carriers is a prerequisite to develop novel therapeutic agents for immune-modulation in the respiratory tract.

456
Protein and MPL adsorption capacities for MCT in candidate therapeutic formulations for use in immunotherapy, compared against existing adjuvants

Bell, AJ; Thomas, NG; Heath, MD; Hewings, SJ; Skinner, MA
Allergy Therapeutics, Worthing, United Kingdom

Background: The World Health Organisation recommends adsorption of 80% or more of tetanus and diphtheria toxoid antigens by aluminium containing adjuvants. The protein adsorption capacities from aluminium and calcium adjuvants are well documented. Modified Allergen Tyrosine Adsorbed-Monophosphoryl Lipid A (MATA-MPL) formulations have been shown to be effective therapeutics in allergy immunotherapy. The micro crystalline tyrosine (MCT) in these formulations has been shown to consistently adsorb both allergoid and MPL on manufacture. However MCT adsorption capacity factors for proteins and MPL have not been measured for direct comparison to aluminium and calcium adjuvants.
Method: Adsorption capacities of MCT for MPL and protein were calculated from quantitative determinations of both in the formulations. A gas chromatography method was used to determine MPL contents and a Bradford method was used to determine protein contents. Adsorption capacities of aluminium and calcium adjuvants were calculated in a similar way for direct comparison to MCT.
Results: MCT demonstrated greater adsorption capacities for MPL than both aluminium and calcium adjuvants.
Conclusion: The ability of MCT to readily adsorb MPL compared to aluminium and calcium adjuvants supports a characteristic association based on both tyrosine's structure and MCT's physical properties. MCT is an effective depot candidate for allergy immunotherapy formulations and vaccines.

457
Combined exposure of intestinal epithelial cells to the dietary oligosaccharide mixture of scFOS/lcFOS and CpG DNA effectively enhances the Th1 and regulatory IL-10 response in human PBMC

Hayen, SM[1,2]; Overbeek, SA[3]; Bruijnzeel-Koomen, CAF[1]; Garssen, J[3,4]; Otten, HG[2]; Willemsen, LEM[3]
[1]Department of Dermatology/Allergology, UMC Utrecht, Utrecht, the Netherlands; [2]UMC Utrecht, Laboratory of Translational Immunology, Utrecht, the Netherlands; [3]Utrecht University, Utrecht Institute for Pharmaceutical Sciences, Pharmacology, Utrecht, the Netherlands; [4]Nutricia Research, Immunology, Utrecht, the Netherlands

Background: Exposure of intestinal epithelial cells (IEC) to a 9:1 mixture of prebiotic short chain galacto- and long chain fructo-oligosaccharides (scGOS/lcFOS) in combination with synthetic CpG DNA (a Toll like receptor 9 ligand), was previously shown to modulate the cytokine response of activated peripheral blood mononuclear cells (PBMC). As scGOS is derived from lactose it may pose a risk of unwanted side effects in severe cow's milk allergic (CMA) patients. Therefore the use of a 9:1 mixture of inulin-derived scFOS/lcFOS as dietary intervention is proposed as adjunct therapy to support allergen specific immunotherapy in CMA. Our aim was to compare the immunomodulatory effect of scGOS/lcFOS or scFOS/lcFOS in combination with CpG DNA in a transwell coculture of IECs and fresh healthy donor PBMC.
Method: IEC (HT-29 cells, human colon adenocarcinoma cell line) were grown on transwell filters until confluence. IEC were apically exposed to scGOS/lcFOS or scFOS/lcFOS either or not combined with CpG DNA, and co-cultured with anti-CD3/CD28 activated human PBMC isolated from fresh whole blood in the basolateral compartment in RPMI with human AB serum. After 24 h, cytokines IFN-γ, IL-10 and IL-13 were measured in the basolateral supernatant. Next, to measure specific epithelial production of galectin-9, an important factor in regulatory T-cell induction and neutralization of IgE, the epithelial inserts were transferred to a new plate, washed and supplied with fresh media. After 24 h, galectin-9 was measured in the basolateral supernatant.
Results: Apical exposure of HT-29 cells to scGOS/lcFOS or scFOS/lcFOS in the presence of CpG DNA enhanced basolateral IFN-γ and IL-10 concentrations ($P < 0.001$), whereas CpG DNA alone was less effective ($P < 0.05$). CpG DNA suppressed IL-13 production by PBMC ($P < 0.001$), while enhancing IEC derived galectin-9 ($P < 0.01$) in the presence or absence of the oligosaccharides.
Conclusion: Epithelial exposure to scFOS/lcFOS shows to be as efficient as scGOS/lcFOS in increasing the CpG DNA induced Th1 and regulatory IL-10 response by underlying immune cells. As such it would be interesting to study whether it could provide as an adjuvant in antigen-specific immunotherapy in CMA patients.

458
Localisation kinetics of aluminium after subcutaneous injection in a rat model

Heath, MD; Hewings, SJ; Kramer, MF; Skinner, MA
Allergy Therapeutics Plc, Worthing, United Kingdom

Background: Subcutaneous immunotherapy is an effective treatment for allergy. It works by helping to re-balance an individual's immune response to allergens and the ability to drive an antibody titre response is greatly improved by the use of adjuvants, the most common being aluminium hydroxide. No data or pre-clinical model on the localisation kinetics of aluminium after subcutaneous injection, based on allergy formulations, currently exists.
Method: Albino rats of the Crl:WI (Han) strain each received a single subcutaneous administration on 4 occasions with a 3 or 4 day intervals of a Birch concentrate formulated with either Alhydrogel or L-Tyrosine as the representative depot adjuvant. Dose sites were extracted and digested up to 6 months after final administration and aluminium (Al3 +) analysed via ICP-MS.
Results: A significant proportion of aluminium (~50%) was retained at the injection site 3 months post final injection. The rate of clearance of aluminium from the dose site was calculated over a 6 month time period. As an estimate (from D14 and D180 data), the terminal half-life for clearance from SC dose site would be approx 240 days (i.e. time taken to remove half of the dose). Therefore, estimated time to clear 95% dose from SC site would take approx 1.2 years in the rat model.
Conclusion: The localisation kinetics of aluminium after subcutaneous injection, based on allergy formulation, has been investigated with the rate of clearance of aluminium from the injection site calculated from a murine model. Granuloma formations are one of the most common unwanted adverse reactions when a patient receives allergy subcutaneous immunotherapy. The results presented herein support current understanding that aluminium has the propensity to form focal accumulations in the body, beginning at the site of administration.

© 2015 The Authors

Poster Discussion Session PDS 20

Diagnosis and treatment of anaphylaxis and hymenoptera venom allergy

459
Allergy to bee venom in Turkish beekeepers

Buyuktiryaki, AB[1]; Vezir, E[1]; Kirli, U[2]; Topal, Y[3]; Cetinkaya, E[2]; Kaya, A[1]; Guvenir, H[1]; Ginis, T[1]; Capanoglu, M[1]; Civelek, E[1]; Kocabas, CN[3]
[1]Ankara Children's Hematology Oncology Training and Research Hospital, Division of Pediatric Allergy and Immunology, Ankara, Turkey; [2]Sitki Kocman University, Training and Research Hospital, Mugla, Turkey; [3]Department of Pediatrics, Sitki Kocman University, School of Medicine, Mugla, Turkey

Background: The prevalence of systemic allergic reactions to bee venom ranges from 0.3% to 8.9% in general population. Beekeepers are heavily exposed to honeybee stings, therefore at an increased risk for developing bee sting allergy. The aim of this study was to evaluate the features of bee sting reactions in beekeepers and to investigate the knowledge and attitudes of this group concerning bee allergy.
Method: Among the 4000 beekeepers registered to the Mugla Beekeepers Association 1070 subjects were selected by randomization. A questionnaire was administered to 818 beekeepers who were accessible by phone.
Results: Of the study group, 97.3% (n = 796) were male with a mean age of 49.5 ± 11.7 (mean ± standard deviation) years and the beekeeping duration was 20 (10–30) [median (interquartile range)] years. The frequency of bee stings was 300 (150–800) in a year. Sixty-nine (8.4%) beekeepers had concomitant allergic disease. Systemic, large local, and local reactions were reported as 32 (%3.9), 39 (%4.8) and 138 (%16.9), respectively. Seventeen (2.1%) of the 32 systemic reactions were anaphylactic. Although 699 (89%) participants were aware that bee stings might cause an allergic reaction, most of them (n = 620) did not keep a medicine available for using in case of need. After a bee sting, 702 (86.8%) beekeepers reported that they did not do any medical intervention, besides 78 (9.6%) of the group preferred alternative treatment modalities. In fact, only 128 (16.1%) subjects have received an education about bee allergy. Five hundred and sixty-eight (72.1%) beekeepers believed that bee sting is beneficial for humans, especially for improving rheumatic diseases (n = 464).
Conclusion: Despite being a high-risk group, the knowledge level of beekeepers about bee allergy is not sufficient and majority of them continue their professional life without receiving any education.

460
Quality-of-life in insect venom allergy: validation of the Turkish version of the 'Vespid Allergy Quality of Life Questionnaire' (VQLQ-T)

Sin, BA[1]; Aydın, Ö[1]; Gelincik, A[2]; Gürlek, F[3]; Baysan, A[4]; Sin, A[3]; Öztuna, D[5]; Mısırlıgil, Z[1]; Oude-Elberink, JNG[6]
[1]Division of Immunology & Allergy, Ankara University, School of Medicine, Istanbul University, Chest Diseases, Ankara, Turkey; [2]Division of Immunology & Allergy, Istanbul Faculty of Medicine, Internal Medicine, Istanbul, Turkey; [3]Division of Immunology & Allergy, Ege University, School of Medicine, Internal Medicine, Izmir, Turkey; [4]Division of Immunology & Allergy, Gülhane Military Hospital, School of Medicine, Internal Medicine, Ankara, Turkey; [5]Department of Biostatistics, Ankara University, School of Medicine, Ankara, Turkey; [6]Department of Allergology, University Hospital of Groningen, Groningen, the Netherlands

Background: Insect venom allergy is one of the most important life-threatening allergies. Both vespid and honey bee allergy may worsely affect health-related quality of life when patients experience systemic allergic reaction after a sting. The only established "Vespid Allergy Quality of Life Questionnaire (VQLQ)" is used to assess the psychological burden of disease in patients with vespid venom allergy.
Method: The study population consisted of 81 adult patients with bee venom allergy and 65 with vespid venom allergy. Patients who had history of systemic reactions confirmed by positive skin test and/or elevated specific IgE were included. The Turkish language Questionnaire (VQLQ-T) consisting of 14 items along with the Expectation of Outcome (EoO) questions (2 items) was administered to patients to achieve cross-sectional validation. The questionnaire was also administered to 36 patients receiving venom immunotherapy or Epi-Pen to establish the longitudinal validity.
Results: In the Turkish version VQLQ-T we could reproduce the validity and reliability of original version. The cross-sectional validation of vespid allergic patients yielded a correlation coefficient of 0.97 (Cronbach alpha). The Spearman's correlation coefficent was 0.55 ($P < 0.001$). In the longitudinal validation, the mean VQLQ score was 5.27 (±1.29) before treatment whereas it was 2.78 (±1.01) after treatment ($P < 0.001$). The cross-sectional instrument validation for bee venom allergy yielded a correlation coefficient of 0.96 (Cronbach alpha). The Spearman's correlation coefficent between pretreatment VQLQ score and EoO questionnaire was 0.49 ($P < 0.001$). After treatment the correlation between these scores was 0.75 ($P = 0.001$). In the longitudinal validation of items for bee venom allergy; while mean VQLQ score was 5.07 (±1.36) in pretreatment, it was 2.76 (±0.98) after treatment ($P < 0.001$). The correlation between mean change in VQLQ score (2.32 ± 1.13) and mean change in EoO score (2.81 ± 1.83) was 0.55 ($P = 0.029$). Also there was a significant difference in mean VQLQ scores between beekeepers and non-beekeepers ($P < 0.05$). The mean VQLQ scores of patients who stopped VIT were significantly lower than patients receiving VIT ($P = 0.028$).
Conclusion: The Turkish version of VQLQ-T enables measurement of quality of life in patients with vespid or bee venom allergy. Furthermore, the responsiveness of this instrument demonstrate the questionnaire's ability to detect changes over time.

461
Characterisation of wasp venom collected via electrostimulation or venom sac extraction

Sinnige, N; Quaak, S; van Deursen, D; Jolanda, M; van den Hout, R; Luykx, D
HAL Allergy, Leiden, the Netherlands

Background: A characterisation study was performed on wasp venom obtained via either electrostimulation or venom sac extraction. Different assays with respect to identity, content and protein structure were applied.
Methods: SDS-PAGE: Reduced samples were applied to 4–12% Bis-tris gels combined with silver staining.

© 2015 The Authors

Immunoblot: After SDS-PAGE, proteins were transferred to a PVDF membrane and stained using pooled sera of wasp venom allergic patients, HRP conjugated antibodies and CN/DAB substrate.

MS: Tryptic digests were prepared from wasp venom samples. Peptides were separated via nano-HPLC before electrospray ionisation. Ionised peptides were fragmented revealing amino acid sequences.

Protein: Spectrophotometric method according to Lowry using BSA as a standard.

Major allergen content: An ELISA was used to quantify Ves v 5.

CD: Far-UV CD spectra were recorded from 260–190 nm.

Results: SDS-PAGE protein profiles revealed that wasp venom collected via sac extraction contains a high variety of proteins in comparison to electrostimulation venom. Via immunoblot and MS allergens Ves v 1 (~35 kDa), Ves v 2 (~45 kDa), Ves v 3 (~ 90 kDa) and Ves v 5 (~27 kDa) were identified in the wasp venom collected via the two different methods. The amount of Ves v 5 in relation to total venom protein was higher in wasp venom collected via electrostimulation (4%) compared to via sac extraction (2%). The CD-spectrum of sac extraction venom indicated a relative higher amount of α-helical proteins in comparison to electrostimulation venom.

Conclusion: The SDS-PAGE profiles and Ves v 5 (%) in total protein indicate that wasp venom collected via venom sac extraction contains more unidentified proteins compared to collection via electrostimulation. Ves v 1, 2, 3 and 5 were identified in both venoms.

462
CD63 and CD203c expression during venom immunotherapy with wasp venom using the basophil activation test (BAT): 2-years and first 3-years follow-up results

Reiß, N[1]; Spornraft-Ragaller, P[2]; Hauswald, B[3]; Nemat, K[4]; Koschel, D[5]; Bauer, A[2]; Wolf, G[1]; Siegert, G[1]; Neumeister, V[1]
[1]Universitätsklinikum C.G.Carus, TU Dresden, Institut für Klinische Chemie und Laboratoriumsmedizin, Dresden, Germany; [2]Universitätsklinikum C.G.Carus, TU Dresden, Klinik und Poliklinik für Dermatologie, Dresden, Germany; [3]Universitätsklinikum C.G.Carus, TU Dresden, Klinik und Poliklinik für HNO-Heilkunde, Dresden, Germany; [4]Universitätsklinikum C.G.Carus, TU Dresden, Klinik und Poliklinik für Kinderheilkunde und Jugendmedizin, Dresden, Germany; [5]Fachkrankenhaus Coswig GmbH, Zentrum für Pneumologie, Coswig, Germany

Background: SIT is an established therapy for wasp venom allergy. The aim of our work is to investigate the progression of surface antigen CD63 and CD203c expression during SIT using BAT.

Method: We included 71 patients in our study (61 wasp, 10 honey bee; 17 aborted). The study was approved by the institutional ethical review board. Here we report on patients with SIT against wasp venom in course of 2 year (40 patients) and 3 years (9 patients). Blood samples were collected before and 3 days (3d), 2 weeks (2w) and 6 months (6 m) after SIT start. Further blood samples were repeatedly collected every 6 months until 3 years. For all samples we determined CD63 and CD203c expression using BAT after stimulation with various wasp venom concentrations. We evaluated the relative proportion of activated basophile granulocytes at 57 µg/l venom concentration (a2) and the calculated concentration c50 to stimulate 50% of total activatable basophile granulocytes.

Results: CD63 expression (and inversely c50) at 2y/3y (CD63 nonresponder: 5) decreased in 24/4 and increased in 5/0 patients, while it was constant in 6/3 cases. Median changes to baseline at 2y/3y were a2 = -49% ($P < 0.01$)/-78% ($P < 0.05$) and c50 = 576% ($P < 0.01$)/761% ($P = 0.24$). CD203c expression (and inversely c50) at 2y/3y (no CD203c nonresponder) decreased in 17/5, increased in 11/1 and did not change in 12/3 patients. Median changes to baseline at 2y/3y were a2 = -16% ($P = 0.01$)/-56% ($P = 0.08$) and c50 = 208% ($P < 0.01$)/426% ($P < 0.01$).

Conclusion: Expression of CD63 and CD203c in BAT do not show a uniform behavior during the investigated time.

Statistically significant differences can be demonstrated for CD63 and CD203c expression after 2 years; for the evaluation after 3 years we need more results. Further work is required to gain inside into long-term stimulation behavior in BAT and correlation with sting challenge.

463
Usefulness of ImmunoCAP inhibition of total extract and molecular components in the diagnosis of double sensitization to vespid venom

Delavalle, B; Labrador-Horrillo, M; Guilarte, M; Luengo, O; Sala-Cunill, A; Cardona, V
Hospital Vall d'Hebron, Allergy Section, Internal Medicine Department, Barcelona, Spain

Background: Double sensitization in patients with vespid venom allergy is common. Although is most cases component resolved diagnosis identifies the causative vespid, in some patients results are not conclusive. Differentiation between cross-reactivity and a genuine double sensitization is crucial for the indication of an effective treatment.

Aim: To identify the genuine vespid sensitization in patients with double (*Vespula spp* and *Polistes dominula*) specific IgE (sIgE) in a geographic area where both species are present.

Method: We prospectively studied patients referred to us from January 2012 to October 2014 because of anaphylactic reactions to vespid stings and double positivity to sIgE to *Vespula* and *Polistes*. All patients underwent a complete study including skin tests (prick and intradermal) with commercial *Vespula spp* and *Polistes dominula* extracts (ALK-Abelló), and sIgE to the whole extracts and molecular components (Ves v1, Ves v5 and Pol d5) by ImmunoCAP (Thermo Fisher). ImmunoCAP inhibition was performed by overnight incubation of 250 µl of patient's sera with 25 µg of *Vespula* and *Polistes* extract and the same volume of extract diluent as control. sIgE against the whole extract and allergen components were determined again. We calculated the percentage of inhibition of vespid venom sIgE after the incubation with the same vespid venom (homologous) and with the opposite (heterologous). An heterologous inhibition ≥75% was considered as a genuine sensitization to one vespid venom whereas heterologous inhibititon under 75% would point to true double sensitization. Homologous sensitizations always had to be ≥75%.

Results: Tewnty two patients (17 males) with a median age of 50 years (range 16–89) were included. According to the CAP inhibition results, 17/22 patients (77.3%) were genuinely sensitized to only one vespid venom (9 *Vespula* and 8 *Polistes*), while 5 patients were considered truly double sensitized. Median percentage of heterologous inhibition was 75.1% (range 75–95.1%) for *Vespula* and 84.3% (range 75.0–92.6%) for *Polistes* sIgE to both Pol d5 and Ves v5 were both positive in 16/17 monosensitizated patients, thus not being useful to identify the genuine sensitizating venom.

Conclusion: In patients with double vespid venom sensitisation, ImmunoCAP inhibition is a useful tool to identify the genuine sensitizer and thus prescribe one specific immunotherapy. In our study Pol d5 and Ves v5 were not useful to identify the relevant sensitisation.

© 2015 The Authors

464
Multi-sensitization to hymenoptera venoms in a group of Brazilian patients

De Amicis, KM[1,2,3]; Watanabe, AS[2,4]; Galvao, CE[2,4]; Figo, DD[1,2]; dos Santos-Pinto, JRA[2,5]; Palma, MS[2,5]; Castro, FF[1,2,4]; Kalil, J[2,4,6]; Ferreira-Briza, F[3]; Gadermaier, G[3]; Santos, KS[1,2]
[1]Allergy and Immunopathology LIM 60, University of Sao Paulo, Sao Paulo, Brazil; [2]Institute for Investigation in Immunology (iii), INCT, Sao Paulo, Brazil; [3]Division of Allergy and Immunology, University of Salzburg, Molecular Biology, Salzburg, Austria; [4]University of Sao Paulo, Clinics Hospital of School of Medicine, Sao Paulo, Brazil; [5]University of Sao Paulo State (UNESP), Institute of Biosciences, Rio Claro, Brazil; [6]University of Sao Paulo School of Medicine, Laboratory of Immunology, Heart Institute (InCor), LIM19, Sao Paulo, Brazil

Background: Double sensitization to both, *Apis mellifera* (honeybee) and *Vespula* ssp. (yellow jacket) venom is common in up to 59% of the European hymenoptera venom allergic patients and this rate is more than 50% in the United States. Reports about double sensitization involving *Apis mellifera* and *Solenopsis* sp. (fire ant) are rare and there is nothing described about multi-sensitization to hymenoptera venoms. Despite the great biodiversity of insects in Brazil where among wasps, *Polistes* sp. and *Polybia paulista* poses the major risk for patients, there is no data available about sensitization to more than one insect.
Method: Nineteen patients with clinical history of anaphylaxis to hymenoptera venoms were tested by skin prick test (SPT), ImmunoCap and IgE ELISA. From those, nine patients sensitized to three or four venoms were selected for western blotting (WB) using commercial venom extracts of *Polistes* sp., *Apis mellifera* and *Solenopsis* sp. as well as *Polybia paulista* venom extract produced by our group.
Results: Symptoms presented by this group included urticaria, angioedema, diarrhea, bronchospasm and loss of consciousness. Sensitization was confirmed by SPT. IgE reactivity to *Polistes* sp., *Apis mellifera* and *Solenopsis* sp. was positive in 9/19 patients (47%) when tested by ImmunoCAP (cut-off 0.35 kUA/l). The specific IgE recognition evaluated by ELISA showed that the most frequent reactivity was observed for *P. paulista* (13/19 - 68%) and fire ant venom (12/19 - 63%), from those the latter one presented higher magnitudes of IgE responses. In IgE WB, the nine multi-sensitized patients recognized multiple bands in each venom extract. Apart from apparently homologous molecules, other IgE-reactive proteins presenting distinct molecular masses unique for each venom were found.
Conclusion: This is the first report of multi-sensitization to hymenoptera venoms. Component-resolved analysis with recombinant species-specific major allergens avoiding CCD reactivity may help to distinguish true double sensitization from cross-reactivity. It is important to remark that *Polybia paulista* venom extract is commercially available neither for diagnosis nor therapy.

465
Immunological differences between subpopulations of Vespa crabro allergic patients

Erzen, R; Silar, M; Bajrovic, N; Zidarn, M; Lalek, N; Kopac, P; Kosnik, M; Rijavec, M; Korosec,P
University Clinic for Pulmonary and Allergic Diseases Golnik, Golnik, Slovenia

Background: Hornet (*Vespa crabro*) and wasp (*Vespula germanica*) belongs to Vespidae family. Their venoms shows marked cross-reactivity so wasp venom is used for specific immunotherapy in patients allergic to wasp or hornet venom. In our prospective study we wanted to evaluate immunological differences between patients who had experienced systemic hypersensitivity reaction to hornet sting but not to wasp sting and in patients who responded with systemic hypersensitivity reaction after sting of both members of Vespidae family.
Method: The study was prospective. We included patients with systemic hypersensitivity reaction after hornet sting. The first subgroup of patients tolerated wasp stings, the second one experienced large local reaction after wasp sting and the third subgroup of patients experienced systemic hypersensitivity reaction after both wasp and hornet stings. In all patients we performed sIge, recombinant sIgE, inhibition tests and basophil activation test with both venoms.
Results: We included 30 patients. There were no differences neither in sIgE and recombinants measurement between different subgroups of patients. Inhibition tests revealed primary sensitization to wasp venom in majority of patients and double sensitization in few of them. None of the patients showed primary sensitisation to hornet sting only. Basophil response to hornet venom was comparable in all three subgroups. However, basophil response to wasp venom was significatly higher in subgroup of patients who experienced systemic reaction.
Conclusion: Wasp venom BAT was the only method which showed some differences between subgroups of patients who experienced systemic hypersensitivity reaction after hornet sting. Patients with low wasp venom basophil sensitivity experienced anaphylaxis only after hornet sting, while patients with high wasp venom basophil sensitivity experienced anaphylaxis after both wasp and hornet stings. Patients who experienced anaphylaxis after hornet sting and showed low wasp venom basophil sensitivity are thus not ideal candidates for specific immunotherapy with wasp venom.

466
Does the severity of systemic reaction depend on insect sting localization? The comparison of children to adults

Cichocka-Jarosz, E[1]; Stobiecki, M[2]; Brzyski, P[3]; Jedynak-Wąsowicz, U[1]; Czarnobilska, E[2]; Lis, G[1]
[1]Jagiellonian University Medical College, Chair of Pediatrics, Kraków, Poland; [2]Jagiellonian University Medical College, Chair of Toxicology and Environmental Disease, Kraków, Poland; [3]Jagiellonian University Medical College, Chair of Epidemiology and Preventive Medicine, Kraków, Poland

Background: It is still a matter of controversy if the site of insect sting (IS) within the head/neck region correlates with the severity of insect sting systemic reaction (SR). There are some data on adult population indicating that such a correlation does not exist[1]. Moreover, in adults, the number of mast cells (MC) within distal body sites grossly outnumbers MC within the proximal area[2]. There are no published data analyzing the relationship between IS place and SR severity in children nor evaluating MC distribution in a child's healthy skin. The aim of our study was to compare the difference between children and adults with respect to the site of a single IS and SR severity.
Method: Questionnaire-based study sample included 103 VIT-treated persons: 48 children (47%) (69% males, mean age 10.6 ± 4.3 years) and 55 adults (53%) (53% males, mean age 41.2 ± 13.6 years). There was predominance of bee venom allergy in children (60%), whereas wasp venom allergy prevailed in adults (55%), $P = 0.032$.
Results: The most frequent IS sites in children were the feet (27%), hands (21%) and head (17%), while in adults - the head (29%), trunk/neck (16%) and feet (15%). There was no difference in IS sites between genders and types of venom allergy group, regardless of age.

There was a predominance of grade III SR in children (48%), whereas grade IV prevailed in adults (49%) ($P = 0.027$), with no difference between genders and venom group types.

In grade IV children, the most frequent IS sites were the hand (33%), foot (33%) and head (25%), contrary to the pattern observed in adults, involving the head (26%), trunk/neck (19%) and arm (19%).

In adults, the lowest percentage of grade IV SR occurred after IS involving the hand

© 2015 The Authors

- 0%, foot - 38% and head - 44%, while the highest percentage involved the arm region (71%).

In children, there were no grade IV reactions after IS situated in the trunk/neck, forearm and leg (thigh and calf), while the highest percentage of grade IV reactions involved the hand or foot (33% each).

Conclusion: Though we again confirmed a significant difference of reaction severity between adults and children[3], there was no significant correlation between sting location and SR severity, regardless of age. However, the percentage of grade IV reactions apparently differed between certain IS sites. A larger sample study would be confirmatory.

467

History of large local reaction to hymenoptera stings: outcome of re-stings (preliminary data)

Severino, M[1]; Bilò, MB[2]; Bignardi, D[3]; Cortellini, G[4]; Nittner-Marszalska, M[5]; Mauro, M[6]; Reccardini, F[7]; Rudenko, M[8]; Patella, V[9]; Pravettoni, V[10]; Ridolo, E[11]; EAACI Interest Group Insect Venom Hypersensitivity
[1]Allergy Unit, S. Giovanni di Dio, Hospital, Firenze, Italy; [2]Allergy Unit, Ancona, Italy; [3]Allergy Unit, Genova, Italy; [4]Allergy Unit, Rimini, Italy; [5]Medical University of Wrocław, Wrocław, Poland; [6]Allergy Unit, Como, Italy; [7]Allergy Unit, Udine, Italy; [8]Allergy and Immunology Centre, London, United Kingdom; [9]Allergy Unit, Salerno, Italy; [10]Allergy Unit, Milano, Italy; [11]Alergy Unit, Parma, Italy

Background: Hymenoptera stings can cause reactions with either toxic or allergic mechanisms and these reactions may largely vary in severity, from local reaction to systemic anaphylaxis. Among the Hymenoptera-induced reactions, the so-called large local reactions (LLR) have been classified as a special aspect for decades, mainly in relation with their possible predictive value for subsequent systemic reactions. The definition of LLR is essentially clinical and empiric. A LLR is defined as a reaction of oedema, erythema and pruritus, with a diameter greater than 10 cm and which peaks at 24–48 h, and then subsides. Despite the general interest, the literature concerning LLR is overall poor and fragmentary. It is estimated that the risk of developing a systemic reaction after a LLR is relatively low, ranging from 5 to 15%.

The aim of this study is to evaluate the outcome of re-stings in patients with a LLR over a period of 5 years (2012–2017); in these patients specific immunotherapy with Hymenoptera venom was not necessary. According to current guidelines.

Method: Of 294 patients were be enrolled in the study up to now. Skin testing (intradermal tests) and specific serum with bee venom, Vespula, vespa crabro (if available) and Polistes spp or Polistes dominulus (if the latter is available or relevant in the geographic area), total IgE and tryptase assay were carried out sera were collected for future studies.

Results: Fifty two out of 294 patients were re stung. 15 (28.8%) patients experienced a systemic reaction, 23 patients experencied a LLR and 14 patients were negative.

Conclusion: By now our preliminary data show that the risk of developing a systemic reaction after a LLR is superior to the previous data from literature, but these results have to be confirmed by the end of our study.

468

Bee and wasp induced anaphylaxis in Southern Denmark in the period 2008–2011

Ruiz Oropeza, A[1]; Mikkelsen, S[2]; Bindslev-Jensen, C[1]; Mortz, CG[1]
[1]Department of Dermatology and Allergy Centre, Odense University Hospital, Odense Research Center for Anaphylaxis (ORCA), Odense, Denmark; [2]Department of Anaesthesiology and Intensive Care, Odense University Hospital, Mobil Emergency Care Unit, Odense, Denmark

Background: Bee and wasp stings are described as one of the three most common cause of anaphylaxis in children and adults. Primary treatment is usually performed at pre-hospital level. This study aims to estimate the severity of anaphylaxis to bee and wasp stings and the correlation to the treatment administrated at pre-hospital level.

Method: A retrospective study based on diagnosis (ICD-10) from The Mobile Emergency Care Unit (MECU) in the Region of Southern Denmark (2008–2011). Discharge summaries from the MECU and acute ward on the Hospitals were reviewed in order to assess the severity of the anaphylactic reaction according to Sampson 2003 and Mueller 1966. Treatment was evaluated in relation to administration of adrenaline and other drugs.

Results: Ninety three patients (26 women, 67 men; mean age 49 years) were evaluated during the study period. Moderate to severe anaphylaxis was found in 63% according to Sampson (47% according to Mueller). One death was registered. Intramuscular (IM) adrenaline was given to the patients with moderate to severe anaphylaxis in 56% of the relevant cases. In the same group, 86% received intravenous (IV) glucocorticoids and 85% IV antihistamine.

Conclusion: More than 50% of the cases were graduated as moderate to severe anaphylaxis (Sampson 3–5 and Mueller 2–4). In discordance with current guidelines, only 56–57% with moderate to severe anaphylaxis received treatment with IM adrenaline, while most of the patients were treated with IV antihistamine and glucocorticoids.

469

The causative and risk factors of hypersensitivity reactions including anaphylaxis in patients with mastocytosis

Górska, A[1]; Niedoszytko, M[1]; Chełmińska, M[1]; Lange, M[2]; Gruchała-Niedoszytko, M[3]; Słomiński, M[4]; Jassem, E[1]
[1]Department of Allergology, Medical University of Gdansk, Gdańsk, Poland; [2]Department of Dermatology, Venereology and Allergology, Medical University of Gdansk, Gdańsk, Poland; [3]Department of Clinical Nutrition, Medical University of Gdansk, Gdańsk, Poland; [4]Department of Pneumonology, Medical University of Gdansk, Gdańsk, Poland

Background: Approximately 30% of patients with mastocytosis suffer from symptoms resulting from the activation and release of mediators from the mast cells which might result from an immune response or non-allergic mechanisms of hypersensitivity after activation of non-specific stimuli.

Objectives: The aim of the study was to analyze the prevalence of the hypersensitivity reactions including anaphylaxis and to identify their causative and risk factors in mastocytosis. Furthermore, we analyzed the response to the treatment of mediator-related symptoms.

Method: The study group included 152 adult patients with mastocytosis. The diagnosis included histopathological examination, KIT mutation analysis, flow cytometry, tryptase analysis. The diagnosis of allergy was confirmed using SPT (skin prick tests) and/or sIgE.

Results: The hypersensitivity reactions were observed in 127 (84%) cases. The prevalence of anaphylactic reactions was 50% and was higher in patients with systemic mastocytosis ($P = 0.007$) and specifically in indolent form ($P = 0.026$) than in cutaneous mastocytosis. The most frequent triggers of anaphylaxis were food (29%), insect stings (22%) and drugs (15%). Tryptase levels were higher in patients with anaphylaxis ($P = 0.029$), also in MC activation symptoms provoked by physical factors ($P = 0.002$). The frequency of those symptoms was reported in 112 (74%) patients and was higher in systemic mastocytosis patients compared to those with cutaneous mastocytosis ($P = 0.026$) as well as in patients with anaphylactic reactions in medical history in comparison to patients without anaphylaxis ($P = 0.0002$).

The treatment was ineffective in 8 (10.5%) patients and gave only a partial remission in a further 14 (18.4%) patients.

© 2015 The Authors

Conclusion: We reported significant incidence of physical factors related symptoms in mastocytosis patients. Risk factors included increased serum tryptase and indolent form of mastocytosis. Serum tryptase level was a significant predictor of MC activation provoked by physical factors. The conventional pharmacological treatment was ineffective in 10% of patients, who may require biological treatment.

470
Anaphylaxis in a Belgian emergency department: discrepancy in guideline adherence between acute management and medication at discharge

Mostmans, Y[1]; Blykers, M[1]; Mols, P[2]; Gutermuth, J[1]; Grosber, M[1]; Naeije, N[3]
[1]Vrije Universiteit Brussel/UZ Brussel, Dermatology, Jette, Belgium; [2]C.H.U. Saint-Pierre, Service des Urgences et du SMUR, Brussel, Belgium; [3]C.H.U. Brugmann, C.I.A., Jette, Belgium

Background: Anaphylaxis is a life-threatening emergency of which reliable epidemiological data on incidence is lacking. In this study data of anaphylactic patients was collected over a period of 4 years (2009–2013). The aim was to analyze how fast an emergency specialist was encountered, and his or her adherence to the current WAO-guideline in managing anaphylaxis.
Method: Data on anaphylactic patients was collected from 2009–2013. During this period emergency doctors completed an extensive questionnaire for adult patients with anaphylaxis presenting in the emergency room. Over this period 100 cases out of 230.878 patients visiting the emergency department of the Sint-Pieters hospital, Brussels were included. Inclusion criteria were based on the Sampson criteria of gradation of anaphylaxis. Data were analyzed using a Microsoft Excel database.
Results: 0.04% of all emergency visits in adults presented with anaphylaxis. In both women and men, dyspnea and urticaria were the most frequently noted symptoms. 72% of pts. received medical help later than 30 min of onset. In 51.7% of cases foodstuff, in 46.1% medications and in 3.4% hymenoptera venom were suspected as elicitor. 67% of all patients were given adrenaline, 85% received antihistamines, 89% were given methylprednisolone. 46% of all patients were discharged directly from the emergency room. 87% of those patients received further recommendations: 67% corticosteroids, 83% antihistamines, 9% Adrenaline IM (Epipen®), 74% were instructed to consult an allergologist.
Conclusion: Our Belgian urban population showed the same characteristics, concerning symptoms and frequent elicitors, as previous cohorts. The majority of patients was treated according to the WAO-guideline for acute management of anaphylaxis, while only a minority of patients received the recommended Adrenaline IM at discharge. 72% of all patients received medical help later than 30 min after onset of symptoms, underlining the necessity of the prescription of Adrenaline auto-injectors. The majority of patients were advised to consult an allergologist: the collaboration between both specialties should improve diagnosis and follow-up.

471
Awareness of physicians and healthcare providers concerning anaphylaxis and epinephrine auto-injector usage

Ozmen, S[1]; Aykan, E[2]; Corut, N[1]; Bostanci, I[1]
[1]Dr. Sami Ulus Training and Research Hospital of Women's and Children's Health and Diseases, Pediatric Allergy and Immunology, Ankara, Turkey; [2]Dr. Sami Ulus Training and Research Hospital of Women's and Children's Health and Diseases, Pediatriae, Ankara, Turkey

Background: Morbidity and mortality due to anaphylaxis can be reduced by both early diagnosis and accurate rapid treatment. Only epinephrine should be administered to treat anaphylaxis. On discharge patients should be prescribed epinephrine auto-injectors and taught how to use them by physicians and other healthcare providers.

This questionnaire study was designed to determine the awareness of physicians and other healthcare providers concerning anaphylaxis and the use of epinephrine auto-injectors.
Method: The study was carried out between February 1 and March 15, 2014 at Dr. Sami Ulus Training and Research Hospital of Women′s and Children′s Health and Diseases, a tertiary care hospital. A total of 166 healthcare providers, including physicians and other staff such as nurses and paramedics, participated in the study. The demographic characteristics, experience of anaphylaxis treatment and epinephrine auto-injector training were obtained using a standardized questionnaire. The participants completed the questionnaire themselves. The obtained data was statistically analyzed.
Results: The participants were composed of specialist doctors (25.9%), pediatric residents (31.9%) and other healthcare staff (42.2%). Eighty-four (50.6%) of the participants were found to have received training about anaphylaxis. Forty-seven (%55.9) of the trained healthcare providers had been educated in the previous 2 years. Among the trained healthcare providers 62 (73.8%), informed us that epinephrine by intramuscular route was applied during anaphylaxis. On the other hand, only 32 (39%) of untrained healthcare providers had chosen the intramuscular administration of epinephrine. When comparing these two results there is clearly a statistically significant difference ($P = 0.00$). While 81 (48.8%) of the participitants had some knowledge about the epinephrine auto-injector, only two specialists and three pediatric residents were found to prescribe the epinephrine auto-injector on discharge.
Conclusion: Post-graduate training is required for the diagnosis and treatment of anaphylaxis and should be given to all healthcare providers in terms of informing children and their carers. All healthcare providers should be trained how to accurately inform patients and their carers about the use of epinephrine in anaphylaxis as well as the prescription epinephrine auto-injectors.

© 2015 The Authors

Poster Discussion Session PDS 21

Pediatric allergy

472

Breast milk fatty acids in relation to sensitization up to 24 months of age - the ALADDIN birth cohort

Rosenlund, H[1]; Mie, A[1]; Fagerstedt, S[1]; Alm, J[1,2]
[1]Department of Clinical Science and Education, Karolinska Institutet, Stockholm, Sweden; [2]Sachs' Children and Youth Hospital, Södersjukhuset, Stockholm, Sweden

Background: Breast milk fatty acids (FAs) have been associated with allergic disease in children. The FA composition of breast milk is dependent on maternal diet, which is strongly influenced by lifestyle. Previous studies found that children of families with an anthroposophic lifestyle have a lower prevalence of allergic disease and sensitization. The aim of this study was to investigate if the lower prevalence of sensitization among children of anthroposophic families could be explained by differences in breast milk FA composition, in particular concentrations of omega-3, omega-6 and ruminant FAs.
Method: The prospective birth cohort ALADDIN includes 330 children from anthroposophic, partly anthroposophic and non-anthroposophic families in Sweden. ALADDIN aims at elucidating the role of specific lifestyle factors that could clarify the lower allergy prevalence in anthroposophic children. For this study, 245 breast milk samples, collected at 2 months of age, were analyzed with GC-FID for FA composition. Allergen-specific IgE against 7 common allergens were obtained from blood samples at ages 6, 12 and 24 months. Data were analyzed longitudinally with generalized estimated equation.
Results: An inverse association was observed between total concentration of omega-3 FA in breast milk and sensitization in the child up to 24 months of age (highest vs. lowest quartile, OR_{adj} 0.22, 95% CI 0.07–0.69, P for trend 0.005). In particular, DHA represented this association (OR_{adj} 0.18, 95% CI 0.04–0.73, P for trend 0.006). No statistically significant associations were observed between omega-6 FAs or ruminant FAs and sensitization. Moreover, in a model adjusting for traditional risk factors for allergy we observed 70% lower risks of sensitization up to 24 months of age among the anthroposophic group compared to the non-anthroposophic group (OR_{adj} 0.30, 95% CI 0.11–0.77). This association remained largely unchanged when breast milk omega-3 PUFA was included in the model.
Conclusion: Our result shows that omega-3 FAs in breast milk have a protective effect on sensitization in children up to 24 months of age, however, omega-3 FAs did not explain the lower risk of sensitization among children of anthroposophic families.

473

Dietary pattern in infancy and the outcomes of food sensitization and atopic dermatitis: COCOA birth cohort study

Lee, S-Y[1]; Kwon, S-O[2]; Shin, H-L[3]; Ahn, K[4]; Suh, DI[5]; Shin, YH[6]; Yang, S-I[1]; Lee, E[7]; Hong, S-J[7]
[1]Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Pediatrics, Anyang, Korea; [2]Korea Health Industry Development Institute Nutrition Policy & Promotion Team, Osong, Korea; [3]Asan Institute for Life Sciences, University of Ulsan College of Medicine, Seoul, Korea; [4]Samsung Medical Center, Sungkyunkwan University School of Medicine, Pediatrics, Seoul, Korea; [5]Seoul National University College of Medicine, Pediatrics, Seoul, Korea; [6]CHA Gangnam Medical Center, CHA University School of Medicine, Pediatrics, Seoul, Korea; [7]Childhood Asthma Atopy Center, Research Center for Standardization of Allergic Diseases, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Background: The role of diet during infancy on the development of allergic diseases later in childhood remains controversial. Reverse causality is always a matter of concern.
Method: Data from 706 children in the ongoing a Cohort for Childhood of Asthma and Allergic diseases (COCOA) study were analyzed at 1, 2 years of age. We studied food diversity at 6 months, duration of breast-feeding, and timing of introduction of solid foods. Multivariate logistic regression analyses were performed for all children and for children without atopic dermatitis and food allergy symptoms within the first 6 months of life to take into account reverse causality.
Results: Total breast-feeding $\geq$ 6 months and introduction of solid food $\geq$ 6 month were positively associated with the risk of sensitization to egg white at 1 year.(aOR 3.74, 95% CI 1.24–11.28; aOR 8.30, 95% CI 1.01–68.19) The introduction of $\geq$ 6 food item at 6 months decreased the risk of atopic dermatitis at 2 years. (aOR 0.18, 95% CI 0.04–0.85).

In children without early atopic dermatitis and food allergy were considered, the risk of sensitization to egg-white at 1 year was associated with longer duration of breast-feeding. (aOR 3.74, 95% CI 1.09–12.86) Atopic dermatitis at 2 years was significantly less frequent in children who received a more diverse diet at 6 months. (aOR 0.14, 95% CI 0.02–0.91).
Conclusion: Exposure to diverse food antigens during early life might enhance the development of immune tolerance.

474

Predictors formation of allergic diseases in infants with gestational age less than 34 weeks

Kyslova, I; Yablon, O; Mazulov, O
Vinnytsia State Medical University, Pediatrics №1, Vinnytsia, Ukraine

Background: There is a hypothesis, that adverse exposures in fetal and early postnatal life might influence the formation of allergic diseases in later life. We retrospectively investigated the diagnostic and prognostic value of perinatal factors and indicators of BDNF, VEGF in serum in infants with gestational age less than of 34 weeks in the neonatal period as predictors formation of allergic diseases in adjusted age of 18 months.
Method: Depending on the results obtained in the in adjusted age of 18 months identified 2 groups of children: 27 children (study group) with manifestations of allergic disease (atopic dermatitis, food allergy) and 15 children without signs of allergic inflammation.
Results: In 44.5% of children with allergic disease mothers were older than 35 years ($P < 0.05$, Sp 98.5%, NPV 82.2%). During pregnancy, mothers of children study group often diagnosed chronic placental insufficiency (29.6% and absence of such a measure in the comparison group, $P < 0.05$, Sp 81.8%, NPV 84.3%), acute viral infection (55.6%), and 33.4% - fetal distress. 22.3% of children were born by cesarean section. Birth weight in children of the study group was significantly lower (1235.7 $\pm$ 34.1 g,

© 2015 The Authors

$P < 0.05$, Sp 66.7%, NPV 88.0%). In the neonatal period in children study group more often were diagnosed congenital pneumonia (22.3%), respiratory distress syndrome (48.1%), bronchopulmonary dysplasia (33.4%), $P > 0.05$. Children from the study group more often after birth need of mechanical ventilation (22.3%, $P < 0.05$), children of comparison - CPAP therapy (20%, $P > 0.05$).

The value of BDNF and VEGF in the serum in the first week of life in children of all investigated groups did not differ ($P > 0.05$).

In the late neonatal period (28 days) in children with allergic disease manifestations observed a significant increase BDNF (846.3 ± 139.6 vs.155.6 ± 55.5 pg/ml in the comparison group, $P < 0.01$, Sp 100%, Se 80%, NPV 88.2%) and VEGF (378.2 ± 120.7 vs. 98.4 ± 16.7 pg/ml in the comparison group, $P < 0.05$, Sp 86.7%, Se 60%, NPV 76.5%).

Conclusion: Perinatal factors (maternal age over 35, placental insufficiency during pregnancy, birth weight less than 1500 g) and high level of BDNF and VEGF in the serum in the late neonatal period are predictors formation of allergic diseases in adjusted of 18 months.

475
Peanut and tree nut allergies in review: from clinical symptoms to component-resolved diagnosis in pediatric patients

Riviere, JG[1]; Garriga, T[1]; Vila, B[1]; Moreno-Galdó, A[1]; Narváez, K[1]; Gomez, M[1]; Guillen, M[1]; Soley, R[1]; Labrador Horrillo, M[2]; Blasco, C[1]
[1]Hospital Vall d'Hebron, Pediatric Allergy Section, Barcelona, Spain; [2]Hospital Vall d'Hebron, Allergy Section, Internal Medicine Department, Barcelona, Spain

Background: Nut allergies, although taxonomically different, are one of the top three leading causes of food-related reaction in industrialized countries and their prevalence is rising. Onset trends to lower ages. Geographic zone may influence individual sensitization. Component resolved diagnosis can help differentiate genuine sensitization from cross-reactivity. Range of manifestations may vary from mild to severe and life-threatening. According to studies in our population, hazelnut may be more prevalent than peanut allergy. However, few pediatric studies describe the prevalence in Spain, especially in Catalonia. The purpose of the study is to describe the age of onset, clinical and molecular characteristics, as well as identify the most prevalent nut allergy in the local pediatric population.

Method: An opened prospective observational study was designed, including patients from outpatients of a tertiary hospital with first time compatible IgE mediated allergy symptoms and sensitization to at least three different taxonomic groups of food, excluding milk and eggs. Inclusion criteria were positive skin prick tests (SPT) to any nut. Age ranged between 0 and 18 years old. SPT with standard standardized food and inhalant allergen extracts and controlled provocation exposure if needed were performed. Moreover, specific IgE (ImmunoCAP®, Thermo-Scientific) and specific IgE to a panel of recombinant allergens by the immunoCAP-ISAC® 112 were measured.

Results: A total of 112 nut allergic patients were included. 84% of patients shown positive SPT for nuts; 82% for hazelnuts, 77% for peanut, 75% for almonds, 66% for chestnut. Hazelnut was the most prevalent tree nut allergy in our area (50%), followed by almonds (28%), peanuts (22%) and nuts (12%), being urticaria/angioedema (16%) and anaphylaxis (12%) the most frequent clinical symptoms. Statistical association between sensitization to hazelnut and systemic symptoms was found ($P < 0.05$). Lipid transfer protein (LTP) was the most prevalent sensitization in our population followed by storage proteins. Regarding LTP sensitization, the most frequent molecule determined was Jug r 3 (82%), followed by Ara h 9 (70%) and Cor a 8 (54%), while Jug r 1 was the most prevalent storage protein sensitization (38%).

Conclusion: Hazelnut is the most prevalent nut allergy in our poly-sensitizated population, being LTP and storage proteins the most prevalent sensitizations. Urticaria/angioedema followed by anaphylaxis were the most frequent clinical features.

476
Intracellular interferon-gamma production by cord blood mononuclear cells is strongly associated with the newborn's season of birth

Tereshchenko, S; Smirnova, S; Novitckii, I; Shubina, M
Scientific Research Institute of Medical Problems of the North, Krasnoyarsk, Russian Federation

Background: Interferon-gamma is the premier cytokine produced by Th1 lymphocytes. Peripheral blood mononuclear cells of atopic patients have been shown to produce lower levels of interferon-gamma. More important, that lower level of interferon-gamma production, in particular by cord blood mononuclear cells, may precede of atopic clinical manifestation. Studies of the relationship between season of birth and allergy have indicated that birth in autumn or winter is associated with a higher incidence of atopic diseases. Seasonal variation of intracellular interferon (IFN)-gamma production by cord blood mononuclear cells (CBMC) is not studied well enough.

Method: Intracellular IFN-gamma expression as well as early activation marker CD69 (separate for total mononuclear cells population and CD4 + lymphocytes) was examined using flow cytometry after PHA stimulation of CBMC obtained from 92 full-term newborns (9 - born in winter, 26 - in spring, 36 - in summer, 21 - in autumn). Statistical analysis was performed using Kruskal-Wallis test. Data are shown as median of cells 10*6/L (25–75 quartiles).

Results: The higher amount of IFN-gamma producing CBMC (CD4 + /IFN-gamma+, $P = 0.0008$ and CD4 + /IFN-gamma+/CD69 + , $P = 0.0078$ subtypes) was in summer season of birth with comparison to other seasons. The lowest amount of IFN-gamma producing CBMC was in winter season, both for CD4 + / IFN-gamma+ and CD4 + /IFN-gamma+/ CD69 + subtypes. For CD4 + /IFN-gamma+ subtype the variation was as: winter - 21.2 (19.7–31.3), spring - 35.2 (25.0–79.4), summer - 66.7 (35.1–95.4), autumn - 23.2 (12.2–44.1).

Conclusion: The seasonal nature of IFN-gamma production by CBMC suggests that maternal exposures might influence cord blood cytokine profiles. We suppose that this association may be explained by the altered photoperiods, which possibly affect hypothalamic cytokine gene expression and/or seasonal changes in maternal vitamin D status.

477
Anthroposophic lifestyle is associated with lower prevalence and later debut of allergic sensitization - the ALADDIN birth cohort

Fagerstedt, S[1]; Marell-Hesla, H[1,2]; Johansson, E[1]; Rosenlund, H[1]; Mie, A[1]; Alm, J[1,2]
[1]Karolinska Institutet, Södersjukhuset, Stockholm, Sweden; [2]Sachs' Children and Youth Hospital, Södersjukhuset, Stockholm, Sweden

Background: Environmental and lifestyle factors are considered to contribute to the global increase in allergic sensitization. Pre- and post natal periods are important time windows for immune system development. We aimed to investigate if lifestyle or specific lifestyle factors affect the development and timing of allergic sensitization in children up to 5 years of age.

Method: IgE-analysis of allergic sensitization to a subset of allergens in children at 6, 12, 24 and 60 months of age from the Swedish prospective birth cohort study

© 2015 The Authors

ALADDIN. Children were divided into 3 lifestyle groups: anthroposophic, partly anthroposophic and non-anthroposophic. Debut age at sensitization was defined by Kaplan-Meier analysis. Sensitization was analyzed longitudinally.

Results: Children from families with an anthroposophic lifestyle had a significantly higher age at debut of sensitization as well as lower prevalence of allergic sensitization, compared to their controls.

Conclusion: Lifestyle affects not only the risk of development of allergic sensitization in children but can also influence the timing of sensitization.

478

High maternal distress scores in pregnancy are associated with development of offspring's atopic dermatitis

Kwon, J-W[1]; Kim, Y-H[2]; Yang, S-I[3]; Lee, E[3]; Kim, HY[4]; Jung, Y-H[5]; Seo, J-H[6]; Kim, B-J[7]; Kim, H-B[2]; Lee, S-Y[8]; Choi, YK[9]; Do, N[9]; Bae, YJ[9]; Hong, S-J[3]
[1]Seoul National University Bundang Hospital, Pediatrics, Seongnam-si, Korea; [2]Sanggye Paik Hospital, Inje University College of Medicine, Seoul, Korea; [3]Childhood Asthma Atopy Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [4]Pusan National University Yangsan Hospital, Yangsan, Korea; [5]Bundang CHA Medical Center, CHA University School of Medicine, Seongnam-si, Korea; [6]Korea Cancer Center Hospital, Seoul, Korea; [7]Haeundae Paik Hospital, Inje University College of Medicine, Busan, Korea; [8]Hallym University Sacred Heart Hospital, Suwon, Korea; [9]Korea Institute of Child Care and Education, Seoul, Korea

Background: The development of allergic disease has been thought to the results of immunologic response to various genetic and environmental factors. Perinatal environments including maternal psychological distress might be related with development of allergic diseases. We evaluated the influences of maternal prenatal distress for the development of offspring's atopic dermatitis (AD).

Method: A total of 2150 pregnant women were enrolled from general population between April 2008 and January 2009. Kessler scores for the probability of mother's distress were calculated at 1 month before and 6 month after delivery. Korean version of ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire was obtained to evaluate the offspring's prevalence of allergic diseases at the age of 5. Logistic regression analyses were performed to analyze the association between prenatal maternal depression scores and the development of atopic dermatitis.

Results: The prevalence (95% CI) of recent itchy eczema and physician diagnosed AD at 5 years old were 23.1% (21.0–25.3) and 25.9% (23.7–28.1). Children with current AD (having both physician diagnosed AD and recent eczema) was 14.1% (12.4–15.8). Offsprings from the mothers with high Kessler score ($\geq$ 19) in 1 month before delivery were more likely to have a history of recent AD treatment (aOR 1.93, 95% CI 1.04–3.59) and current AD (aOR 1.72, 95% CI 0.94–3.13) at age 5. Persistent maternal distress (Kessler score $\geq$ 19 at 1 month before and 6 months after delivery) was also associated with physician diagnosed AD (aOR 2.61, 95% CI 1.02–6.67) and current AD (aOR 2.52, 95% CI 0.94–6.74).

Conclusion: Prenatal maternal distress might influence the development of offspring's AD. This finding suggests the possibility of AD prevention according to control of maternal depression in pregnancy.

This study was funded by the Panel Study of Korean Children (PSKC) of Korea Institute of Child Care and Education (KICCE), and developed based on PSKC dataset of 2008–2013.

479

High serological cross-reactivity between grass and wheat in 73 wheat tolerant children with grass pollen allergy

Nilsson, N[1]; Berthold, M[2]; Ekoff, H[2]; Hedlin, G[1]; Borres, MP[2]; Nilsson, C[3]; Sjölander, S[2]
[1]Karolinska Institutet and University Hospital, Lung and Allergy, Stokholm, Sweden; [2]Thermo Fisher Scientific, Uppsala, Sweden; [3]Sachs' Children's Hospital, Lung and Allergy, Stokholm, Sweden

Background: IgE sensitization to wheat is far more common than wheat allergy. Wheat is a grass and patients with IgE to grass pollen often display IgE antibody (IgE-ab) binding to different cereals including wheat. It is often unclear if wheat sensitization reflects a specific IgE-response to wheat or a cross-reactivity with pollen allergens. Also, it is unclear how common sensitization to wheat is among grass pollen allergic individuals. Aim To study IgE sensitization to wheat, grass pollen and allergen components of these allergen sources among wheat tolerant children with a diagnosis of grass pollen allergy.

Method: Seventy-three Swedish children (0–18 year) with a doctor′s diagnosis of grass pollen allergy, IgE-ab to grass and currently eating wheat were included in the study. Serum samples were analyzed for IgE-ab to timothy, wheat, seven grass- and three wheat specific allergen components as well as cross-reacting carbohydrate determinants (CCD) using ImmunoCAP. An IgE-ab level of $\geq$ 0.35 kU_A/l was considered positive.

Results: As expected, all children were sensitized to timothy and the median level of timothy-IgE-ab was 20 kUA/l. Sensitization to timothy components was most commonly seen to Phl p 4 (96%) followed by Phl p 1 (88%), Phl p 5b (53%) and Phl p 6 (51%). Phl p 2, Phl p 11 and Phl p 12 were minor components in this population (19–38% were sensitized) while only on percent had IgE-ab to Phl p 7. Forty-four children (60%) were sensitized to wheat and the median IgE-ab level was 1.2 kU_A/l. Sensitization to specific wheat components was analyzed in all children, showing that 15% percent had IgE-ab to Tri a 14 (LTP), 4% to Gliadin and 3% to Tri a 19 (ω-5-gliadin). IgE-ab to the cross-reactive CCD marker was seen in 5% and in total, 22% of the children were sensitized to the cross-reactive components CCD and/or Phl p 12.

Conclusion: Close to two-thirds of 73 wheat tolerant children with grass pollen allergy were also sensitized to wheat, although the level of IgE-ab to wheat was significantly lower than that to grass. As expected, the frequency of IgE-ab responders to the three wheat specific allergens; LTP and two allergens of the gliadin family, was low. The number of children with IgE-ab to the two known cross-reactive allergens, profilin and CCD could not explain the entire cross-reactivity and thus further cross-reacting allergens remain to be identified.

480

Extensive hydrolyzed formula with prebiotic mixture efficiency in infants with cow's milk allergy

Pampura, A[1]; Makarova, S[2]; Lavrova, T[3]; Filatova, T[1]; Abelevich, M[4]
[1]Russian National Research Medical University, Moscow, Russian Federation; [2]Scientific Center of Children's Health, Moscow, Russian Federation; [3]Institute of Nutrition, Moscow, Russian Federation; [4]Niznij Novgorod State Medical Academy, Niznij Novgorod, Russian Federation

Background: Cow's milk protein (CMP) is the major food allergen in infancy. Besides the skin symptoms in patients with Cow′s milk allergy (CMA) there are some differences in gut microbiota with bifidobacter and lactobacillus deficiency. Using prebiotics in extensive hydrolyzed formula is one of the possible perspective strategies in atopic dermatitis associated with CMA.

The aim of the open prospective multicenter clinical study was to evaluate the effect of extensive hydrolyzed whey formula (EHWF) with short chain galacto- and long chain fructooligosaccharides 0.8 g/100 ml on skin symptoms, stool characteristics and gut microbiota.

Method: Fifty one bottle fed infants with moderate atopic dermatitis (SCORAD <

© 2015 The Authors

40) associated with CMA were enrolled in the study. They were fed with EHWF with scGOS/lcFOS 0.8 g/100 ml during 4 weeks. We evaluated SCORAD dynamics, stool frequency and its characteristics and needs in topical therapy. Gut microflora evaluated at first visit and after 4 weeks by qPCR.

Results: After 4 weeks of EHWF with scGOS/lcFOS using the severity of skin symptoms was significantly reduced (SCORAD decreased from 26.66 (SD 9.19) to 6.63 (SD 1.20), $P < 0.001$). Stool frequency was 1.88 (SD 0.94) times/day and 88% infants had normal soft stool. The need of topical glucocorticosteroids, zink-conteining agents and antihistamines ($P < 0.01$) was reduced.
The tendency for Bifidobacter growth ($P = 0.1$) and significant growth of bacterial mass of Lactobaccillus ($P < 0.02$) and Eubacter ($P < 0.01$) in gut microflora was observed by qPCR.
Conclusion: The EHWF with prebiotic mixture is effective in infants with moderate atopic dermatitis associated with CMA. The studied formula can modulate microflora profile in atopic children.

482
Experiences of parents administering eye drops to their children - thematic analysis of online blog entries and forum discussions

Bulbul, A[1]; Jones, CJ[1]; Mukhopadhyay, S[1,2]; Smith, HE[1]
[1]Brighton and Sussex Medical School, Brighton, United Kingdom; [2]Royal Alexandra Children's Hospital, Brighton, United Kingdom

Background: Blogs and forum websites are a frequent source of advice for parents of young children. The aim of this study was to explore the questions asked and the difficulties encountered administering eye drops to children, together with the advice being offered to help overcome these difficulties.
Method: Simple and advanced google search was used to find websites with blog entries and forum discussions about administering eye drops to children. A thematic analysis was used to identify challenges and coping strategies used.
Results: Administering eye drops poses a major problem in babies and toddlers as illustrated by such messages as: '...every time, it's her screaming and crying bloody murder!'; '...we looked like a couple of WWE wrestlers on the living room floor. I'm happy to report I came out of that one the victor.', 'She develops Incredible Hulk strength and somehow was always able to prevent me from doing what I needed to do'. This creates emotional discomfort for parents ('I feel like I'm torturing him'). Coping strategies used include: demonstration ('Once we showed him on us then he warmed to the idea of keeping his eyes open.'); distraction ('I usually administer eye drops in the bath....so preoccupied with water and their squeaky toys that they barely notice it'); stealth ('We let her watch something on TV... and then we would sort of cuddle next to her (eye drop bottle hidden) and then just kind of jump her real quick'); reward ('I give him stickers each time we get it done'); waiting until child sleeps ('If she sleeps good just pry her eye open a little and put it in that way.'); play ('We used to sing to her and when she let us do it we would jump around and clap!'); force ('I used to pin Dear Daughter on the floor with me knees either side of her, so I was practically sitting on her... then lean over her with my arms gripping her head still whilst at the same time trying to prise her eyes open'); burrito-technique ('So, I started wrapping her up in a bath towel like a giant baby burrito..., I lean over her, slide my arm under her head, and put the drops in the corner of her eye.').
Conclusion: The tactics used to administer eye drops to young children are diverse, some so physical there is a risk of parental rejection. When prescribing eye drops to children with rhino conjunctivitis we need to be aware of the potential practical challenges to good administration techniques and advise parents appropriately.

483
Prolonged exclusive and partial breastfeeding reduce milk sensitization and the risk of eczema in early childhood: a birth cohort study

Yeh, K-W[1]; Chiu, C-Y[2]; Liao, S-L[2]; Tsai, M-H[2]; Hua, M-C[2]; Lai, S-H[1]; Yao, T-C[1]; Huang, J-L[1]; Prediction of Allergies in Taiwanese Children (PATCH)
[1]Chang Gung Memorial Hospital at Linkuo, Pediatrics, Kweshan, Taiwan, Province of China; [2]Chang Gung Memorial Hospital at Keelung, Pediatrics, Keelung, Taiwan, Province of China

Objectives: The evidence is insufficient and no conclusion for the relationship of exclusive breastfeeding and/or duration of any breastfeeding on the risk of allergic outcomes later in childhood. The aim of this study was to determine the effects of different patterns of breastfeeding in real life on the development of atopic diseases in early childhood.
Method: Children aged 0 through 3 years from a birth cohort in the Prediction of Allergies in Taiwanese Children (PATCH) study were enrolled. Detailed information on breastfeeding was obtained by well-trained investigators at 6 months of age. Specific IgE antibody against food and inhalant allergens were measured sequentially and their relation to different patterns of breastfeeding and the development of atopic diseases were assessed.
Results: A total of 198 children were regular followed up at clinics for a 3-year follow-up period. The rate of exclusive and partial breastfeeding remained low and was 29.3% and 14.6%, respectively, at 6 months postpartum. The total serum IgE levels appeared to be increased gradually with increasing age but were not related to different patterns of breastfeeding across different years of age. A significantly lower prevalence of sensitization to milk was found in children with exclusive and partial breastfeeding for 6 months at age 1 and 1.5. Breastfeeding longer than 6 months was significantly associated with reduced risk of eczema but not allergic rhinitis and asthma at age 1 and 2. In comparison to children with exclusive breastfeeding for at least 6 months, children with partial breastfeeding for less than 6 months were significantly associated with increased risk of eczema at age 1 and 2.
Conclusions: Both exclusive and partial breastfeeding for at least 6 months may not only reduce the prevalence of sensitization to milk but also the risk of developing eczema in early childhood.

484
Maternal vitamin D levels inversely related to allergic sensitization and atopic diseases in early childhood

Huang, J-L[1]; Chiu, C-Y[2]; Tsai, M-H[2]; Liao, S-L[2]; Hua, M-C[2]; Lai, S-H[1]; Yao, T-C[1]; Yeh, K-W[1]; Prediction of Allergies in Taiwanese Children (PATCH)
[1]Chang Gung Memorial Hospital at Linkuo, Taoyuan, Taiwan, Province of China; [2]Chang Gung Memorial Hospital at Keelung, Keelung, Taiwan, Province of China

Background: There are limited studies addressing the impact of maternal vitamin D status on the vitamin D levels in offspring, their sensitization to common allergens and atopic disease development.
Method: Children aged 0 through 4 years from a birth cohort in the Prediction of Allergies in Taiwanese Children (PATCH) study were enrolled. Time series of serum 25-hydroxyvitamin D (25(OH)D) levels were measured in maternal blood before delivery, cord blood and at age 1.5, 3 and 4 using an electrochemiluminescence-based assay. Specific IgE antibodies against food and inhalant allergens were measured at 6 months, and 1, 1.5, 2, 3, and 4 year of age.
Results: A total of 164 mother-child pairs from a birth cohort were recruited in this

© 2015 The Authors

study. The mean levels of maternal 25(OH) D was 23.2 ± 7.7 ng/mL with a high (up to 80%) prevalence of insufficient vitamin D status (< 30 ng/mL). A significant correlation was seen between maternal and cord blood 25 (OH)D levels ($P < 0.001$) and a persistent lower 25(OH)D level was found in children born to mothers with deficient 25(OH)D levels. Deficient maternal 25 (OH)D levels (< 20 ng/mL) appeared to have a higher prevalence of allergen sensitization before age 2. Higher maternal 25 (OH)D levels were significantly associated with lower risk of eczema ($P = 0.006$) and asthma ($P = 0.032$) at age 4.

Conclusions: Low maternal 25(OH)D levels appear not only to be associated with an increase in the prevalence of allergic sensitization but also the risk of eczema and asthma in early childhood.

485
Educational and supporting program for pediatric atopic dermatitis improves parental treatment compliance and has positive impact on disease control

Olariu, SV[1]; Putere, E[2]; Bumbacea, RS[1,3]
[1]Novo Medica Clinic, Bucharest, Romania; [2]Gamma Institute, Bucharest, Romania; [3]Carol Davila' University of Medicine and Pharmacy, Allergology, Bucharest, Romania

Background: Atopic dermatitis is a chronic inflammatory skin disease that is characterized by a chronic course of exacerbations and remissions. Parental understanding of the disease management is important for the treatment of atopic dermatitis in pediatric patients. Lack of information, inadequate treatment compliance, parental alternative health belief and the "corticosteroid phobia" in pediatric atopic dermatitis lead to poor control and negative outcomes. Therefore, providing an educational program for the management of pediatric atopic dermatitis can be of benefit for both the patients and their parents.

Objective: To determine the usefulness of our educational and supportive program, to understand and validate parental concerns about topical treatment safety and to implement methods for managing parental adherence to treatment, emphasizing the role of emollients.

Method: Twenty-five pediatric patients (ages 2 to 8 years old) and their parents were included in our pilot-study. Fifteen parents received informative sessions and supportive psychotherapy, while the remaining ten were the control group. The program consists of three interdisciplinary group sessions of 3 h each, covering medical and psychological issues. The parents were asked to complete a 25-item standardized questionnaire designed to asses parents knowledge about atopic dermatitis, adherence to treatment, level of feeling of control, sleep disturbance, before the sessions and 1 month after monitored treatment.

Results: For the group receiving educational and supportive sessions, the mean scores about atopic dermatitis knowledge and the feeling of control were significantly improved ($P < 0.05$). Also our educational program showed enhanced compliance to treatment by increased use of emollients (by 70 g/week) and reduced use of unconventional therapies (to none). The parents night sleep has improved with 0.4 h/night.

Conclusion: This educational and supportive program proved to be an useful tool to improve clinical outcomes in pediatric atopic dermatitis and parental adherence to treatment by offering medical information and psychological support to parents.

486
Predictive equations of pulmonary function for healthy children in Portugal

Martins, C[1]; M Pinto,[2]; Silva, D[1,2]; Moreira, P[3]; Padrão, P[3]; Oliveira Fernandes, E[3]; Madureira, J[3]; Delgado, L[1,2]; Severo, M[2]; Moreira, A[1,2]
[1]Centro Hospitalar de São João, Porto, Portugal; [2]Faculty of Medicine of the University of Porto, Porto, Portugal; [3]Faculty of Nutritian and Food Sciences, University of Porto, Porto, Portugal

Background: There is a scarcity of data on lung function reference values for healthy children in Portugal. This study aims to develop predictive equations for spirometric reference values for children aged 8 to 12 years in North Portugal.

Method: A random sample of 352 children from 10 primary schools in Oporto were screened by a health questionnaire and physical examination and those found "normal" underwent spirometry according to the standardized procedure recommended by the ATS/ERS task force in 2005. Exclusion criteria were inability to correctly perform spirometry, premature birth (< 37 weeks), reported having wheeze in last 12 months or any history of asthma. Each subject's weight and height were measured, and lung function was performed using a MIR Spirobank, with WinSpiro-PRO software. Spirometric parameters recorded were forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), forced expiratory flow between 25 and 75% of exhalation (FEF2575) and peak expiratory flow (PEF). All computer derived flow-volume curves were reviewed for technical acceptability and the best flow-volume curve was selected to derive the reference equations using multiple regression analysis.

Results: A total of 111 subjects, aged 8.09 (0.75) years (mean (SD)), 51 (45.9%) girls, were included in the study. Both in boys and girls, FVC, FEV1 and PEF showed moderate to strong correlations with height (CC = 0.736, $P < 0.001$; CC = 0.699, $P < 0.001$; CC = 439, $P < 0.001$ respectively), weight (CC = 0.593, $P < 0.001$; CC = 0.615, $P < 0.001$; CC = 0.412, $P < 0.001$) and age (CC = 0.302, $P < 0.001$; CC = 0.403, $P < 0.001$; CC = 276, $P = 0.003$); while FEF2575 only with height (CC = 0.309, $P = 0.001$) and height (CC = 0.420, $P < 0.001$). Using *Student's T* test and confirmed by linear regression, all spirometric parameters were independent of gender. For FVC, FEV1 and PEF the predictive variables are height and weight. For FEF2575 only weight was statistically significant, but the authors opt to maintain the variable height in the model. Results are summarized in table 1.

Table 1: Predictive equations of pulmonary function

Spirometric Index	β (C.I.)	Height (C.I.)	Weight (C.I.)	R^2
FVC	-1.929 (-2.079-(–1.067))	0.028 (0.021–0.036)	0.006 (0.000–0.013)	0.542
FEV1	-1.237 (-2.002-(–0.529))	0.021 (0.015–0.027)	0.008 (0.002–0.013)	0.511
FEF2575	0.386 (-1.917–2.311)	0.010 (0.005–0.028)	0.018 (0.007–0.030)	0.171
PEF	-0.490 (-2.884–1.858)	0.029 (2.962–0.004)	0.017 (-0.002–0.035)	0.229

Conclusion: In conclusion, we present newly developed predictive equations regression for spirometry variables that may be applied to calculate lung function in Portuguese children aged 8 to 12 years.

© 2015 The Authors

Poster Discussion Session PDS 22

Primary and secondary immonodeficiences

487
CD19 complex and memory B cells in patients with Down syndrome

Artac, H; Seckin, AN; Ozdemir, H; Ceylan, A
Pediatric Immunology and Allergy, Selcuk University Medical Faculty, Konya, Turkey

Background: Down syndrome is the most common chromosomal abnormality. Mortality and morbidity with the infections in these patients are much more than the healthy children. We aimed to evaluate the role of CD19 complex and memory B cells in the pathogenesis of immunodeficiency in patients with Down syndrome.
Method: We studied the expressions and median flourescein intensity (MFI) of CD19, CD21, CD81 molecules in B cells of 39 patients with Down syndrome and 40 healthy controls to assess CD19 complex. Memory B-cell subsets was determined by using CD27, IgM and IgD monoclonal antibodies in the flow cytometry. Demographic data, infections, hospitalization and comorbidities in these patients were recorded and compared with CD19 complex and memory B-cell subsets.
Results: The study population consisted of 20 male and 19 female patients with a mean age at admission of 69 ± 61 months. Thirty-one of them had congenital cardiac diseases. Thirty-one patient had low IgM, 21 had low IgA and eleven had low IgG. The ratio of B cell in patients was found to be low (11.9 ± 5.8% in patients; 16.1 ± 6.3% in controls, $P < 0.01$). While the expressions of CD19 (97.5 ± 1.8%; 98.6 ± 0.8%) and CD21 (83.4 ± 7.7%; 90.3 ± 3%) ($P < 0.05$) were lower than controls, the expression of CD81 were not different than controls. CD19 MFI (1060 ± 193; 1601 ± 407), CD21 MFI (11558 ± 2662; 10213 ± 1861) and CD81 MFI (2672 ± 607; 2139 ± 683) were significantly different in patients. The expressions of total memory cells (27.4 ± 13.3%; 33.8 ± 12.9%) and natural effector B cells (5.1 ± 4.2%; 9.4 ± 9.1%) were significantly low in patients with Down syndrome in compared with controls ($P < 0.01$). No significant difference between study patients and controls was observed in naive B cells and class switched memory B cells.
Conclusion: This study showed that there was a meaningful change in CD19 complex. Total memory cells and natural effector B cells were significantly decreased in Down syndrome. Considering that the CD19 complex regulates the events following antigen stimulation, the change is suggested that there is an intrinsic defect in signaling of B cells.

488
Long-term follow-up of lymphocyte subpopulations in common variable immunodeficiency (CVID)

Litzman, J[1]; Trávničková, S[1]; Tkačuková, N[1]; Kadlecová, P[2]; Nechvátalová, J[1]; Vlková, M[1]
[1]Department Clin Immunol Allergol, St. Anne's University Hospital, Brno, Czech Republic; [2]International Clinical Research Centre, St. Anne's University Hospital, Brno, Czech Republic

Background: Patients with the most common symptomatic primary humoral immunodeficiency - common variable immunodeficiency (CVID) - are characterized by frequent abnormalities in lymphocyte subpopulations. This includes a decrease of both absolute and relative numbers of CD4 + cells, also an increase in the absolute number of CD8 + cells was described, leading to the decreased CD4/CD8 ratio. CD4 + cells are frequently activated (increase in the expression of HLA-DR, CD45RO, decreased number of cells expressing CD45RA). Also, a decreased number of NK cells was repeatedly observed, numbers of B-lymphocytes are variable, in some patients the numbers are markedly decreased. It is not clear whether these abnormalities are secondary due to antigen stimulation or dysregulation of the immune system, or are primary and contribute to the pathogenesis of the disease.

The goal of this study was to determine how the major lymphocyte subpopulations changed during long-term follow-up of CVID patients.
Method: Thirty-nine CVID patients (24 females, 15 males aged 26 to 80 years at the time of the study) with B cells > 2% of peripheral blood lymphocytes were included in the study. The length of the follow-up ranged from 3 to 12 years, the intervals between the investigations ranged from 3 to 12 months. Linear regression analysis was used for statistical evaluation.
Results: A decrease of absolute and relative numbers of lymphocytes in differential blood count was observed ($P = 0.0068$ and $P < 0.001$, respectively), while the total number of leukocytes did not change. When assessing relative numbers, a significant increase of CD3 + and CD3 + CD8 + cells was observed (in both cases $P < 0.0001$), while the relative number of NK cells decreased ($P = 0.001$). With absolute numbers, a decrease of CD3 + CD4 + cells ($P = 0.0001$), B lymphocytes ($P = 0.0003$), and NK cells ($P < 0.0001$) was recorded.

Concerning activation markers, no change in the expression of CD45RA, CD45RO and HLA-DR on CD4 + lymphocytes was observed, while an increase of HLA-DR on CD8 + lymphocytes ($P =< 0.001$) was recorded in our study.
Conclusion: Abnormalities in lymphocyte subpopulations in CVID seem to be unstable and have a tendency to deteriorate with time. The most important group of changes seems to be the decrease of the absolute number of lymphocytes, leading to the decrease of absolute numbers of CD4 + , NK and B-cells. Compared to this, the expression of the activation markers does not seem to change significantly with time.

489
Clinical features of immunodeficiency in CLL patients, and effectiveness of immunoglobulin replacement therapy on clinical outcome

Compagno, N; Visentin, A; Cinetto, F; Semenzato, G; Trentin, L; Agostini, C
Department of Medicine, University of Padova, Padova, Italy

Background: Infective complications account for up to 50% of chronic lymphocytic leukemia (CLL)-related deaths. Their aetiology is multifactorial, due to disease-related immune defects and/or chemo-immunotherapy. Hypogammaglobulinemia is the most common immune defect in patients with CLL, and correlates with duration and stage of the disease. The defect is usually irreversible. Immunoglobulin replacement therapy (IgRT) is a safe and effective way to prevent infections in

© 2015 The Authors

CLL patients, but indications to their use are not strictly defined.

Method: We retrospectively analyzed data of 706 patients, to identify a clinical phenotype associated with high infectious risk, in which Ig replacement therapy is appropriate. We identified patients with at least one episode of non neutropenic major infections (MI). Clinical and biochemical data were collected at the time of the MI; for patients who didn't complain any MI we recorded the last available data.

Results: Seventy nine patients had 98 MI: 67 pneumonia, 27 sepsis, 3 central nervous system infections, 1 endocarditis. Patients who experienced infections (PwI) had a shorter overall survival (OS) than patients who did not (PwoI): 10-years OS were 65% and 83%, respectively. PwI had 2.3 higher risk of death for any cause than PwoI.

Clinical-biological characteristics associated with the occurrence of MI were high-risk cytogenetic, unmutated IGHV, $CD38^+$, advance stage disease (Rai III-IV) and previous chemotherapy. We observed a higher risk of MI in subjects with at least 2 Ig isotypes deficiencies (Odds Ratio 10.1). By contrast, patients with only 1 Ig deficiency did not show a higher risk of MI. Multivariate analysis showed that previously treated patients with at least 2 Ig isotypes deficiencies carried the highest risk of contracting a MI during their clinical history. 71 out of 126 patients with such a high risk of MI were treated with IgRT. In these patients we observed a reduction of the incidence of MI (0.044 vs 0.019 MI/patient-year) and an improvement of overall survival (250 vs 206 months).

Conclusion: In our work, we described the prognostic role of MI in CLL patients. We identified the clinical profile of patients at higher risk of major infections, confirming the higher risk of infections in CLL patients with antibody deficiency. Finally, we described the reduction of the incidence of MI and the improvement of the OS with IgRT in patients with higher risk of infections.

490
A new STAT3 mutation in a family with a primary antibody deficiency

Suratannon, N[1,2]; Swagemakers, S[1]; Dik, W[1]; van de Vosse, E[3]; van der Burg, M[1]; Dalm, V[1]; van der Spek, P[1]; Driessen, G[1]; van Hagen, PM[1]
[1]Erasmus MC, Rotterdam, the Netherlands; [2]Faculty of Medicine, Chulalongkorn University, Pediatrics, Bangkok, Thailand; [3]Leiden University, Leiden, the Netherlands

Background: Primary antibody deficiencies (PADs) are the most prevalent inherited immunodeficiencies in human. The use of novel approaches, such as whole-genome sequencing (WGS) has helped to identify new gene variants that are involved in the pathogenesis of PADs. Characterization of these variants identifies molecular pathways that are involved in B cell differentiation and function.

Method: The index case (IC) is a 37 year old woman who has a history of autoimmune hypothyroidism, retinal neovascularization, recurrent colon polyps and PAD (age of 14) which was later defined as granulomatous common variable immunodeficiency (CVID). Serum IgE level was normal. She has two daughters, one developed PAD at the age of 9 and she has a brother with PAD. We performed WGS and B cell analysis in both, IC and affected daughter (AD).

Results: Data analysis revealed 5717215 variants in total. After filtering for inheritance and protein affecting variants, 509 unique variants were selected. Analysis of known primary immunodeficiency genes (including ingenuity search (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity)) revealed a variant in STAT3 (Hg18: chr 17, location 37735170–37735171); missense mutation A - C resulting in L387R. This mutation was absent in the control cohorts; Huvariome ($N = 190$), Wellderly ($N = 481$) and 1000 referee genomes. Polyphen-2 analysis revealed the probably damaging variant (HumDiv score; 0.998, sensitivity 0.27, specificity 0.99, Humvar score; 0.993, sensitivity 0.47, specificity 0.96). PCR analysis confirmed the same mutation in her brother but not in the non-affected daughter and mother. Phosphorylation of STAT3 by flow cytometry analysis showed comparable results as controls. B cell analysis revealed normal transitional B cells ($CD38^{high}/CD24^{high}$), naive mature B cells ($CD38^{dim}/CD24^{dim}/IgD^+/CD27^-$) and natural effector B cells ($Cd38^{dim}/CD24^{dim}/IgD^+/CD27^+$). Memory B cells ($CD38^{dim}/CD24^{dim}/IgD^-/CD27^+$) were slightly decreased; 11 (IC) and 7 (AD) cells/ul (normal; 13–122 and 13–-100, corrected for age). IgG+ class switch memory B cells were reduced; 7% (IC) and 36% (AD) respectively (normal >75% of memory B cells).

Conclusion: We present a new STAT3 mutation detected by WGS in a family with PAD, including B cell class switching impairment. The retinal and gastro-intestinal complications in the patient are suspected to result from this mutation.

491
Knowledge, perceptions and quality of life of immunodeficient patients on home based self-infused immunoglobulin replacement therapy

Sloan, AJ; Mahabir, S
Immunology Department, St James Hospital, Dublin, Ireland

Background: Home based self infusion is a popular method of providing immunoglobulin replacement therapy. Patients need motivation, knowledge about their condition and treatment satisfaction to comply with long term self infusion. We sought to assess knowledge and perceptions about self infusion in a cohort of patients and to evaluate their quality of life.

Method: We conducted an observational study in immunodeficient patients on home based self-infused immunoglobulin replacement therapy. Patients were assessed via a questionnaire designed to evaluate quality of life, knowledge and attitudes to their treatment. Responses were anonymous.

Results: Eleven patients on intravenous therapy and 9 patients on subcutaneous therapy participated. All patients knew the name and dose of the product they were using, 60% knew the treatment was IgG and 85% were aware it was a multi donor blood product. 85% could name their condition. 65% were aware of our recommendation for prolonged antibiotic courses to treat infections. Education was attained from healthcare professionals (85%), websites (55%), own reading (20%) or patient information documents (20%).

All patients believed timely infusions were important, with 85% stating it was never okay to miss an infusion. Causes of missed infusions were lack of time (25%), forgetting (25%), or tiredness (15%). 40% had never missed an infusion. 85% felt that their infusions kept them quite well or very well. Overall, 95% liked self infusion quite a bit or very much with 70% finding them convenient. A minority reported that the treatment took too long (1 patient), interrupted social/family life (2 patients), or limited the ability to travel (3 patients). Thirty percent stated that they didn't like others to know about their infusions.

The typical state of health was described as not ill or slightly ill in 80%, with 90% perceiving their condition as slightly or not at all difficult to manage. 25% expressed feeling quite a bit tired. 80% felt not at all to somewhat worried. All patients reported feeling happy most or for a moderate amount of time, with one reporting feeling occasionally depressed.

Conclusion: Patient knowledge about their infusions and condition was satisfactory. Infusions were perceived as contributing to

© 2015 The Authors

good health and infusing on time as important. Patients have a good quality of life and are generally happy, do not perceive themselves as significantly ill and find their condition easy to manage.

492
The comparison of computed tomography and magnetic resonance imaging findings of parenchymal lung diseases in patients with primary immune deficiency

Arslan, S[1]; Poyraz, N[2]; Ucar, R[1]; Yesildag, M[3]; Yesildag, A[2]; Caliskaner, AZ[1]
[1]Department of Immunology and Allergic Disease, Meram Faculty of Medicine, Necmettin Erbakan Universiy, Konya, Turkey; [2]Department of Radiology, Meram Faculty of Medicine, Necmettin Erbakan Universiy, Konya, Turkey; [3]Division of Pulmonary Disease, Konya Training and Research Hospital, Konya, Turkey

Background: Primary immunodeficiencies (PIDs) constitute a heterogeneous group of disorders caused by defects in immunity, leading to recurrent infections, autoimmunity, lymphoproliferation and malignancies. Respiratory tract symptoms and complications present a significant cause of morbidity and mortality among these patients. Computed tomography (CT) play an important role in characterizing lung damage. The magnetic resonance imaging (MRI) is a radiation-free alternative to CT for the evaluation of several lung diseases. We describe and correlate the MRI and CT features of several common patterns of parenchymal lung disease.
Method: Twenty three consecutive patients with PID were included in the study. The diagnosis was based on European Society for Immunodeficiencies. All patients were evaluated with a low-dose chest CT scan and MRI study on the same day. MRI and CT scan viewing were scored in a random order by two independent observers in consensus, adopting a previously validated CT scan scoring system (Bhalla) for lung alterations. The bronchial pathology (bronchiectasis extension, bronchial wall thickening, bronchiectasis severity, mucus plugging, bronchial generations involved) and related to parenchymal abnormalities (consolidations, abscesses, bullae, nodules and nodules dimension) were assessed. Wilcoxon test was used for comparison of changes between methods.
Results: MRI was compared with CT scan on a cohort of 23 patients with PID for identification of morphologic abnormalities. MRI and CT scan gave similar results in detecting the bronchial wall thickening, bronchiectasis severity, mucus plugging, abscesses, consolidations, bullae, nodule and nodule dimension. However, CT scan demonstrated a better identification of bronchial generations involved (z: -2.6, *P*: 0.008) and bronchiectasis extension (z: -2.5, *P*: 0.011).
Conclusion: Repeated imaging assessments over time by chest CT scan are required for identification of pulmonary complications in PIDs. Although recent CT techniques have substantially reduced radiation exposure, it remains high. Immune-compromised patients have an increased risk of lymphoproliferative disease and higher incidence of chromosomal aberrations after x-ray irradiation. The MRI is a radiation-free alternative to CT for evaluation of several lung diseases. Our study demonstrated that MRI represents a promising technique to evaluate lung alterations in patients with PID.

493
Clinico-radiological correlation in children with ataxia telangiectasia in Qatar

Ehlayel, MS[1]; Elsaid, MF[2]; Shami, R[3]; Salem, K[4]; Abdulbari Bener, A[5]
[1]Section of Pediatric Allergy Immunology, Weill-Cornell Med. College, Hamad Medical Corp, Doha, Qatar; [2]Section of Pediatric Neurology, Weill-Cornell Med. College, Hamad Medical Corp, Doha, Qatar; [3]Section of Pediatric Neurology, Hamad Medical Corp, Doha, Qatar; [4]Radiology, Hamad Medical Corp, Doha, Qatar; [5]Department of Biostatistics & Medical Informatics, Istanbul University, Istanbul, Turkey

Introduction: Ataxia telangiectasia (AT) is a rare disease characterized by immunodeficiency and neurological manifestations. Ataxia, resulting from cerebella atrophy, runs a progressive incapacitating course. Clinical monitoring of the disease course is mandatory for early treatment.
Aim: To study clinical severity of AT and correlate it with the degree of cerebella atrophy.
Patients and Method: We retrospectively studied all children (less than 14 years) with AT seen at Hamad General Hospital Clinics between 1998–2013. We collected basic demographic data, parental consanguinity, family history, AT clinical severity scores, and reviewed CBC with differential counts; alpha-fetoprotein, serum immunoglobulins and lymphocyte subsets. Cranial MRI scans of each subject were reviewed by a neuroradiologist. Cerebellar atrophy visually and semi-quantitatively was scored.
Results: We analyzed data on 18 AT children (10 males and 8 females), mean age of 76.9 months. 77.8% had positive family history of AT and 41.7% parental consanguinity. *Lymphopenia* was observed in 77.8% and high serum *alpha-fetoprotein in 87.5% of* children. Clinical severity of ataxia was 17.1 ± 8.4 (mean±SD), with 13/15 (86.7%) were moderate-severe. MRI cerebellar atrophy score was 1.9 ± 1.3 *(mean ± sd)*, and moderate in 51% of patients. AT clinical severity score correlated (coefficient r = 0.566) but not statistically significantly $P = 0.088$) with MRI cerebellar atrophy scores.
Conclusions: Moderate to severe ataxia and marked cerebellar atrophy are quite common in AT children. There is a correlation between AT clinical severity and cerebellar atrophy. Larger prospective studies will further determine the significance of our observations and help practicing practitioners monitor the progression of the disease.

494
Hereditary angioedema: report from the Czech registry

Hakl, R[1]; Kuklínek, P[1]; Krčmová, I[2]; Hanzlíková, J[3]; Vachová, M[3]; Zachová, R[4]; Strenková, J[5]; Litzman, J[1]
[1]Department of Clinical Immunology and Allergology, St. Anne's University Hospital in Brno, Masaryk University, Brno, Czech Republic; [2]Department of Clinical Immunology and Allergology, University Hospital in Hradec Králové, Charles University, Hradec Králové, Czech Republic; [3]Department of Immunology and Allergology, University Hospital in Plzeň, Charles University, Plzeň, Czech Republic; [4]Department of Immunology, University Hospital in Motol, Charles University, Praha, Czech Republic; [5]Institute of Biostatistics and Analyses at the Faculty of Medicine and the Faculty of Science of the Masaryk University, Brno, Brno, Czech Republic

Background: Hereditary angioedema (HAE) is a rare genetic disorder caused by a deficiency in functional C1 inhibitor (C1-INH) activity. In patients with HAE, plasma proteolytic cascades normally regulated by C1-INH become activated, resulting in episodic, recurrent attacks of angioedema. These attacks are characterized by subcutaneous and submucosal oedema in various anatomical locations. The goal of this study is to present the results of the acute HAE attacks treatment obtained from the Czech national registry of primary immunodeficiencies.
Method: We present data collected between March 2012 and December 2014. Data were collected in 4 centres intended for the diagnosis and therapy of HAE.
Results: The registry contains data of 139 HAE patients (women: 77, men: 62; HAE type I 86.3%, HAE type II 13.7%), showing HAE prevalence 1.32 per 100 000 inhabitants. 1084 attacks in 104 patients were recorded. The factors described by the patients as being associated with triggering HAE episodes included stress (10.4%), trauma (9.2%) and infection (3.6%). However, in most attacks triggering factor was not identified (68.8%). The most frequent were abdominal (47.7%) followed by peripheral oedema (38.5%). Laryngeal oedema was presented in 10.1% of attacks. Prodromal symptoms (most

© 2015 The Authors

often erythema marginatum, weakness or nausea) were reported by 13.1% of patients. 874 attacks (80.6%) were actively treated (64.2% icatibant, 22.9% recombinant C1-INH, 2.6% plasma derivate C1-INH, 0.3% fresh frozen plasma, 6.8% increase in androgens dosage, 3.1% increase in tranexamic acid dosage). Treatment had to be repeated in 102 attacks (11.7%). Hospitalization was necessary in 7 attacks (0.9%), Emergency medical service (EMS) was used in 5 attacks (0.6%).

Conclusion: Our results show marked clinical variability in HAE patients. The fact that in more than 10% of patients required repeated treatment of single attack shows that although various therapeutic approaches are available, is still difficult to choose the best therapeutic approach for a concrete patient.

495

Screening protocols to detect subclinical respiratory infections in PID: findings from a European survey

Jolles, S[1]; Sánchez-Ramón, S[2]; Quinti, I[3]; Soler-Palacin, P[4]; Agostini, C[5]; Florkin, B[6]; Couderc, L-J[7]; Brodszki, N[8]; Jones, A[9]; Longhurst, H[10]; Warnatz, K[11]; Haerynck, F[12]; Matucci, A[13]; de Vries, E[14]
[1]Immunodeficiency Centre for Wales, University Hospital of Wales, Cardiff, United Kingdom; [2]Department of Immunology, Hospital Clínico San Carlos, Madrid, Spain; [3]Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy; [4]Pediatric Infectious Diseases and Immunodeficiencies Unit, Hospital Universitari Vall d'Hebron, Barcelona, Spain; [5]Department of Clinical and Experimental Medicine, University of Padua, Padua, Italy; [6]CHR de la CITADELLE, Liège, Belgium; [7]Université de Versailles, Hopital FOCH, St Quentin en Yvelines, France; [8]Skåne University Hospital, The Children's Hospital, Lund, Sweden; [9]Great Ormond Street Hospital, Institute of Child Health, London, United Kingdom; [10]Barts Health NHS Trust, London, United Kingdom; [11]University Medical Centre Freiburg, Centre for Chronic Immunodeficiency, Freiburg, Germany; [12]Department of Paediatric Immunology and Pulmonology, Ghent University Hospital, Ghent, Belgium; [13]Department of Internal Medicine, University of Florence, Florence, Italy; [14]Department of Paediatrics, Jeroen Bosch Hospital, Hertogenbosch, the Netherlands

Background: Patients with primary immunodeficiency disease (PID) often develop progressive lung disease due to underlying subclinical infection and inflammation, despite apparently adequate levels of replacement immunoglobulin (Ig). Findings from a meeting of EU experts (Paris, June 2013) suggested that subclinical infection was not adequately monitored and screening protocols vary by centre, disease and patient.

Method: To better understand screening protocols used across Europe, a survey was conducted to identify how patient population, prevalence of lung disease, screening methods and guidelines used to combat underlying infection differ between centres and patient groups. A survey was sent to 14 PID experts in centres located in Belgium, France, Germany, Italy, The Netherlands, Spain, Sweden and the UK. Participants were asked to consider how frequently they undertake different types of monitoring tests, divided into the following five categories: clinical monitoring, laboratory monitoring, lung function testing, imaging and other.

Results: Responses were received for a total of 1539 PID patients (351 paediatric, 1188 adult). There is a marked increase in the incidence of lung disease from paediatric patients (15%) to adult PID patients (51%), which occurs despite current monitoring and treatment interventions. The majority of clinical monitoring tests were performed at frequencies reflecting clinic visits, and were more frequently carried out in adult patients. In patients without lung disease, lung function tests were carried out more often in adults than children while in patients with lung disease, testing was more frequent in children. Bronchoscopy, biopsy or bronchoalveolar lavage are only used rarely, if indicated, with no set frequency per year. There is a lack of local and national guidelines for screening and treating lung disease in PID, with only two participants stating they follow national guidelines, and only three having access to local guidelines.

Conclusion: We have observed a great deal of variation across Europe in how frequently different monitoring methods are carried out in PID patients with or without lung disease. These results highlight a need for evidence based guidelines on how to monitor and treat lung disease encountered in primary antibody deficiency, and may represent a starting point for development of a European standard of best practice for detecting and monitoring subclinical infection in PID patients.

496

Spleen embolization as a strategy for controlling cytopenia in patients with Common Variable Immunodeficiency (CVID)

Duarte Ferreira, R[1]; Ornelas, C[1]; Silva, SP[1]; Lopes Silva, S[1]; Baldaia, C[2]; Tavora, I[3]; Barbosa, MP[1]
[1]Immunoalergology, Hospital de Santa Maria, CHLN, E.P.E., Lisboa, Portugal; [2]Gastroenterology, Hospital de Santa Maria, CHLN, E.P.E., Lisboa, Portugal; [3]Imagiology, Hospital de Santa Maria, CHLN, E.P.E., Lisboa, Portugal

Background: Nodular regenerative hyperplasia (NRH) has been considered the main hepatic abnormality in patients with CVID. In some patients, this condition might lead to serious portal hypertension, hypersplenism, and various cytopenias, most commonly neutropenia and thrombocytopenia. Treatment usually consists of potentially harmful steroids and antimetabolite agents that further increase the susceptibility for infection. Splenectomy is not frequently considered, as the spleen is believed to act as a vascular buffer of portal pressure, but it has been used in CVID patients to improve refractory autoimmune cytopenias.

Case: We present the case of a male patient who was diagnosed with gangrenous appendicitis at age 30, complicated with peritonitis. He thus underwent several surgeries, including a partial colectomy. Both hypogammaglobulinemia and later specific antibody deficiency were identified in this context, leading to the diagnosis of CVID (Euroclass B^+; SmB^+; Tr^{norm}; 21^{lo}). The patient then initiated intravenous IgG replacement therapy (700 mg/Kg every 4 weeks) at our hospital. Six years after diagnosis, blood tests showed increased liver enzymes, a cholestatic pattern without hyperbilirrubinemia, as well as worsening thrombocytopenia and leukopenia with neutropenia. A splenic (due to enlarged spleen) vs. immune etiology was considered for cytopenias. Transjugular liver biopsy was compatible with NRH and the portal hypertension was documented.

Simple splenectomy was not an option due to the patient's previous surgical history. Partial embolization of the main splenic artery was therefore considered and the procedure was carried without complications. However, only a small area of necrosis was obtained and improvement of the cytopenias was not achieved.

Conclusion: In the presence of NRH with portal hypertension, embolization of the main splenic artery could be considered as an alternative procedure in patients in whom simple splenectomy is not an option.

498

Pulmonary complications in patients with common variable immunodeficiency

Erdem Bahceci, S; Genel, F; Gulez, N; Nacaroglu, HT; Can, D
Division of Pediatric Immunology and Allergy, Dr Behcet Uz Children's Hospital, Izmir, Turkey

Background: In the follow-up of patients diagnosed with common variable immunodeficiency (CVID), pulmonary complications such as chronic airway disease, interstitial lung disease (ILD), bronchiectasis are commonly seen; they are the most common causes of increased morbidity and mortality. In our study pulmonary complications in patients with CVID were analyzed retrospectively.

© 2015 The Authors

Method: In the study, 28 patients followed with CVID in Pediatric Allergy Immunology Department of our hospital were involved. The patients were divided into two groups as with and without pulmonary complications; pulmonary complications were split into three with respect to their complication types such as bronchiectasis, asthma and ILD. Clinical and laboratory data of patients were evaluated retrospectively.

Results: Of the patients, 67.9% of them were male and 32.1% were female. Pulmonary complications were observed in 13 patients (46.4%). Asthma was seen in 54%, bronchiectasis in 23% and ILD were observed in 23% of these patients. Mean age was 112 ± 61 months, mean age at diagnosis was 80 ± 45 months, and mean follow-up period was 39 ± 35 months. In cases with pulmonary complications, age at diagnosis, the age at onset of symptoms and IgG levels were significantly lower and duration of IVIG treatment was determined as significantly longer ($P < 0.05$). Spesicific antibody responses could not be found in any of the patients with bronchiectasis and ILD. No difference was determined between cases with asthma and cases without any pulmonary findings. While patients with bronchiectasis applied to our clinic with mostly upper and lower respiratory infection, 85.7% of the cases with asthma referred to us with wheezing attacks. In all patients with ILD, there was splenomegaly at the time of the diagnosis. TACI mutation was determined as 23% in cases with pulmonary complications and 20% without complications.

Conclusion: As a result, both airway and parenchymal pulmonary diseases are frequently observed in CVID. Low serum IgG level increases pulmonary complication risk. Early diagnosis and appropriate management of pulmonary complications are of importance in terms of the quality of life and long term morbidity.

499

Three neutrophilic granulocyte′s subpopulations $CD62L^{dim+}CD63^{dim+}$, $CD62L^{bright+}CD63^{dim+}$ and $CD62L^{dim+}CD63^{mid+}$ in newborns with congenital pneumonia

Nesterova, IV[1]; Chudilova, GA[2]; Lomtatidze, LV[2]; Kleschenko, E[3]; Kovaleva, S[2]; Smerchinskaj, T[3]; Sapun, O[3]

[1]Department of Allergology and Immunology, The Peoples' Friendship University of Russia, Moscow, Russian Federation; [2]Department of Clinical Immunology, Kuban State Medical University, Krasnodar, Russian Federation; [3]Department of Neonatology, Kuban State Medical University, Krasnodar, Russian Federation

Background: Neutrophilic granulocytes (NG) are most important effector cells of the antimicrobial defense. Studies of NG′s subpopulations $CD62L^{dim+}CD63^{dim+}$, $CD62L^{bright+}CD63^{dim+}$ and $CD62L^{dim+}CD63^{mid+}$ in newborns with congenital pneumonia has not yet been carried out.

Method: We have studied 32 infants (19 boys, 13 girls) aged 3–15 days, body weight 2880–4370 g, with a gestational age of 38–40 weeks, with a diagnosis of congenital pneumonia (CP) that in 36% of cases was complicated of neonatal sepsis (NS). The control group (C) consisted of 22 healthy newborns. The multicolor cytoflowmetric method with mAbs panel CD62L- FITC, CD16-ECD, CD63-PE was used.

Results: 3 NG subpopulations: $CD62L^{dim+}CD63^{dim+}$, $CD62L^{bright+}CD63^{dim+}$, $CD62L^{dim+}CD63^{mid+}$ were allocated in healthy newborns and newborns with CP and NS. The number of NG with the phenotype of $CD62L^{+}CD63^{+}$ was in 95.15 ± 0.99% in the control group. In infants with CP the number of $CD62L^{+}$ $CD63^{+}$ NG was 93.91 ± 0.61% and did not significantly differ from C. In infants with NS the number of $CD62L^{+}$ $CD63^{+}$ NG was reduced to 85.7 ± 2.88% in comparison with C and CP. In C group subpopulation $CD62L^{dim+}CD63^{dim+}$ was detected in 50.44 ± 4.38% of NG, subpopulation $CD62L^{bright+}CD63^{dim+}$ - in 46.58 ± 0.55%, minor subpopulation $CD62L^{dim+}CD63^{mid+}$ NG was detected in 1.4 ± 0.55%. In infants with CP the number NG phenotype $CD62L^{dim+}CD63^{dim+}$ was increased to 72.7 ± 3.5% (1.5 times), and NG with phenotype $CD62L^{bright+}CD63^{dim+}$ was decreased to 21.95 ± 3.65% (2 times), NG phenotype $CD62L^{dim+}CD63^{mid+}$ was 4-fold increased to 5.98 ± 0.74%. In newborns with NS the number of $CD62L^{bright+}CD63^{dim+}$ NG was only 11.2 ± 3.0%, which was 4 times less, than in the C group, and 2 times higher, than in the group with CP. Draws attention to a sharp increase in the number of NG phenotype $CD62L^{dim+}CD63^{mid+}$ to 14.5 ± 2.5% against 1.4 ± 0.55% in C and 5.98 ± 0.74% in the group with CP. At the same time, the number of NG subpopulation $CD62L^{dim+}CD63^{dim+}$ was slightly higher, than in control – 67.34 ± 4.33%, but lower, than the rate in the group with CP.

Conclusion: We have demonstrated 3 new subpopulations of NG in healthy newborns and newborns with CP and NS. Those subpopulations had different profiles in C group, CP and NS. The detection of the degree of decreasing the subpopulation $CD62L^{bright+}CD63^{dim+}$ NG is very important for differential diagnostics of CP and NS and can be use as the differential criteria.

500

An occurrence of bronchial asthma in patients with common variable immunodeficency

Milota, T[1]; Svobodova, T[2]; Zachova, R[1]; Vernerova, E[1]; Sediva, A[1]

[1]Department of Immunology, University Hospital in Motol, Prague, Prague, Czech Republic; [2]Department of Paediatrics, University Hospital in Motol, Prague, Prague, Czech Republic

"Common Variable Immunodeficiency" (CVID) is a heterogenous group of disorders characterised by a disturbed immunoglobulin production and a dysregulation of immune system. Recurrent respiratory tract infections are the most common manifestation in the patients with CVID. Consequently, chronic inflammation in lower airways can result in „Chronic Obstructive Pulmonary Disease" (COPD). According to many clinical studies aimed at CVID the symptoms of COPD can be observed in 45–68% of patients. On the other hand there are just a few studies regarding allergic diseases, particularly "Bronchial Asthma" (BA).

Bronchial obstruction is a typical hallmark for the BA patients as well as for the COPD patients. Both entities differ particularly in underlying pathophysiological mechanisms. Eosinophilic inlammation prevails in BA, on contrary neutrophilic inlammation is typically found in COPD patients. These findings are then also reflected in therapeutic approach. The response to corticosteroids is much more effective to downregulate inflammation in the eosinophilic inflammatory terrain than in the diseases with neutrophilic inflammation.

Therefore we performed the study to distinguish CVID patients with bronchial obstruction phenotypically corresponding to BA from COPD based on the performance of a bronchodilator reversibility test with salbutamol or a bronchial challenge test with methacholine. Additionally we assessed predicting factors for BA such as positive allergic family history, serum eosinophilic cationic protein, exhaled nitric oxide, eosinophil blood count, total and antigen - specific IgE in serum.

In the study we in total analysed data from18 CVID patients. In 11 patients (61.1%) with normal ventilation parameters we revealed bronchial hyperreactivity. For those the therapy with beta - 2 - agonists and/or inhaled corticosteroids was initiated. For 2 patients (11.1%) with obstructive ventilation disorder we performed bronchodilator test with negative result in the first patient, thereby the diagnosis of COPD was confirmed. For the second patient with positive result we assessed the diagnosis of „Asthma and

© 2015 The Authors

Chronic obstructive pulmonary disease Overlap Syndrome" (ACOS). The rest of patients (27.8%) had spirometry with normal ventilation parameters and the negative bronchial challenge test. The tailored therapy based on our results led to significant release of symptoms and improvement of disease control proved by „Asthma Control Questionnaires".

© 2015 The Authors

Poster Discussion Session PDS 23

Asthma: Mechanisms

501

The TLR7 agonist R-837 relaxes guinea pig airways through a TLR7-independent, airway smooth muscle-mediated mechanism

Larsson, O[1]; Manson, M[2]; Starkhammar, M[1,2]; Fuchs, B[2]; Adner, M[2]; Kumlien Georen, S[1,2]; Cardell, L-O[1]
[1]Division of ENT Diseases, Karolinska Institutet, CLINTEC, Stockholm, Sweden; [2]Experimental Asthma and Allergy Research, Karolinska Institutet, Institute for Environmental Medicine, Stockholm, Sweden

Background: Toll-like receptor (TLR) 7 receptor agonists are of interest in the treatment of allergic disease, due to their ability to reduce allergic airway inflammation and rapidly relax airways.
Method: TLR7 agonists R-837, R-848 or CL264 were administered to guinea pig airways following establishment of stable pre-contractions with carbachol in vitro or histamine in vivo. Pharmacological inhibitors were applied to study involvement of conventional pathways of bronchodilation. Bilateral vagotomy was performed to investigate neuronal involvement. Isolated human airway smooth muscle cells (HASMCs) were pre-incubated with R-837 and the effect of histamine-induced calcium flux was determined.
Results: R-837 rapidly relaxed carbachol-pre-contracted airways *in vitro* and histamine-pre-contracted airways *in vivo*. A small role for prostanoids could be detected, but otherwise relaxation occurred independently of nitric oxide and carbon monoxide signalling, cAMP signalling and neuronal innervation. The TLR7 antagonist IRS 661 did not alter R-837-induced relaxation and the structurally dissimilar TLR7 agonist CL264 did not relax airways. R-837 concentration-dependently inhibited histamine-induced calcium flux in HASMCs.
Conclusion: The data demonstrate that R-837 can act directly on ASM to induce bronchorelaxation, via a TLR7 independent mechanism. The bronchorelaxant properties of R-837 parallel other bronchorelaxant compounds (e.g. chloroquine), which like R-837, but unlike CL264, contain the chemical structure quinoline, and act by inhibiting intracellular calcium flux. Developing compounds with a quinoline and TLR7-binding moiety may be of interest in the development of future multifunctional drugs for treatment of asthma.

502

Role of dsDNA in rhinovirus-induced allergic asthma exacerbations

Toussaint, M; Jackson, DJ; Edwards, MR; Johnston, SL
Airway Disease Infection Section/MRC Centre for Allergic Mechanisms of Asthma, Imperial College London, Paddington, United Kingdom

Background: Currently, a major outstanding question in asthma research is what are the important virus induced signals or pathways that boost T_H2 responses during allergic asthma exacerbations (AAE). It is well-know that double stranded (ds) DNA released from dying cells mediates aluminum adjuvant activity by promoting T_H2 sensitization. Furthermore, it has been shown in several mouse models that during respiratory viral infections, the level of extracellular dsDNA into the airways increased significantly. This increase of dsDNA is predominantly derived from neutrophils by the NETosis process. During respiratory viral infections, neutrophil recruitment could be responsible of T_H2 exacerbation of allergic asthma by releasing high amount of dsDNA in the airways. Until now, the role played by host dsDNA in the exacerbation of T_H2 immune response during AAE has never been assessed.
Method: We used a human experimental model of rhinovirus infection to measure T_H2 cytokines (IL-4, IL-5 and IL-13) and dsDNA levels in nasal lavage from atopic asthmatic ($n = 21$) and healthy ($n = 10$) during rhinovirus infection. Subjects were seen on Days 2, 3, 4, 5, 7 and 10 postinoculation for clinical assessment and nasal sampling, perfomed by nasosorption. dsDNA was measured in the acellular fraction of the nasal lavage using Quant-iT PicoGreen dsDNA reagent (Invitrogen) and T_H2 cytokines were measure after nasosorption by using the Meso Scale Discovery array platform.
Results: At baseline, there were no differences in nasal dsDNA levels between the 2 groups. Following RV infection nasal dsDNA level was significantly increased on day 3 and 4 in the asthmatic subjects compare to healthy subjects. Peak post-infection nasal dsDNA levels correlated positively with lower and upper respiratory symptom score, a clinical marker of asthma exacerbation severity, and with peak post-infection nasal IL-4, IL-5 and IL-13 levels ($P < 0.01$; $P < 0.05$; $P < 0.05$).
Conclusions: Extracellular dsDNA levels in nasal lavage are increased in virus-induced allergic asthma exacerbations and are associated with higher disease severity and greater T_H2 inflammation. Extracellular dsDNA may contribute to the pathogenesis of asthma exacerbations and treatments targeting dsDNA such as DNase or poly-L-lysine may have clinical benefits in asthma exacerbations.

503

Passively sensitized human organotypic tissue as asthma model to study mast cell-nerve interaction

Jiménez Delgado, SM; Schindler, S; Sewald, K; Armin, B
Fraunhofer Institute for Toxicology and Experimental Medicine, Airway Immunology, Hannover, Germany

Background: In the pathogenesis of asthma mast cells play a key role. It is well known that mast cell activation and degranulation is mediated through the aggregation of their high-affinity IgE-receptor (FcεRI) by IgE and antigen. But, newly evidences suggest that mast cells can also respond to stimuli that are independent of FcεRI, such as tachykinin peptides. The tachykinin substance P has been referred as a pivotal neuropeptide involved in activation and degranulation of mast cells. But, there is a lack of in vitro models to study the local mast cell-nerve interaction. Thus, we aimed to analyze, if C-fibers activation and the release of neuropeptides lead to mast cell degranulation and bronchoconstriction, using passively sensitized human Precision-Cut Lung Slices (PCLS) as an ex vivo model. Moreover, the role of key molecules such as substance P receptor, histamine 1 receptor and soluble IgE were evaluated.
Method: To mimic an acute allergic phase, human precision cut lung slices (PCLS) were passively sensitized with plasma from allergic patients. Subsequently, bronchoconstriction was provoked with the allergen house dust mite (HDM) or with C-fiber agonist capsaicin ± histamine 1 receptor antagonist, NK1 receptor inhibi-

© 2015 The Authors

tor and IgE antagonist. Bronchoconstriction was visualized by videomicroscopy and the reduction of the airway area [% of initial airway area] was analysed. Additionatly, cryosection preparations from human bronchus were immunostained using antibodies against the mast cell tryptase. Mast cell morphology after ± capsaicin stimulation was illustrated in confocal images.

Results: HDM– and capsaicin-induced bronchoconstriction showed reduction of the airway area to 30% and 47% respectively. PCLS incubated with allergic plasma ± the histamine 1 receptor antagonist, the NK1 receptor inhibitor and IgE antagonist and stimulated with capsaicin showed no reduction of the airway area. Regarding cryosection preparations from human bronchus, immunostaining of mast cells revealed degranulation as evidenced by loss of cell-stored and appearance of freely located mast cell tryptase.

Conclusion: Here we show a functional communication of C-fiber nerves and mast cell mediators in human lung tissue leading to strong bronchoconstriction from passively sensitized human PCLS in response to capsaicin. Thus, passively sensitized human organotypic tissue expose the importance of neuroimmunologic characteristics involved in allergic asthma.

504
IL13 controls nitric oxide production through phosohatidylinositol 3-kinase in human bronchial epithelial cell

Yamamoto, M[1]; Trudeau, JB[2,3]; Wenzel, SE[2,3]
[1]Allergology and Respiratory Medicine, Showa University, Tokyo, Japan; [2]University of Pittsburgh Medical Center, Asthma Institute, Pittsburgh, United States; [3]University of Pittsburgh Medical Center, Pulmonary, Allergy and Critical Care Medicine, Pittsburgh, United States

Background: Exhaled nitric oxide (FeNO) is a non-invasive marker of airway eosinophilic and Type-2 inflammation in airway diseases like asthma. Previous studies have suggested that the majority of the FeNO arises from inducible nitric oxide synthase (iNOS) expressed by human bronchial epithelial cells (HBEC). IL13 is a key mediator in allergic inflammation which is involved in development of airway hyperreactivity, mucus hypersecretion, and recruitment of eosinophils into tissue. IL-13, when added to primary HBECs in air liquid interface (ALI) induces the expression and activation of iNOS mRNA and protein. However, the pathway(s) by which, iNOS is induced in response to IL-13 are not clear. IL-13 is known to signal through the Type II IL-4Ra which has both JAK/STAT and insulin receptor binding sites (IRS). IRS is believed to regulate phosphatidyl-inisitol-3 kinase (PI3K). We therefore hypothesized that IL-13 regulates iNOS expression and activation through the PI3K pathway in primary HBEC.

Method: HBEC were obtained from bronchial brushings of 21subjects (12 severe asthma, 4 mild to moderate asthma, 5 healthy subjects). HBEC were passaged into transwells and at confluence into ALI. The cells were cultured with IL13 for 8 days. 24 h before the harvest, those epithelial cells pretreated with PI3K inhibitor LY294002, NVP-BEZ235 or ZSTK474 for 30 min, and IL-13 added for 24 h. Cells were lysed for mRNA/protein analysis and supernatants collected for nitrite/nitrate (NOx).

Results: INOS mRNA expression was significantly decreased by LY294002 ($n = 10$, mean = 24.9 ± 53.8 AU vs. 10.1 ± 24.2 AU, $P = 0.0007$). INOS protein expression was also significantly decreased ($n = 10$, mean = 0.9 ± 0.8 AU vs. 0.3 ± 0.3 AU, $P = 0.03$). Moreover, NOx levels from upper supernatants were lower following inhibition of PI3K ($n = 10$ mean = 46.0 ± 24.6 vs. 31.9 ± 13.8, $P = 0.01$). Nitration of proteins in cell lysate was significantly lower after PI3K inhibition ($n = 8$ mean = 1.15 ± 0.9 AU vs. 0.97 ± 0.8 AU, $P = 0.01$). Another PI3K inhibitor (NVP-BEZ235) decreased iNOS mRNA/protein expression and NOx levels, but nitrotyrosine levels were not changed. No differences have been observed between asthmatic and normal subjects.

Conclusion: These results suggest that IL13 regulates iNOS expression and nitric oxide production through the PI3K pathway in primary HBEC perhaps through the PI3K gamma and/or mTOR pathways. Targeting these pathways could improve some to the downstream consequences of Type-2 inflammation.

505
Protease allergens are capable of degrading surfactant proteins to varying degrees and this efffects can be inhibited with specific protease inhibitors

Birben, E[1]; Karaaslan, C[2]; Kalayci, O[1]
[1]Pediatric Allergy Unit, Hacettepe University School of Medicine, Ankara, Turkey; [2]Department of Biology, Molecular Biology Section, Faculty of Science, Hacettepe University, Ankara, Turkey

Background: Surfactants are mixtures of lipids and surfactant-specific proteins that form a lining layer between the aqueous airway liquid and the inspired air. In addition to their biophysical role, they have immunomodulatory properties. The effect of protease allergens on airway surfactants is not well known.

Aim: To investigate the effects of protease allergen on degradation of Surfactant A and D.

Method: Surfactant A and D were incubated with Der p1, Der p serine proteases, Lolium perenne extract and Bla g2 allergens with and without specific protease inhibitors and electrophoresed on PAGE.

Results: All allergenic extracts were found to degrade both SP-A and SP-D to varying degrees. This effect was aborted with specific protease inhibitors. Der p1 (a cysteine protease) degraded both SPA and D completely. Der sp (a serine protease) degraded SP-A completely but SP-D only partially. These effects of Der p1 and Der sp were totally reversed by using specific protease inhibitors. Bla g2 (an aspartic protease) degraded both and also this effect was partly reversed by pepstatin A. Lolium perenne (a serine protease) degraded SP-D only and this effect of was aborted only by a combination of cysteine and serine protease inhibitors. Lolium perenne had no effect but had no effect on SP-A.

Conclusion: Allergenic proteases are able to degrade surfactants of the airways to varying degrees by their protease effects. These effects can be reversed by specific protease inhibitors. This may have important implications with respect to inception of the inflammatory airway diseases such as asthma.

506
Exosomes from eosinophils of asthmatic patients produce alterations on structural lung cells

Cañas, JA[1]; Mazzeo, C[1,2]; Guerra, A[1,2]; Barranco, P[3]; Quirce, S[3]; Ruiz Hornillos, J[4]; Izquierdo, M[5]; Sastre, J[6]; del Pozo, V[1,2]
[1]Inmunology, IIS-Fundación Jiménez Díaz-UAM, Madrid, Spain; [2]Ciber de Enfermedades Respiratorias, CIBERES, Madrid, Spain; [3]Hospital La Paz Institute for Health Research (IdiPAZ) and CIBER of Respiratory Diseases CIBERES, Madrid, Spain; [4]Hospital Universitario Infanta Elena, Madrid, Spain; [5]IIB-'Alberto Sols'-UAM, Madrid, Spain; [6]Fundación Jiménez Díaz, Madrid, Spain

Background: Asthma is a disease of great importance given the high medical and social impact and the enormous health costs. Exosomes are nanovesicles that play an important role in intercellular communication releasing diverse molecules (RNA, miRNAs, proteins and lipidic mediators). Recently, our group has described that eosinophils from asthmatic subjects release exosomes. The aim of this study is to investigate the effect of these eosinophil exosomes from asthmatic patients in structural lung cells.

Method: The study was performed in two types of structural lung cells: *Bronchial Smooth Muscle Cells (BSMCs)* and *Small*

© 2015 The Authors

Airway Epithelial Cells (SAECs). These cells were cultured with exosomes, previously purified from eosinophils from asthmatic and healthy individuals, and functional studies were carried out: apoptosis, necrosis, wound healing assays and also, we analyzed relative gene expression of important genes in asthma pathology (TSLP, Periostin, TGF-β, TNF-α, MMP9, VEGF and CCR3, CCL26, TLR3, NFκβ).

Results: When *BSMCs* were cultured in the presence of exosomes at different times, apoptosis/necrosis rate and relative gene expression showed changes compared to cells without exosomes. The rate of apoptosis and necrosis in structural lung cells was higher in presence of exosomes, being its maximum rate at 24 h. Similarly, when *SAECs* were cultured in the presence of exosomes, they needed more time to close the wound completely than *SAECs* without exosomes. Regarding the relative gene expression, we observed that the profile of gene expression is different between cells with or without exosomes and also, this profile appeared to be different when an epithelial cells layer presents a wound.

Conclusion: The presence of exosomes from eosinophils of asthmatic patients in bronchial smooth muscle cells and airways epithelial cells, appear to affect its functionality compared to cells lacking the presence of these exosomes.

507
Plasma concentration of hepatocyte growth factor in asthma patients correlates with impairment of lung function

Kowal, K; Pampuch, A; Bodzenta-Lukaszyk, A
Allergology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland

Background: Hepatocyte growth factor (HGF) is involved in regulation of inflammation and tissue remodeling. The aim of this study was to evaluate plasma concentration of HGF in asthmatic patients in relation to selected clinical and immunological parameters.

Method: Citrate anticoagulated plasma samples were obtained from 60 asthmatic patients (APs) and 10 healthy controls (HCs). HGF concentration was evaluated using ELISA method. In asthmatic patients lung function tests, exhaled nitric oxide concentration (FeNO), serum total IgE concentration (t-IgE), peripheral blood eosinophilia and platelet count were evaluated.

Results: The mean HGF plasma concentration in APs (336.3 ± 72.4 pg/ml) did not differ significantly from that in HCs (379.7 ± 93.8 pg/ml; $P = 0.1$). In mild APs ($n = 27$) plasma HGF concentration (302 ± 48 pg/ml) was significantly lower than in HCs ($P = 0.002$) and in moderate-severe APs ($P = 0.004$). In APs plasma HGF concentration correlated significantly with lung function expressed as FEV1 ($r = -0.52$; 95% CI −0.689 to -0.298; $P < 0.0001$) or MEF50 ($r = -0.393$; 95% CI -0.595 to -0.145; $P = 0.003$). No correlation of plasma HGF concentration with FeNO, tIgE, blood eosinophilia, or platelet count could be demonstrated.

Conclusion: Dysregulated production of HGF in asthmatic patients may be involved in pathogenesis of airway remodeling leading to progression of impairment of lung function.

508
Is swimmers' asthma a distinct asthma endotype?

Seys, SF[1]; Hox, V[2]; Van Gerven, L[2]; Dilissen, E[2]; Marijsse, G[2]; Peeters, E[2]; Kasran, A[2]; Aertgeerts, S[3]; Troosters, T[4]; Vanbelle, V[5]; Peers, K[6]; Ceuppens, J[2]; Hellings, P[2]; Dupont, L[4]; Bullens, D[7]
[1]KU Leuven, Leuven, Belgium; [2]KU Leuven, Clinical Immunology, Leuven, Belgium; [3]KU Leuven, Academic Center for General Practice, Leuven, Belgium; [4]KU Leuven, Pneumology, Leuven, Belgium; [5]Flemish Swimming Federation, Merelbeke, Belgium; [6]KU Leuven, Sport Medical Advice Center, Leuven, Belgium; [7]KU Leuven, Pediatric Immunology, Leuven, Belgium

Background: Asthma and airway hyperreactivity is more common in elite swimmers compared to other elite athletes and controls. Several cytokines (Th1, Th2, Th17) were reported to be implicated in asthma pathogenesis. The immunological airway pattern of swimmers' asthma still has to be elucidated.

Method: Sputum cytokine mRNA levels (IL-4, IL-5, IL-6, IL-17F, IL-22, IFN-gamma and TNF) and cell counts were measured at baseline and after intensive training in elite swimmers ($n = 38$), indoor athletes ($n = 15$) and controls ($n = 15$). Sputum cytokine-high levels were defined based on normal levels (below 90th percentile) in controls. Eucapnic voluntary hyperventilation test was used to assess exercise-induced bronchoconstriction.

Results: Most swimmers ($n = 18$) and indoor athletes ($n = 10$) had a cytokine-low profile. Thirteen elite swimmers and 5 indoor athletes were identified with a sputum IL-6-high profile. Sputum IL-6 was also significantly increased in both swimmers and controls after intensive swimming. Higher sputum neutrophil percentages were found in indoor athletes and swimmers compared to controls. On top, eight elite swimmers had a sputum 'IL-4-high' profile which was not related to previously diagnosed asthma or atopy. Exercise-induced bronchoconstriction was detected in 11/34 (32%) swimmers compared to 0/13 indoor athletes and 1/11 (9%) controls.

Conclusion: One third of elite swimmers and indoor athletes show a unique sputum molecular pattern characterized by high IL-6 and neutrophil levels. This is probably induced by sustained heavy exercise and not by exposure to by-products of chlorination. On the other hand, one in five elite swimmers show typical Th2-mediated inflammation. This has implications for choosing the optimal treatment for elite swimmers with asthma.

509
Endoplasmic reticulum stress is implicated in the development of allergic airway remodeling via the induction of VEGF expression

Lee, YC; Kim, SR; Park, HJ; Lee, KB
Chonbuk National University Medical School, Jeonju, Korea

Background: Vascular endothelial growth factor (VEGF) is a key player in the tissue fibrosis, especially airway disorders. Recent studides have demonstrated that endoplasmic reticulum (ER) stress has been reported to be implicated in various chronic pulmonary disorders such as chronic obstructive pulmonary disorder (COPD), bronchial asthma, and pulmonary fibrosis. However, little is known concerning the role of ER stress in the pathogenesis of bronchial asthma, particularly airway remodeling.

Method: We used a long-term exposure murine model of allergic airway disease to evaluate the effect of 4-PBA, an ER stress regulator, on hyperresponsiveness, inflammation, and remodeling of the airways.

Results: This study with the chronic model of allergic airway disease revealed the typical pathophysiological features; increased the expression of ER stress markers and the protein levels of unfolded-protein response (UPR)-related markers in lung tissues, increased numbers of airway inflammatory cells, increased plasma exudation, airway hyperresponsiveness, and increased levels of Th2 cytokines, TGF-β1, and VEGF. In addition, the mice chronically exposed to ovalbumin (OVA) developed features of airway remodeling, including thickening of the peribronchial smooth muscle layer, subepithelial collagen deposition, and increased airway mucus production. Administration of 4-PBA reduced the pathophysiological symptoms of asthma including airway remodeling, plasma exudation, Th2 cytokines, TGF-β1,

© 2015 The Authors

and VEGF in lungs as well as the increased expression of ER stress markers and the protein levels of UPR-related markers after OVA inhalation.
Conclusion: These results indicate that inhibition of ER stress may attenuate chronic antigen-induced airway inflammation, hyperresponsiveness, and airway remodeling through the regulation of VEGF.

511
The profile of protease Inhibitors produced by the primary bronchial epithelial cells is a function of the epithelial cell source (healthy vs. asthma) and the type of allergen protease

Karaaslan, C[1]; Birben, E[2]; Kalayci, O[2]
[1]Department of Biology, Molecular Biology Section, Hacettepe University, Ankara, Turkey; [2]Pediatric Allergy and Asthma Unit, Hacettepe University School of Medicine, Ankara, Turkey

Background: We have previously shown that protease inhibitors produced by A549 cells have a wide range of action beyond protease inhibition. The factors that stimulate the secretion of protease inhibitors from primary epithelial cells and the role of allergens in this regard are largely unknown.
Aim: To determine the profile of protease inhibitors that is produced in response to allergen stimulation by bronchial epithelial cells.
Method: Primary bronchial epithelial cells obtained from three asthmatic and three healthy individuals were grown in BEGM, than differentiated in an Air Liquid Interface (ALI) culture for 4 days in B-ALI medium. When a trans-epithelial resistance of >1000 ohm was reached, cells were stimulated with Der p1 (cysteine protease) and Dsp (serine protease) both at 2 μg/ml for 24 h. Global gene expression was determined with Affymetrix U133 Plus2 array and qPCR method was used for confirmation of microarray results.
Results: Summarized in Table 1. Microarray results were confirmed with qPCR (data not shown).
Conclusion: The profile of protease inhibitors produced by primary bronchial epithelial cells in response to protease antigens is a function of the source of the epithelial cells (healthy vs. asthma) and the biochemical property of the protease stimulant.

Table 1: Protease inhibitor genes showing ≥2.5 fold change above unstimulated cells after Derp1 and Dsp stimulation

	Increased		Decreased	
	Der p1	Dsp	Der p1	Dsp
Healthy	None	None	SERPINS: E1, B2, B4, E2, B1, B3, CSTB	SERPINS: E1, B2, B9, B1, B3, B4
Asthma	SERPINS: B13, B3, TFPI2, MBIP1	SERPIN I2, WFDC6, SPINLW1	None	None
Healthy & asthma	SERPIN B3	None	None	None

[Protease inhibitor genes showing ≥2.5 fold change].

512
Evaluation of the lung ventilation in patients with uncontrolled asthma

Iashyna, L; Feshchenko, Y; Opimach, S; Moskalenko, S; Polianska, M
State Organization "National Institute of Phthisiology and Pulmonology named after F.G. Yanovsky National Academy of Medical Sciences of Ukraine", Kiev, Ukraine

Background: Airway obstruction in bronchial asthma (BA) patients can lead to gas exchange abnormalities. This study aimed to investigate lung ventilation pecularities in patients with BA depending on asthma control level.
Method: The study involved 30 BA patients without exacerbation (11 men and 19 women) with mean age (57.3 ± 2.3) years and the average FEV_1 (72.3 ± 1.8) %. Asthma Control Test (ACT) and capnometry (the volume of dead space ventilation (Vde) and its part in the tidal volume (Vde%VT), the volume of alveolar ventilation (Va) estimation) were performed for all participants. Data are presented as mean±SD.
Results: Based on ACT the patients were divided into 2 groups: with controlled (ACT > 20 points, 10 subjects) and uncontrolled (ACT < 20 points, 20 subjects) course of disease. In patients with uncontrolled asthma Vde was (211 ± 8) ml and Vde%VT - (30.5 ± 1.5)%, statistically significant greater than in patients with controlled asthma - (176 ± 11) ml and (24.6 ± 0.5)% respectively, $P < 0.05$. Va in patients with uncontrolled asthma is lower, namely (7.3 ± 0.4) l/min., than in controlled asthma - (8.3 ± 0.2), $P < 0.05$. The value of alveolar ventilation is reduced by 14% in uncontrolled asthma patient where one possible factor may be high waste "dead" space ventilation.
Conclusion: The uncontrolled course of asthma may accompanying with relative alveolar hypoventilation, which can be assessed by capnometry, and so with gas exchange abnormalities compared to controlled asthma.

513
The effect of Der p and $1,25(OH)_2D_3$ on TLR4 and vitamin D receptor expression and the release of IL-4, IL-10 in P815 mast cells

Meng-Rong, L[1]; Ruxia, L[2]
[1]Center for Asthma and Allergy Immunotherapy, The Second Affiliated Hospital & Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China; [2]The Second Affiliated Hospital & Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China

Objectives: To observe the effect of Dermatophagoides pteronyssinus (Der p) and $1,25(OH)_2D_3$ on the expression of TLR4, Vitamin D receptor (VDR) and the release of IL-4, IL-10 in P815 mast cells.
Methods: (1) The expression of TLR4 and VDR on P815 cells was detected by RT-PCR and by Immunofluorescence (IF).

(2) The expression of TLR4 and VDR mRNA and protein in 0.1 μg/ml Der p treated P815 cells for 36 h was detected by Western Blotting and Real-time PCR.

(3) The concentrations of IL-4 and IL-10 in the supernatants were measured by ELISA.

(4) P815 cells were cultured in 6-well cell culture plate for 24 h, then cultured for the next 36 h by dividing into control group, Der p 0.1 μg/ml group, Der p 0.1 μg/ml + $1,25(OH)_2D_3$ 10^{-10}M group, Der p 0.1 μg/ml + $1,25(OH)_2D_3$ 10^{-8}M group.
Results: (1) The expression of TLR4 mRNA and protein in 0.1 μg/ml Der p group was significantly higher than that in the control group. The expression of VDR mRNA and protein was significantly lower than that in the control group ($P < 0.01$).

(2) The expression of TLR4 mRNA and protein in various concentrations of 1,25 $(OH)_2D_3$ treated was no significant difference. The expression of VDR mRNA and protein in 10^{-8}M concentrations of 1,25 $(OH)_2D_3$, there was significantly higher than that in control group ($P < 0.01$).

(3) The expression of TLR4 and VDR mRNA and protein in Der p 0.1 μg/ml + $1,25(OH)_2D_3$ 10^{-8}M group was signifi-

© 2015 The Authors

cantly higher than that in control group ($P < 0.01$).

(4) The concentration of IL-4 in various concentrations of $1,25(OH)_2D_3$ treated was no significant difference. The concentrations of IL-10 in 10^{-8}M and 10^{-10}M concentrations of $1,25(OH)_2D_3$ treated group was significantly higher than that in group control ($P < 0.01$).

(5) The concentrations of IL-4 in Der p treated group 36 h incubation period was significant higher than that in control group ($P < 0.01$), in Der p and 1,25 $(OH)_2D_3$ combined treated group, there was a dose-dependent down-regulation of IL-4 protein.

Conclusions: (1) Der p can direct up regulation the expression of TLR4, down regulation the expression of VDR, and increase the secretion of IL-4 in P815 mast cells.

(2) The effect of $1,25(OH)_2D_3$ on mast cells may be through up regulation the expression of VDR and increase the secretion of IL-10 and inhibition the secretion of IL-4 which caused by Der p.

514
Circulating miRNAs signatures in patients with various asthma phenotypes

Kowalski, ML[1,2]; Makowska, JS[1]; Pawelczyk, M[1]; Wardzynska, A[1]; Piechota-Polanczyk, A[1]; Niedzielska, T[1]
[1]Department of Immunology, Rheumatology and Allergy, Medical University of Łódź, Lodz, Poland; [2]Healthy Aging Reasearch Centre, Medical University of Łódź, Lodz, Poland

Background: MicroRNAs are small, 20 to 25 nucleotide, noncoding RNAs that are expressed in tissues and body fluids. They are regarded as molecules important in controlling inflammatory processes, but little is known about their role in airway inflammation. The aim of the study was to assess selected circulating microRNAs in serum of asthmatic patients and healthy individuals with regard to asthma phenotypes.

Method: The study group consisted of 30 patients with bronchial asthma (mean age 58 ± 2 year) and 10 healthy individuals (mean age 55 ± 5 year). In all patients the following procedures were performed: allergy/asthma questionnaire, spirometry, FeNO measurement, skin prick tests and blood sampling. Nasal swabs were taken for microbiolological culture. Asthma severity was assessed based on ATS 2000 criteria. Serum expression levels were evaluated for miRNA-16, -126a, -29a, -29b, -29c and -21 with Real Time PCR.

Results: We have found no significant differences in the expression pattern of selected miRNAs between asthmatic patients and healthy controls. No changes were also observed between subjects with and without aspirin hypersensitivity. MiRNA-29b was significantly upregulated in subjects with a history of asthma exacerbation within the last year ($P < 0.05$). Expression of miR-126a was significantly upregulated in 6 patients with severe/refractory asthma, when compared with mild model of asthma ($P < 0.05$). Moreover, miRNA-29b was upregulated in subjects with Staphylococcus aureus in nasopharyngeal cultures ($P = 0.05$), and miRNA-126a and miRNA-29a expression levels were upregulated in subjects receiving antileukotriene therapy ($P < 0.05$ and $P < 0.01$ respectively).

Conclusion: The results suggests that expression of some miRNA may be associated with specific asthma phenotypes.

© 2015 The Authors

Poster Discussion Session PDS 24

Diagnosing drug hypersensitivity

515
Anaphylaxis to local anaesthetics: a clinical-laboratory integrated approach

Emma, R; Polosa, R; Amaradio, MD; Caruso, M
Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy

Background: Allergy to local anaesthetics (LA) is considered rare (1–2%), but potentially more serious. After an anaphylactic reaction to LA, correct determination of the responsible agent(s) is essential for the management of patient. Unfortunately, none of the available tests demonstrates absolute diagnostic accuracy, in particular for non-immediate reactions. Thus, novel reliable diagnostic tests would be useful for this purpose. Here we studied subjects reporting adverse reactions to Lidocaine (LID) with a clinical-laboratory integrated approach.
Method: Ten subjects addressed to our centre for unexpected reactions to LID were screened by medical history collection, Skin Prick Test (SPT) and laboratory tests: two Basophils Activation Tests (BATs) by CD63 and CD203c, and a novel Lymphocyte Stimulation Test (LST) by CD69/IL-4. All samples were stimulated by LID in peculiar conditions, marked with a distinctive mix of anti-human Antibodies (CD63/CCR3 for CD63-BAT, CD3/CD203c/CRTH2 for CD203c-BAT and CD4/CD69/IL-4 for LST), and then analysed by Flow Cytometry. Positivity were established using the Stimulation Index (SI), a ratio between the percentage of positive cells in stimulated samples and in Background Controls. When SI≥2 we consider the test as positive.
Results: On 10 subjects, only 5 resulted positive by BATs, of which 2 by CD63/CD203c, one only by CD63 and 2 only by CD203c. Of these positivity only 3 was confirmed by skin prick tests. Instead 4 patients resulted positive only by LST (40% of subjects), of which 2 by CD69/IL-4, 1 only by CD69 and 1 only by IL-4. 1 subject was negative to all tests. None of the LST positive subjects evidenced positivity to SPT.
Conclusion: In conclusion 9 on 10 subjects (90%) obtained a complete diagnosis of sensitisation to LID. The subject negative to all tests probably experienced a non-immunologic reaction. A diagnostic approach based on medical history and that take into consideration more variables can be crucial in the management of anaphylaxis to LA. The BATs could be a good method to screen under safe conditions the suspected sensitisation to LA prior to perform the *in vivo* tests. Moreover subjects who reported a history of NIR evidencing positivity by the LST, and not by BATs nor by SPT, highlighted the hypothesis that this method could fill the gap in the *in vitro* allergy diagnosis.

516
Immediate and non-immediate allergic reaction to sulfonamides in children

Atanaskovic-Markovic, M[1,2]; Medjo, B[1,2]; Radovic, P[1]; Petrovic, M[1]
[1]University Children's Hospital, Beograd, Serbia; [2]Medical Faculty University of Belgrade, Beograd, Serbia

Background: Sulfonamides are among the most common causes of drug allergy. The prevalence of parent or self reported allergy to sulfonamides was found to range from 0.2 to 2.2% for different age groups in children. Sulfonamides rarely cause immediated reactions such as urticaria, angioedema and anaphylaxis and more commonly result in different types of non-immediate reactions such as maculopapular eruption, delayed appearing urticaria, fixed drug eruption (FDE) and Stevens-Johnson syndrome (SJS).

The purpose of this study was to confirm or rule out the diagnosis of allergy to sulfonamides in children.
Method: Of 18 children referred to the Drug Allergy Unit of University Children′s Hospital of Belgrade, between January 2005 and December 2014, 9 children had immediate and non-immediate allergic reactions to sulfonamides. Various clinical reactions were described as being induced by drugs (the number of patients affected is shown in parentheses): urticaria (6), urticaria and angioedema (3), delayed appearing urticaria (3), maculopapular eruption (4), FDE (1) and SJS (1). The reason for the prescription was an upper respiratory or urinary infections. The time period that had elapsed from the occurrence of reaction to the allergologic workup varied from 4 months to 10 years. We perfomed skin tests (prick and early reading intradermal tests (ID) for immediate, and patch tests (PT) and delayed reading ID for non-immediate reaction). Maximum concentration of 0.8 mg/ml for ID of sulfonamides had used. In case of FDE, PT applied to the site of the lesion. In children where skin tests were negative drug provocation test (DPT) were performed. In case of positive skin tests further examinations were interrupted and the children were considered allergic to that drug. Ten children were negative control group which had previously tolerated these drugs.
Results: Out of the total of 18 tested children, 7 (38.88%) were girls and 11 (61.11%) were boys. The ages ranged from 1.5 years to 18 years. Two children had positive family history of allergy to sulfonamides. Only one child had food allergy. We found 15 positive skin tests (9 for immediate and 6 for non-immediate reactions: 4 positive patch tests and 2 positive delayed reading ID tests). We did not search for cross-reactions among sulfonamides.
Conclusion: According to our results, skin tests remains the most sensitive and reliable test to ascertian the diagnosis of hypersensitivity to sulfonamides in children.

517
Selective hypersensitivity reactions to paracetamol

Touati, N[1]; Schrijvers, R[1,2]; Demoly, P[1,3]; Chiriac, AM[1,3]
[1]Allergy Unit, Département de Pneumologie et Addictologie, Hôpital Arnaud de Villeneuve, University Hospital of Montpellier, Montpellier, France; [2]General Internal Medicine, Allergy and Clinical Immunology, University Hospitals Leuven, Leuven, Belgium; [3]Sorbonne Universités, UPMC Paris 06, UMR-S 1136, IPLESP, Equipe EPAR, Paris, France

Background: Paracetamol (acetaminophen) is generally considered a safe medication and is the most used over-the-counter painkiller worldwide. In large series of patients, hypersensitivity reactions to paracetamol are mainly described in cross-intolerant patients with hypersensitivity to acetylsalicylic acid (ASA) and non steroidal anti inflammatory drugs (NSAIDs). However, selective reactions to paracetamol have been described, as both immediate and non immediate hypersensitivity.

© 2015 The Authors

Method: We performed a retrospective analysis of the hospital charts of the patients that underwent an allergy work up for paracetamol, over a 15-year period, in the Allergy Unit of the University Hospital of Montpellier.
Results: The study included 343 patients, accounting for 438 clinical histories involving paracetamol. Almost half of the events involved isolated urticaria/angioedema, followed by maculopapular exanthema (25%) and anaphylaxis (20%). The sex ratio was 1/3 and the mean age was 40.6 years (2–85). Thirty-eight patients (11%) were considered hypersensitive to paracetamol based on a positive drug provocation test. Eight of them were children. There were significantly more immediate reactions and anaphylactic shocks (but not anaphylaxis without shock) in the hypersensitive group ($P < 0.05$), and more maculopapular exanthemas in the tolerant one. The mean eliciting dose was 989 mg paracetamol (1–1911) and half of the patients reacted at rather high doses (> 900 mg).
Conclusion: Our study provides data regarding hypersensitivity to paracetamol. Overall, 90% of the patients describing a reaction after paracetamol intake were not allergic. Over 80% of the hypersensitive patients in our series had selective hypersensitivity to paracetamol and were tolerant to ASA/NSAIDs.

518
The basophil activation test in the diagnostic performance in allergy to neuromuscular blocking agents

Aguiar, R[1]; Lopes, A[1]; Melo, A[2]; Pereira-Barbosa, MA[1]; Santos, MC[2]
[1]Immunoallergology Department, Hospital de Santa Maria- Centro Hospitalar Lisboa Norte, Lisbon, Portugal; [2]Instituto de Medicina Molecular-Faculdade de Medicina da Universidade de Lisboa, Unidade de Imunologia Clínica, Lisbon, Portugal

Background: The incidence of allergic reactions during surgical and medical procedures has increased in recent years. It is estimated that there is an affair between 10,000–20,000 anesthetic procedures and in 6500 administrations of neuromuscular blocking agents (NMBA). The aim of this study was to evaluate the usefulness of BAT in sensitized NMBA patients and to investigate the role in cross-reactivity between them.
Method: We carried out a 5-year prospective study (July 2009–July 2014) that included 15 patients presenting an intraoperative anaphylactic reactionThe patients provided written informed consent. As controls we included 10 volunteers with no remarkable medical history, who underwent surgery and tolerated intravenous NMBA. All patients and controls were submitted to skin prick and intradermal tests according to ENDA/Drug Allergy Interest Group position paper with a battery of muscle relaxants (atracurium, cisatracurium, rocuronium, vecuronium and pancuronium) and Basophil Activation Test (BAT) for the same drugs, performed by Flow2 ® CAST method (Bühlmann) using three concentrations 0.05, 0.5 and 5 μg/ml.
Results: In our study the sensitivity of BAT ranged from 36.1% to 91.7%. 15 patients (pts) were studied with the following Results.

For Cisatracurium, Rocuronium, Pancuronium 13 pts had concordant results between SPT and BAT; 2 pts had no consistent Results.

2 positive SPT and 2 negative BAT. For Atracurium 12 pts had concordant results and 3 pts had positive SPT and negative BAT. For Vecuronium, 12 pts had concordant results and 3 pts had negative SPT and positive results for BAT.
Conclusion: In our study the sensitivity of BAT varied from 36.1% to 91.7%. In patients with proven NMBA anaphylaxis, the BAT sensitivity was primarily 36.1%, which increased to 85.7% when allergies with an onset of less than 3 years were separately considered. The time elapsed between the anaphylaxis and in vitro BAT is a significant parameter for analysing BAT sensitivity.

519
Direct comparison of 3 commercial *in-vitro* assays for anti-penicillin specific IgE

de Boer, D[1]; Bons, JA[1]; Nieuwhof, CM[2]; Menheere, PP[1]
[1]Maastricht University Medical Center, Central Diagnostic Laboratory, Maastricht, Netherlands; [2]Department of Internal Medicine, Immunology and Allergology, Maastricht University Medical Center, Maastricht, Netherlands

Background: Three commercial *in-vitro* assays for anti-penicillin specific IgE (PEN IgE_{spec}) are available (ImmunoCAP, 3gAllergy and Hytec). According to literature only ImmunoCAP assays have been validated by publicly accessible sources. We have studied all the PEN IgE_{spec} assays in order to compare the non-validated with the validated assays.
Method: Information supplied by the manufacturers of PEN IgE_{spec} assays [Phadia, Thermo Fisher Scientific (ImmunoCAP), Siemens Healthcare Diagnostics (3gAllergy) and Hycor Biomedical (Hytec)] were compared in respect to analytical design and specifications. Additionally, the analytical performance was tested by reanalysing 24 serum samples, which were prescreened at least one ImmunoCAP PEN IgE_{spec} assay. Reanalysis was achieved by the penicillin G, penicillin V, ampicillin and amoxicillin IgE_{spec} assays of all 3 manufactures. Total concentration of IgE (IgE_{tot}) was performed by the ImmunoCAP assay.
Results: Based on the information supplied, PEN IgE_{spec} assays proved to differ considerably in design and software lowest reporting levels (ImmunoCAP [0.01 kU/l], 3 g Allergy [0.10 kU/l] and Hytec [0.35 kU/l]) and less in analytical cut-off values (ImmunoCAP [0.10 kU/l], 3 g Allergy [0.10 kU/l] and Hytec [standard 0.35 kU/l; optional 0.10 kU/l]). In respect to the clinical cut-off value of 0.35 kU/l and reanalysis of samples, 3 g Allergy assays showed 22% and Hytec 23% of conflicting results when compared to ImmunoCAP. Additionally, 3gAllergy showed 15% of disagreement when compared to Hytec. According to information in the leaflet of ImmunoCAP, the results of of PEN IgE_{spec} should be interpreted cautiously if IgE_{tot} >500 kU/l. Those of the other manufacturers did not contain such warnings. Two samples had IgE_{tot} >500 kU/l. These 2 samples also had IgE_{spec} >0.35 kU/l after reanalysis and accordingly should have been treated cautiously. The clinical profiles of the respective 2 patients (severe atopic dermatitis) and a systematic review of the literature indicated that the ImmunoCAP results of the samples must have been interfered by specific IgE, IgA and IgG against D-Ala-D-Ala, rather than non-specific IgE.
Conclusion: *In-vitro* assays for PEN IgE_{spec} of ImmunoCAP, 3gAllergy and Hytec differ significantly in analytical design, specifications and performance. When analysing for PEN IgE_{spec}, the simultaneous measurement of IgE_{tot} seems to be required for all commercial assays and should not be limited to ImmunoCAP assays.

520
Analysis of the basophil activation test to quinolones using two different activation markers

Ariza, A[1]; Fernandez, TD[1]; Rodriguez, MJ[1]; Montañez, MI[1]; Guerrero, MA[2]; Blanca, M[2]; Mayorga, C[1]; Torres, MJ[2]
[1]Research Laboratory, IBIMA, Regional University Hospital of Malaga - UMA, Málaga, Spain; [2]Allergy Unit, IBIMA, Regional University Hospital of Malaga - UMA, Málaga, Spain

Background: Quinolones are well-tolerated antibiotics increasingly prescribed. Hypersensitivity reactions as immediate urticaria and anaphylaxis have been reported. These reactions can be evaluated in vitro by basophil activation tests (BAT) although with not optimal sensitivity. Many factors could influence the results among them the

© 2015 The Authors

election of basophil activation markers. The objective of this study was to evaluate the influence of two different activations markers, CD63 and CD203c, in the sensitivity of BAT for the evaluation of immediate allergic reactions to quinolones.

Method: We studied 20 patients with immediate allergic reactions to quinolones. BAT was performed with moxifloxacin and ciprofloxacin at 2 different concentrations (2 and 0.2 mg/ml) using CD193 (CCR3) for basophil selection and CD203c or CD63 as activation markers.

Results: In general, the percentage of activation was lower when using moxifloxacin than ciprofloxacin. Comparing both activations markers, CD63 or CD203c, we found a higher percentage of positive cases using CD63 (64.3%) than using CD203c (28.6%) when ciprofloxacin was used in the test ($P = 0.025$). No differences were found when moxifloxacin was used in BAT (14.3% for both markers). Similar results were found when patients were separated according to the culprit drug in the reaction.

Conclusion: These results indicate that the use of CD63 as basophil activation marker in the evaluation of immediate reactions to quinolones shows a higher or equal sensitivity than the other widely used activation marker, CD203c.

521
Allergological work-up in the suspicion of drug hypersensitivity in patients undergoing allergen-specific immunotherapy

Furlan, C; Brandini, D; Pereira-Aun, V; Aun, WT; Mello, JF; Tanno, LK
Allergy and Clinical Immunology, Hospital Servidor Público Estadual de São Paulo, São Paulo, Brazil

Background: Self-reported drug allergy (DA) is a frequent problem in daily clinical practice and has a considerable impact on prescription choices. Atopy has been described as a risk factor for drug hypersensitivity (DH). The aim of this study was to better investigate the cases of suspicion of DH reported by patients undergoing subcutaneous allergen-specific immunotherapy (SCIT).

Method: In this prospective study conducted since 2013, we firstly evaluated the self-reported cases of DH in patients undergoing SCIT in the Allergy Department of our Hospital. The SCIT has been indicated to atopic patients based on the dust mite sensitization (*Dermatophagoides pteronyssinus* and/or *Blomia tropicalis*) and clinical relevance of these allergens. We excluded cases with unrelated history of DH as well as severe cutaneous drug adverse reactions and used the *European Network on Drug Allergy* (ENDA) diagnostic questionnaire to evaluate the suspected DH. The DA work-up followed the ENDA recommendations.

Results: Of all 1400 patients undergoing SCIT evaluated on May/2013, 691 (49.3%) replied the first questionnaire, 133 (19.2%) self-reported having drug allergies. Of the 133 reports, 65 (49%) were evaluated by ENDA questionnaire and 68 (51%) lost the follow-up or refused to go through the DA evaluation. Forty-five (33.8%) indicated hypersensitivity to antibiotics (ATB), 46 (34.6%) to NSAIDs, 5 (3.7%) to both ATB and NSAIDs, 31 (23.3%) to other drugs and 6 didn't remember the medication involved. Of the 65 evaluated, 42 (64.6%) were women and the mean age was 28 (4 to 70 years). Thirty (46.1%) cases reported immediate reaction and the mean time between the reaction and the evaluation was 10.5 years. Eleven (16.9%) cases were excluded and the DH tests have been offered to 54 (83%) patients, from whom 38 (58.46%) had possible/probable clinical history of DH. Twenty-five (38.46%) cases refused or were not interested in undergoing the DH tests and 12 (18.46%) are still under investigation. Of 17 who completed the investigation, 11 were with NSAIDs and 6 with antibiotics, all negative.

Conclusion: The results of this study confirmed that DH reactions occurred in less than one quarter of patients with a history suggesting possible DA. Negative results on DA work-up may have occurred due to the loss of sensitization and absence of cofactors in the diagnostic procedure. Diagnostics tests in individuals with self-reported DA can exclude these conditions.

522
Positive predictive value of benzyl-penicillin skin testing for beta-lactam allergic patients

Lacombe Barrios, J[1,2]; Salas, M[1]; Gomez, F[1]; Barrionuevo, E[1]; Doña, I[1]; Bogas, G[1]; Herrero, L[1]; Sanchez, MI[1]; Ruiz, MD[1]; Blanca, M[1]; Torres, MJ[1]
[1]Allergy Service, IBIMA-Regional University Hospital of Malaga UMA, Malaga, Spain; [2]Clinical Immunology and Allergy Service, CHU Sainte-Justine, Montreal, Canada

Background: Skin testing with benzyl-penicillin (BP) is sometimes performed alone, when penicilloylpolylysine (PPL) is not available, or together with other penicillin determinants. However, the positive predictive value of BP skin testing determined by drug provocation test (DPT) has not yet been established. The aim of the present study was to determine the diagnostic value of BP skin testing in patients with a consistent clinical history of beta-lactam allergy.

Method: Patients presenting a history of hypersensitivity to beta-lactam were evaluated by prick and intradermal skin tests with PPL (5×10^{-5}), BP (10,000 U/ml), minor determinant mixture (MDM, 2×10^{2}), amoxicillin and clavulanic acid. All cases with negative PPL and MDM, independently of the BP skin test result, underwent a graded drug provocation test (DPT) with BP (600,000 U) followed, if negative, by a 2 day home DPT with penicillin V.

Results: We evaluated 97 patients with a mean age of 41.7 years (15.1 – 84.1) and a suggestive history of mostly immediate (68.0%) hypersensitivity reaction to beta-lactams. The penicillin workup confirmed hypersensitivity for 23 patients: 7 patients were cross-reactive to penicillin, 9 patients were selectively allergic to amoxicilin, 5 patients to clavulanic acid and 2 to cephalosporins. Skin testing with BP was positive for two patients and they both tolerated the DPT. BP skin test sensitivity and specificity were 0.0% [95%CI;0.0–21.9] and 97.5% [95%CI;90.3–99.6] respectively, and positive and negative predictive value were 0.0% [95%CI;0.0–80.2] and 81.1% [95%CI;71.4–88.1] respectively.

Conclusion: In our population the addition of BP skin testing do not increase specificity of the overall workup. Moreover, possible false positive results have to be taken into account in the decision of performing this test.

523
Eyedropa allergy

Vázquez de la Torre Gaspar, M; Blanca-López, N; Pérez-Alzate, D; Garcimartin Galicia, MI; Ruano Pérez, FJ; Somoza Álvarez, ML; Canto Diez, G
Allergy, Hospital Infanta Leonor, Madrid, Spain

Background: Allergic reactions to eyedrops are rare in spite of the extensive use of these drugs by ophthalmologists. The percentage of this kind of adverse drug reactions (ADR) is reported to be less than 6%.

Method: A total of 62 patients with suspect of ADR to eyedrops were evaluated in a period of 6 years (2008–2014). The allergological evaluation included: clinical history, patch tests (PTs) and conjunctival drug provocation test (CDPT). PTs with delayed readings at 48 and 96 h were performed with the culprit eyedrops, alternative eyedrops and, in addition, the preservatives of the drugs. The culprit drugs used were: mydriatics (phenylephrine 100 mg/ml, tropicamide 10 mg/ml, cyclopentolate 10 mg/ml and atropine 10 mg/ml), local anesthetics (tetracaine 1 mg/ml, oxibuprocaine 4 mg/ml), solutions for glaucoma theraphy (brimatro-

© 2015 The Authors

prost 0.3 mg/ml, travoprost 0.4 mg/ml, brinzolamide 10 mg/ml and timolol 5 mg/ml) and fluorescein 20 mg/ml. The preservatives included were: benzalkonium chloride 0.1%, ethylenediamine 1%, phenylmercuric acetate 0.01%, parabens 16%, polymixin B 3% and thimerosal 0.1%. The CDPT was carried out by instilling one drop of the ophthalmic solution in the eye, with readings made at 20 min and 24 h later.

Results: From the total group, 40 patients (64.5%) were finally diagnosed of allergy to eyedrops. In 67.5% several mydriatics were involved in the reaction, and in 17.5% of these, a local anesthetic was also implicated. The clinical history reported by these 40 patients was: blepharoconjunctivitis in 23 cases (57.5%), conjunctivitis in 16 (40%) and cutaneous erythema only in one case. PTs were positive in 32 (80%) patients: 100% to phenylephrine and, in only one subject cyclopentolate in addition to phenylephrine. The CDPT was necessary for the diagnosis in the remaining 8 (20%) patients, being 7 positive to phenylephrine, and one to timolol. Phenylephrine was positive in 95% of patients finally diagnosed of eyedrop allergy. All patients with allergy to phenylephrine tolerated tropicamide, atropine, and cyclopentolate, except one that had a positive PT with cyclopentolate.

Conclusion: Phenylephrine is responsible for most of the allergic reactions induced by eyedrops. PTs are a safe and useful tool for the diagnosis. Tropicamide, atropine and cyclopentolate seems to be good mydriatic alternatives for these patients.

524
Comparison of diagnostic sensitivity of basophil activation test (BAT) and specific IgE in patients with confirmed immediate amoxicilline hypersensitivity

Kosnik, M[1,2]; Silar, M[1]; Kopač, P[1]; Bajrović, N[1]; Eržen, R[1]; Suntar Erjavšek, A[1]; Korošec, P[1]; Zidarn, M[1]; Golnik,[1]
[1]University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia; [2]Medical Faculty, University of Ljubljana, Ljubljana, Slovenia

Background: Because of low sensitivity and specificity of in-vitro tests for penicillin allergy, skin testing (ST) and drug provocation tests (DPT) are the mainstays of the diagnostics of hypersensitivity reactions to those antibiotics. The aim of this study was to compare diagnostic sensitivity of BAT and sIgE against amoxicillin.

Method: BAT and sIgE (ImmunoCAP, Thermo Fisher Scientific, Phadia) to amoxicillin were performed in 17 patients with positive immediate ST (14 patients) or DPT (3 patients) to amoxicillin. BAT was performed by Bühlmann's drug allergens as well as commercially available drugs. BAT was considered positive when stimulation index was >2 and at least 5% of basophils were activated by allergen.

Results: Amoxicilin BAT was positive in 9/17 patients, but only at allergen concentration of 833 μg/ml. Concentration of 83.3 μg/ml newer gave a positive result. One patient was only positive in BAT with commercially available antibiotic. Diagnostic sensitivity was 52.9%. BAT was positive in 1 of three patients with negative ST. Specific IgE were positive in 3 patients (including one BAT negative-skin prick test positive patient). Diagnostic sensitivity was 17.6%.

Conclusion: BAT had much higher diagnostic sensitivity in patients with immediate hypersensitivity to amoxicillin compared to ImmunoCAP sIgE. Further analyses are necessary to identify groups of patients who might benefit from BAT. We hypothesize, that BAT might be used to confirm diagnosis in skin test negative patients with a history of severe immediate reaction to amoxicillin thus obviating the need of DPT.

525
Development of validated method for CYP2D6 phenotyping in studies of correlation between drug allergy and cytochrome P450 activity

Abdrashitov, RK[1]; Egorenkov, EA[1,2]; Smirnov, VV[1,2]; Ramenskaya, GV[1]; Khaitov, MR[2]
[1]Department of Pharmaceutical and Toxicological Chemistry, I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation; [2]National Research Center - Institute of Immunology Federal Medical-Biological Agency of Russia, Moscow, Russian Federation

Background: Generally, drug allergy may be caused either by a drug itself or its metabolite. If allergenic potential of drug depends on its properties, transformation of drug to its allergenic metabolite strictly depends on metabolism activity. Therefore it is important to get information about metabolic activity in order to minimize the possibility of allergic response to drugs. Biotransformation is controlled by several enzymes, but the most active and dominant one is cytochrome P450, which has got a lot of isoforms with their specific role in metabolic process. Since metabolic activity depends not only on genetic factors but also on such phenotypical factors as sex, age, weight, diet, concurrent conditions and drug therapy, phenotyping with probe drugs should be used to determine the up-to-minute catalytic activity of metabolizing enzymes.

One of CYP isoforms, CYP2D6, deals with several tricyclic antidepressants, antipsychotics and beta-blockers, which are known for their drug allergy potential, so this CYP isoform is of interest for this study.

Method: Method is based on simultaneous determination of endogenous pinoline as a CYP2D6 substrate and its metabolite,6-hydroxy-1,2,3,4-tetrahydro-beta-carboline, in urine using LC-MS/MS. Endogenous chemical substances were used as reference substrate in order to protect patients from different adverse drug reactions. Urine was used as a bioliquid in order to minimize invasiveness and discomfort of method for patients.

Results: A quantification method for simultaneous pinoline and its metabolite detection was developed. Method was validated, and its selectivity, linearity, precision and accuracy were proved. Validated analytical range is 50–500 pg/ml for both compunds. Lower limit of detection of pinoline and its metabolite is 50 pg/ml for each. Worked out method was tried out on a number of patients who have been treated with beta-blockers and showed a great result in assay of pinoline and its metabolite and further CYP2D6 activity determination.

Conclusion: We expect to put this method into clinical practice and use it in studies of correlation between drug allergy and cytochrome P450 activity.

526
Negative predictive value of skin tests to iodinated contrast media

Soyyigit, S[1]; Göksel, Ö[2]; Aydın, Ö[1]; Gençtürk, ZB[3]; Erdoğan, BD[3]; Bavbek, S[1]
[1]Department of Immunology and Allergic Diseases, Ankara University, School of Medicine, Ankara, Turkey; [2]Department of Pulmonary Medicine, Asthma and Allergy Unit, Ege University, School of Medicine, İzmir, Turkey; [3]Department of Biostatistics, Ankara University, School of Medicine, Ankara, Turkey

Background: Iodinated contrast media (ICM) causes both immediate and non-immediate type hypersensitivity reactions. Skin tests are useful for the diagnosis of CM allergy, but data about negative predictive value (NPV) of ICM skin tests is limited.

Objective: To determine NPV of skin tests to ICM.

Method: Seventy-three patients with a reliable history of hypersensitivity reactions to ICM were included as patient group. The severity scale of Ring and Messer was used for classification of immediate reactions. Non-immediate reactions were graded as mild, moderate and severe. A total of 136 subjects who never-exposed ICM, and 47 subjects who had tolerated ICM exposure served as control group. All patients and

© 2015 The Authors

controls were tested with the culprit and/or alternative ICM with prick/intradermal and/or patch tests. All subjects were questioned whether they re-exposured/reacted to the skin test negative ICM.

Results: Mean age was 54.5 ± 13 years, F/M ratio was 54/19 in patient group. Fifty-nine patients (80.8%) and 14 (19.2%) had a history of an immediate and non-immediate reaction respectively. The prevalence of Grade 1, 2, 3, and 4 reactions were 31.7%, 35%, 28.3%, and 5% respectively. Most of non-immediate reactions were moderate (53.8%). Skin tests (prick and/or intradermal) were positive in 18.3% of patients with immediate-reaction and 23.1% of patients with non-immediate reaction resulting in 18% sensitivity in patients with immediate and 23% sensitivity in non-immediate reaction. In overall group (n = 256), skin test specificity and sensitivity were 96%, 19%, respectively. Of 236 patients with negative skin tests, 206 were contacted by phone and asked further ICM administration (87.3%). Among them, 158 patients (84.9%) reported subsequent ICM administration either with premedication including antihistamine and systemic steroid in 16 (10.1%), or without premedication in 143 (89.9%). Of 143 patients, only three (1.9%) reacted to ICM with mild non-immediate (itch&maculopapular eruption) in two, and Grade 1 immediate reaction (redness, hypotension) in one case representing a NPV of 98% (95% CI:93–99).

Conclusion: The NPV of skin tests with ICM was very high. None of the reactions in skin-test-negative patients was severe. This result reassures physicians who might hesitate to perform further evaluation with CM in patients with negative skin tests.

527
Is it justified referring patients with history of drug allergy, atopy and atopic disease for testing radio contrast media allergy (RCM)?

Tepetam, FM[1]; Ciftaslan, N[2]; Oruc, O[2]; Colakoglu, B[3]; Keren, M[2]
[1]Sureyyapasa Chest Diseases and Thoracic Surgery Training and Research Hospital, Immunology and Allergfy, Istanbul, Turkey; [2]Sureyyapasa Chest Diseases and Thoracic Surgery Training and Research Hospital, Istanbul, Turkey; [3]Istanbul University, Istanbul Faculty of Medicine, Immunology and Allergfy, Istanbul, Turkey

Background: Hypersensitivity reactions to radio contrast media (RCM) are common in the daily practice. The main risk factor for hypersensitivity reactions is a previous reaction with RCM. Atopy, pollinosis, urticaria, drug allergy, food allergy, female cender, treatment with beta blocking agents, age (20–29 or >55) may also increase the risk of RCM hypersensitivity reactions. In recent years, we have experienced an increase in the referral of patients to an allergy clinic for allergy testing with RCMs, Our aim was to evaluate are these factors at an increased risk of developing allergic reactions to RCMs and the diagnostic value of performing skin tests with RCMs.

Method: The study was conducted among patients referred by physicians to our Immunology and Allergy department, concerned about RCM use and allergy testing with RCM. The participants were evaluated, a standard questionnaire regarding demographic data, history of atopic disease and drug adverse reactions was filled out. Skin prick test (SPT) was performed on the volar forearm with aeroallergens and food allergens and with doctor-recommended RCM or with non iyonic monomers (iohexol, iopamidol, iomeprol) and was read after 20 min. Afterward intradermal test (IDT) was performed with 10-fold diluted RCM. After completion of tests the patients were asked with telephone while any reaction occured.

Results: Total 102 patients (65 women, 37 men; mean age: 55.04) formed the study population. 17 patients were suffering from chronic urticaria, 32 have asthma, 11 have rhinosinusitis, 2 have atopic dermatitis. A total of 19 subjects described intolerance reactions to NSAİDs, 38 antibiotics, 8 RCMs and 7 other drugs. All of this patients SPTs and IDTs were negative. Atopy ratio was 40.19% in the study group; mite allergens were the most common cause of allergy. Only 1 of the 102 phoned patients (0.98%) presented urticaria within 1–2 min after administration of RCM, although prick and intradermal tests were negative. This patient doesnt have atopy, atopic disease and history of drug allergy but only has chronic urticaria.

Conclusion: There isnt any increased risk to perform allergy testing in patients with atopy, pollinosis, urticaria, drug allergy, food allergy, female cender and any age. But reaction can be masculated in patients with history of RCM allergy because of recommended premedication and we can also suggest premedication in patients with history of chronic urticaria.

© 2015 The Authors

Poster Discussion Session PDS 25

Immunotherapy vaccines

528

Improvement in the average adjusted daily symptom score after 12 months of house dust mites subcutaneous immunotherapy: ALUMITES, a randomized, controlled, multicenter, phase IV study

Sánchez, J[1]; Vidal, C[2]; Hernández, D[3]; Pelaez, A[4]; Azpeitia, A[5]; Valero, A[1]
[1]Hospital Clinic Universitari Barcelona, Barcelona, Spain; [2]Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain; [3]IIS Hospital La Fe, Valencia, Spain; [4]Hospital Clinico Universitario de Valencia, Valencia, Spain; [5]Stallergenes Ibérica, S.A., Barcelona, Spain

Background: ALUMITES study was designed to assess 1 year treatment efficacy of house dust mites (HDM: D. pteronyssinus + D. farinae) 10 IR/ml subcutaneous immunotherapy (SCIT) for allergic rhinitis patients.
Method: In this controlled multicenter phase IV study, HDM allergic adult patients were randomized (2:1) to receive 10 IR/ml depot extract plus symptomatic treatment (group A) or symptomatic treatment only (group B). Here we report Average Adjusted daily Symptoms Score (AAdSS) after 12 months of treatment. The total daily score of the symptoms scale was adjusted with the rescue medication (recorded in the patient diary) following the algorithm published (Clin Exp Allergy. 2011;41(9):1282–8). The first day, the total score and the total adjusted score, were equal. If the patient does not take rescue medication on d-1 and day d, the total score and the total adjusted score were equal. If the patient took rescue medication on d, then: Total adjusted score d = max (Total score d, Total adjusted score d -1). Total adjusted score d + 1 = max (Total score d + 1, Total adjusted score time-d). If the total adjusted score was missing, the total adjusted score was also missing. AAdSS of the following 7 days of each visit (recorded in the patient diary) was analysed per patient and for each of the visits.
Results: Forty seven of 57 patients (33 A, 14 B) were evaluated for the AAdSS at 12 months. Baseline AAdSS was 4.0 ± 2.86 in the active group (A) and 4.1 ± 2.76 in the control group (B). AAdSS in active group (A) at final visit was 2.2 ± 2.26 with a reduction of 1.8 ± 3.52 (45%) ($P = 0.0008$). In control group (B) the 12 months value was 3.2 ± 3.28 with a non significative reduction of 0.9 ± 3.08 (22%) (P = NS).
Conclusion: Twelve months treatment with a 10 IR/ml depot HDM extract, leads to significant improvements in the average adjusted daily symptom score taking into consideration both symptoms and rescue medication intake reduction. This improvement was not achieved by the control group.

529

Accelerated and conventional up-dosing with a registered allergoid birch pollen SCIT preparation: a comparison of tolerability between adults and adolescents

Buczyłko, K[1]; van der Werf, JF[2]; Boot, D[2]; van Ree, R[3]; Majorek-Olechowska, BM-O[4]
[1]NZOZ Centrum Alergologii, ŁÓDŹ, Poland; [2]HAL Allergy, Leiden, Netherlands; [3]Department of Experimental Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; [4]Alergo-Med Specjalistyczna Przychodnia Lekarska Sp. z o.o., Tarnow, Poland

Background: Recently we compared accelerated (A-reg.) and conventional (C-reg.) up-dosing regimens with an allergoid birch pollen SCIT preparation. The A-reg. was found to be non-inferior thus providing a safe option to up-dose patients within 2 weeks. In the study both adults and adolescents were included, below an overview of all safety parameters will be given, comparing both populations.
Method: In birch pollen allergic rhinitis/rhinoconjunctivitis patients with or without mild asthma (FEV1 > 70%) an A-reg. (0.1-0.3-0.5 ml at weekly intervals) was compared to the C-reg. (0.05-0.1-0.2-0.3-0.4-0.5 ml at weekly intervals) using a glutaraldehyde-modified birch pollen preparation adsorbed to aluminium hydroxide. After up-dosing 3 maintenance doses (0.5 ml) were given. The multi-centre study was performed according to an open randomized, parallel group design. Early and late (within 30 min and within 24 hrs after injection) local (ELRs, LLRs) and systemic reactions (ESRs, LSRs) as well as general adverse events (AEs) were recorded. Vital signs were also monitored.
Results: A total of 123 birch pollen allergic patients were randomized to either the C-reg. (62 patients, 41 adults) or A-reg. (61 patients, 40 adults). The majority of the subjects did not show ELRs, only wheals ≤ 5 cm diameter were found with similar frequencies for both age groups. Regarding LLRs, 24.4% ($n = 30$) of the subjects reported reactions with wheals ≤ 5 cm and 16.3% ($n = 20$) with wheals > 5 cm ≤ 12 cm. One reaction with wheal > 12 cm was observed in the adult group (C-reg.). For the LLRs no consistent differences between age groups were found. Concerning ESRs the vast majority of subjects experienced no symptoms or non-specific symptoms (grade 0). None of the adolescents and only one adult patient had an ESR grade I. LSRs of grade I occurred in 3.3 (C-reg.) to 6.5% (A-reg.) of the subjects. No consistent differences between the adult and adolescent groups were found. 146 treatment-emergent (TE) AEs related to the medication were reported. The percentage of patients with a related TEAE appears somewhat lower in the adolescent compared to the adult population.
Conclusion: In general good tolerability was observed for both A-reg. and C-reg. and only small differences in the frequencies of local and systemic reactions and general AEs between the two age groups were observed. In both treatment groups there is a tendency towards better tolerance by the adolescent patients as compared to the adults.

530

Persistence of anti-Bet v 1 IgG4 prior and during a second pollen season after AllerT ultra-fast immunotherapy (phase IIb follow up)

Reymond, C[1]; Kettner, A[1]; Boand, V[1]; Duc, S[1]; Della Corte, G[1]; Spertini, F[2]
[1]Anergis SA, Epalinges, Switzerland; [2]CHUV, Division of Immunology and Allergy, Lausanne, Switzerland

Background: Allergic subjects receiving 5 subcutaneous injections over 2 month of AllerT™, a mix of three Contiguous Overlapping Peptides (COPs) derived from Bet v 1, showed an improvement in rhinoconjunctivitis symptoms and medication scores during the first and second birch pollen

© 2015 The Authors

seasons. The level of immunoglobulins against either AllerT or the Bet v 1 allergen was measured during the second pollen season.

Method: Blood was collected before, at peak and after the 2014 birch pollen season. Bet v 1 specific as well as AllerT specific IgG4 and IgE were quantified by ELISA.

Results: Upon AllerT administration, Bet v 1 specific IgG4 had been previously shown to increase by about 20 fold over placebo and to remain elevated during the first birch pollen season. Specific IgG4 remained significantly elevated compared to placebo and rose during the second pollen season reaching about 4–5 fold pre-treatment level. AllerT specific IgG4 peaked after treatment to decrease back to basal level in most subjects already within the first pollen season. Except in a few subjects, AllerT specific IgG4 remained below detection limit before and during the second season. AllerT specific IgE correlated with IgG4 levels. No significant difference was observed in Bet v 1 specific IgE levels between placebo and treated groups.

Conclusion: A single course of 2 month immunotherapy with AllerT induced an IgG4 response against Bet v 1 which remained significantly elevated before and after the second pollen season compared to both pre-treatment and placebo levels. These results are in line with previous phase I/IIa immunological results after two and four birch seasons. AllerT specific IgE and IgG4 were only transiently elevated in most subjects as a consequence of AllerT administration. Efficacy in symptom reduction during first and second pollen seasons, as well as persistent IgG4 responses, indicate a long term effect of AllerT treatment.

531
Aluminum pharmacokinetics in serum and urine following 1 year high dose SCIT with a depigmented polymerized birch pollen extract

Sager, A
Medical Department, LETI Pharma GmbH, Witten, Germany

Background: AL(OH) is widely used as adjuvant in subcutaneous immunotherapy. As part of the development programme of a high-dose depigmented polymerized birch pollen extract PK investigation of Aluminum was required by the EMA.

Method: In a randomized placebo-controlled multi-national multicenter study 48 patients (mean age 41.3 years) underwent PK analysis. Patients with allergic rhinoconjunctivitis with/without asthma were treated with 0.5 ml depigmented polymerized birch extract (5000 DPP/ml) or matching placebo every 4–6 weeks for 1 year. Each injection contained 1.5 mg AL(OH). PK of Aluminum was analysed using flame atom absorption spectrometry (FAAS) before and after the first application -after 1 month and after 1 year of treatment in serum before and 1, 2 4 and 8 hrs after injection as well as cumulative over 24 h in urine.

Results: At baseline the mean AL serum level was 1.98 µg/l (SD 1.17) and in urine 9.69 µg/l (SD 8.46). After 1 month AL serum level did not increase except for 1 patient who developed after 4 hrs an isolated increase of serum Al of 278.44 µg/l (no serum increase after 2 and 8 h: 1.62 µg/l and 9.43 µg/l in 24 h urine). This increase was considered as external contamination. Urine AL levels increased by 5.8%. After 1 year there was a slight increase in serum AL level to 2.14 µg/l (SD1.25) after 4 hrs and 13.99 µg/l (SD7.39) in 24 h urine. Both values remained clinically irrelevant since upper limits of normal ranges were 7.5 µ/l and 60 µg/l for serum or urine respectively. Results are presented in a blinded way since active and placebo treatment contains the same amount of Al(OH).

Conclusion: During 1 year of AL(OH) adsorbed SCIT with a high-dose depigmented polymerized birch pollen extract there was no clinically relevant increase in Aluminum level in serum or cumulative in 24 h urine compared to baseline.

532
Pre-clinical study on the use of micro crystalline tyrosine (MCT) adjuvants in allergy immunotherapy

Weiss, S[1]; Johansen, P[1]; Heath, MD[2]; Kramer, MF[2]; Skinner, MA[2]; Kündig, T[1]
[1]Department of Dermatology, University of Zurich Hospital, Zurich, Switzerland; [2]Allergy Therapeutics Plc, Worthing, United Kingdom

Background: Vaccines and allergen-specific immunotherapy typically contain adjuvants that facilitate immune responses in humans and animals. For almost a century, salts of aluminium (hydroxide and phosphate) were the only approved adjuvants in humans. One major problem of aluminium adjuvants is that they are not biodegradable and that they typically stimulate so-called T-helper type 2 (Th2) as opposed to Th1 immune responses, which again affects the type of antibody responses produced. The goals of new adjuvants are therefore (i) to facilitate recognition of antigen/allergen, (ii) to be biodegradable and biocompatible, (iii) to be without toxic or inflammatory side effects, and (iv) to trigger protective Th1-like immune responses as well as allergen-neutralising antibodies.

Co-precipitates of micro crystalline tyrosine (MCT) and proteins have been suggested as candidate adjuvants for allergen-specific immunotherapy.

Method: Immunogenicity testing of MCT-ovalbumin vaccines in naïve BALB/c and C57BL/6 mice was undertaken. Three injections were performed at 2 week intervals, and the mice were tail bled prior to each injection as well as at different time points after the last injection. The obtained sera were analysed for OVA-specific antibodies, while spleen cells were tested for T-cell responses including cytokine secretion after re-stimulation of the cells in vitro with ovalbumin.

Results: *MCT* has good adjuvant properties, comprising a high adsorptive power for proteins, and enhancement of Th1-like and associated immune responses, highlighting its potential of action as a biodegradable depot adjuvant in allergen-specific immunotherapy. MCT is naturally metabolised and the pharmacokinetics of MCT present a half-life at the injection site of 48 h; this is a particular benefit for allergy SCIT, a traditionally long course treatment, minimising the need for accumulation of non-biodegradable adjuvant.

Conclusion: Results from pre-clinical immunogenicity and MCT mode-of-action studies in mice have demonstrated the potential of MCT as a non-toxic and biodegradable alternative to conventional adjuvants for use in allergen immunotherapy.

533
Experimental sublingual allergen immunotherapy with monomeric allergoid in a murine model of allergic rhinitis

Laskin, AA; Babakhin, AA; Kamishnikov, OY; Chuvirov, DG; Andreev, SM; Khaitov, MR
National Research Center - Institute of Immunology of Federal Medico-Biology Agency of Russia, Moscow, Russian Federation

Background: The purpose of this study was to investigate the efficacy of sublingual allergen immunotherapy (SLIT) with monomeric allergoid obtained by succinylation of ovalbumin (OVA) in a mouse model of allergic rhinitis (MAR).

Method: To obtain monomeric allergoid OVA was modified with succinylation (98.9% modification). BALB/c mice were i.p. immunized three times in 1 week interval with OVA (25 µg/mouse) together with $Al(OH)_3$. 4 weeks after the last immunization mice were challenged by intranasal applications (INA) with OVA (10 µl into

© 2015 The Authors

each nostril of 25 g/ml OVA solution) during 5 consecutive days and then 7 times every other day. Mice were divided into 4 groups: group 1 was immunized with OVA, challenged by INA and received sham SLIT with PBS; group 2 was immunized and challenged with the same doses of OVA and received SLIT in increasing doses of OVA (10, 100, 1000, 2000 μg/mouse) for 4 consecutive days with 1 day interval between doses; group 3 was immunized and challenged with the same doses of OVA and received SLIT in increasing doses of sOVA (500, 1000, 2000 μg/mouse) 2 times per dose in 3 day interval; group 4 was sham immunized and challenged with PBS (negative control).

After the last challenge sneezing were counted. 24 h after the challenge airway hyperresponsiveness (AHR) to methacholine was measured by whole-body plethysmography. Nasal tissues were removed for histological examination. Serum anti-OVA IgE, IgG1 and IgG2a antibodies before SLIT and after the challenge were measured by ELISA.

Results: Mice in group 1 demonstrated maximal count of sneezing after the last challenge that was significantly higher than that of other groups received SLIT. There was no significant difference in the levels of OVA-specific IgE among groups 1–3. Serum levels of OVA-specific IgG1 were similar in groups 1–3 while OVA-specific IgG2a antibody level in group 2 was a little bit lower than that of group 3 which received SLIT with monomeric allergoid sOVA. There was no difference in AHR in groups 1–4. Histological picture of allergic inflammation in nasal cavity (mucus hyperplasia, infiltration with eosinophils and lymphocytes, hyperemia, secretory activity, exudative reaction) was improved significantly in group 3 compared to groups 1, 2.

Conclusion: These data indicate that SLIT with monomeric allergoid more effective in compare to SLIT with non-modifed allergen that may be safe and effective approach for allergen immunotherapy of allergic diseases.

534
Immunological adequacy of the mix 5 grasses for polcalcin and profilin

Scala, E[1]; Frati, F[2]; Buttafava, S[2]; Incorvaia, C[3]
[1]Experimental Allergy Unit, IDI-IRCCS, Roma, Italy; [2]Medical and Scientific Department, Stallergenes Italia, Milan, Italy; [3]Allergy/Pulmonary Rehabilitation, ICP Hospital, Milan, Italy

Background: The molecular allergy diagnostic technique allows the detection of single allergen molecules and improves the understanding of patients sensitization profiles. We investigated the presence of immunoreactive molecules in raw immunotherapy grasses extracts by means of an inhibition assay.

Method: For the assay, sera were obtained from Italian patients with grass pollen-induced rhinoconjunctivitis, requiring antihistamines and local corticosteroid treatment during the pollen season. In all cases sera were immunoreactive with all grasses molecules present on the microarray. Sera from each patients was preincubated with undiluted commercial extract of a mixture of *P. pratense*, *Dactylis glomerata*, *Anthoxanthum odoratum*, *Lolium perenne*, and *Poa pratensis*, prior to assaying grasses molecules-reactivity in the ImmunoCAP-ISAC platform. The pollen extract was from Stallergènes SA (Antony, France). After incubation, sera were tested for IgE binding inhibition to Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11, and Phl p 12, using the ImmunoCAP-ISAC 112 microarray.

Results: The 5-grass-pollen mixture extract totally inhibited the IgE binding of genuine molecules from *P. pratense* (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 11), and also of polcalcin (Phl p 7) and profilin (Phl p 12).

Conclusion: This inhibition experiment can be considered as an indirect proof that 5-grass-pollen extract contains all genuine and panallergen molecules thus far available for molecular allergy diagnosis, indicating its usefulness in grass pollen specific immunotherapy by working on all kind of grass molecule reactivities.

535
Characterization and production of *Alternaria alternata* polymerized extracts

Abel-Fernández, E[1]; Hernández Arbeiza, J[2]; Nieto Cid, M[3]; Nieto García, A[3]; Casanovas, M[4]; Fernández-Caldas, E[4]
[1]Universidad Complutense de Madrid, Madrid, Spain; [2]Hospital Nuestra Señora de la Montaña/Complejo Hospitalario de Cáceres, Cáceres, Spain; [3]Hospital Universitari i Politècnic La Fe, Valencia, Spain; [4]Inmunotek S.L, Alcalá de Henares, Spain

Background: The production of allergenic extracts modified with glutaraldehyde has supposed an advance in the treatment of allergenic diseases. Polymerization is a strategy to decrease the allergenicity of the extracts. This process has been successfully used with pollen and mite extracts, but has not been well investigated in molds. The aim of the present study was to polymerize and characterize allergen extracts of *Alternaria alternata*.

Method: Different raw material sources were evaluated previous to polymerization, distinguishing between metabolic or somatic phases. Two allergenic *A. alternata* extracts were selected and treated with glutaraldehyde. Polymerization was evaluated by SDS-PAGE, Gas Chromatography and free primary amines determination with fluorescamine (Fluram). The IgE and IgG binding capacity of the polymer was studied by Immunoblot, ELISA and ELISA competition, using sera from allergenic patients. The allergenic composition was analyzed by mass-spectrometry (MALDI-ToF-ToF) and the major allergen content, Alt a 1, was quantified by ELISA.

Results: SDS-PAGE and immunodetection assays revealed the presence of high molecular weight bands and an absence of less than 100 kDa allergens. Gas chromatography and the fluorescamine reaction revealed a diminution of free lysine (78%) and arginine (47%) residues and free primary amines (90.6%) in the polymer. Competition assays showed decreased IgE, but mantained IgG binding capacity of the polymer when compared with the native extract. Mass-spectrometry confirmed the presence of allergenic and non-allergenic proteins.

Conclusion: Obtaining safe and effective mold allergoids is important for mold immunotherapy. SDS-PAGE, Immunodetection, Gas Chromatography and Fluram results suggest that the *A. alternata* proteins have polymerized. *A. alternata* allergoids show a decreased biological activity but maintained immunogenicity, when compared to the native extract.

536
Development and validation of a rabbit specific IgG test to evaluate the biological activity of allergoids

Tudela, JI[1]; Fernandez-Caldas, E[1]; Subiza, JL[1]; Casanovas, M[2]
[1]R&D, Inmunotek SL, Alcala de Henares, Spain; [2]Medical Department, Inmunotek SL, Alcala de Henares, Spain

Background: Industrial Establishments Regulation for modified allergen preparations states that antibody-based assays, or other appropriate test methods, have to be established to identify the relevant allergens in the modified form. These assays could be used to demonstrate consistency of the production process. These assays can be used to demonstrate specificity of the products and demonstrate that the allergoids contain all the relevant allergens.

Method: Production of antisera: New Zealand white rabbits were immunized with an In House Reference Preparation of P. pratense allergoids. Rabbit anti-allergoid specific IgG titers were periodically checked until reaching an appropriate

© 2015 The Authors

level. ELISA competition: Rabbit serum specific IgG polyclonal antibodies were titrated to develop and optimize an ELISA competition system. Specificity and cross-reactivity between *P. pratense*, *B. verrucosa* and *D. pteronyssinus* was also evaluated by ELISA competition.

Immunoblot inhibition: the assay was developed using native allergen extracts in the gel and rabbit *P. pratense* anti allergoid anti-serum. Immunoblot was conducted by inhibiting the rabbit antiserum with *P. pratense* allergoids prior to the incubation with the membrane containing a native extract.

Once the assays were developed, they were validated.

Results: The assay was specific for P. pratense, since minimal inhibition was obtained with D. pteronyssinus and B. verrucosa. The 50% inhibition mean value obtained for one batch repeated 10 times was 0.191 μg/well, ranging from 0.143 to 0.350; 50%: 0.095; 200%: 0.382. The mean value obtained for 6 different batches was 0.142 μg ranging from 0.092 to 0.215; 50%: 0.071; 200%: 0.284. We have been able to demonstrate the presence of Phl p 1 and Phl p 5 in several batches of *P. pratense* allergoid by Immunoblot inhibition. The degree of inhibition is consistent and can be measured objectively using scanning densitometry. We have obtained a mean % reduction of 93 ± 1.97 for Phl p 1 and of 84.32 ± 2.16 for Phl p 5.

Conclusion: We have developed several ELISA and Immunoblot assays using rabbit serum containing high specific IgG titers against allergoids of *P. pratense*. These assays are species specific and can be used to monitor the IgG biological activity and the presence of specific allergens in the allergoids.

537
Low-dose interleukin 2 induces regulatory T-cell mediated control of food allergy in mice

Bonnet, B[1,2]; Vigneron, J[1]; Levacher, B[1]; Vazquez, T[1]; Pitoiset, F[1]; Brimaud, F[1]; Klatzmann, D[1,2]; Bellier, B[1,2]
[1]UPMC INSERM UMRS959, Laboratory i3, Bat CERVI, Paris, France; [2]Department of Biotherapies and the Clinical Investigation Center in Biotherapy, AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

Background: Regulatory T cells (Tregs) are pivotal for maintenance of immune self-tolerance, and evidence also suggests that they are important for promoting immune homeostasis following response to exogenous antigens, including allergens. We sought to evaluate the role of low-dose of interleukin 2 (ld-IL2), which has been shown to induce selective expansion of Tregs and promote the reestablishment of immune tolerance, for allergy treatment.

Method: Mice were daily injected with ld-IL2 (50,000 UI) during 5 days before or after allergy induction mediated by OVA intraperitoneal sensitization and repetitive oral challenges. Efficacy of preventive or therapeutic treatment was evaluated by monitoring the severity of allergic responses as a clinical score and measuring the specific immune responses (cell immunophenotyping and cytokine assays).

Results: Ld-IL2 induces specific but transient systemic (i.e. spleen) and local (i.e. in mesenteric lymph node and Peyer's Patches) Treg expansion. In preventive or therapeutic treatment, ld-IL2 elicits long-term protection against clinical manifestations of food allergy in mice since more than 70% of treated mice were protected over a 7- or 3-month period with repetitive exposure to allergens, respectively. We showed that this clinical effect is Treg mediated since Treg-depleted mice cannot be protected from allergic symptoms by ld-IL2. Moreover, the ld-IL2 clinical effect on allergic symptom can be correlated (i) with a local alteration of Th1/Th2 balance, due to the IFN-γ secreting cell number increase, (ii) an expansion of IL-10 secreting cell numbers in Peyer's Patches and (iii) an inhibition of mast cell degranulation revealed by low serum levels of MCPT-1.

Conclusion: Altogether, we demonstrate for the first time that ld-IL2 is efficient to prevent and to treat allergic immune responses and thus represents a promising alternative treatment strategy for managing allergic diseases.

© 2015 The Authors

Poster Discussion Session PDS 26

Allergy epidemiology - allergens and environment

538

Olive grove cultivation surface and allergic disease severity in patients with seasonal allergic rhinitis and positive skin prick test to both grass and olive pollens. Results of the GRAMOLE study

Dávila, I[1]; Iglesias-Cadarso, A[2]; Moreno-Ancillo, A[3]; Moreno, C[4]; Quiralte, J[5]; Azpeitia, A[6]
[1]Hospital Clínico, Salamanca, Spain; [2]Hospital Puerta de Hierro-Majadahonda, Madrid, Spain; [3]Hospital Nuestra Señora del Prado, Talavera de la Reina, Spain; [4]Hospital Reina Sofía, Córdoba, Spain; [5]Hospital Vírgen del Rocío, Sevilla, Spain; [6]Stallergenes Ibérica, S.A., Barcelona, Spain

Background: Grass and olive tree pollens have overlapping pollination periods in Spain. Identification of the etiological agent in patients sensitized to both allergens is difficult if only skin-prick tests (SPT) are used. The GRAMOLE study aim is to determine major allergens sensitization to grass (Phl p 1 + 5) and olive tree (Ole e 1) pollens in patients with seasonal allergic rhinitis (SAR) and positive SPTs to both pollens (Allergy 2014;69(10):1357–63). In addition the olive grove (OG) surface of the province of residence was considered. We have evaluated the relationship between the olive grove cultivation surface and allergic disease severity.
Method: Patients, aged 5–65 years, with SAR clinically associated to grass and olive pollinosis and positive skin SPTs to both pollens were recruited. Study endpoint was the serum determination of specific-IgE levels to Ole e 1 and Phl p 1 + 5. Patients were divided in 5 areas accordingly to the province surface dedicated to OG cultivation (< 1%; 1–5%; 6–10%; 11–20%; >20%); in any case a minimum of 1000 Ha of province area should have been dedicated to OG cultivation. The relationship between OG cultivation surface area and allergic disease severity (asthma prevalence, asthma severity, rhinitis severity -modified ARIA classification by Valero et al-, allergic conjunctivitis and number of sensitizations) were evaluated.
Results: Of 1263 patients were recruited by 88 allergists. Mean age was 28.8 ± 12.3 years and 50.8% were males. Intermittent allergic rhinitis was present in 21.3% of patients (15.1% mild, 6% moderate, 0.2% severe) whereas persistent rhinitis was present in 78.7% (28.7% mild; 42.9% moderate; 7.1% severe) A total of 59% of patients had asthma and 90.4% conjunctivitis. For a sIgE cut-off ≥ 0.35 kU/l, In vitro sensitization only to Phl p 1 + 5 was 14%; only to Ole e 1 was 12%; to both Phl p 1 + 5 and Ole e 1 was 71% were positive and and 3% of patients were negative to both of them. There was an increase in asthma prevalence (from 50% to 75%) and severity (17.4% to 40.9% moderate persistent asthma), in rhinitis severity (41.3% to 52.2% moderate to severe persistent), and in the prevalence of conjunctivitis (89.4% to 95.9%) in relation to the surface dedicated to OG cultivation.
Conclusion: We observed a relationship of the olive growth cultivation and asthma prevalence and severity, the severity of rhinitis and the prevalence of conjunctivitis. This could be related to olive pollen exposure.

539

A 6-year study on the changes in pollen counts and sensitization rates in South Korea

Kim, YJ[1]; Park, HJ[1]; Park, KH[1]; Lee, J-H[1]; Park, J-W[1]; Hong, C-S[1]; Kim, HR[2]; Han, MJ[2]; Choe, H[2]; Oh, J-W[3]
[1]Severance Hospital, Internal Medicine, Seoul, Korea; [2]National Institute of Meteorological Research, Jeju, Korea; [3]Department of Pediatrics, Hanyang University College of Medicine, Guri, Korea

Background: The amount of airborne pollen is considered to be related to sensitization rates. The sensitization rates to tree pollen significantly increased and those to grass and weed pollen decreased over the last 30 years in South Korea. We evaluated short term changes of sensitization rates and the correlation with pollen counts.
Method: We assessed the number of pollen grains collected in Seoul. Tree pollen of the 15 species, weed pollen of the 8 species and grass were studied from January 1, 2007 to December 31, 2013. We retrospectively reviewed the results of 4442 skin-prick tests (SPTs) to 33 common inhalant allergens conducted in the Severance Hospital allergy clinics in Seoul from January 1, 2008 to December 31, 2013.
Results: The pollen counts of tree, especially pine and corylus, and grass significantly decreased, as years go by. SPT results showed that the skin reactivity to grass significantly increased from 6.9% in 2008 to 14.0% in 2013. The sensitization rates to tree and weed pollen had no changes. In the details, the sensitization rates to Walnut, Popular, Bermuda, Timothy and Dandelion significantly increased. There was significant correlation between rate of change in pollen counts and sensitization rates to oak and hop J (*Humulus japonicus*). However, the others showed no correlation.
Conclusion: Decrease tendency of pollen counts was observed in Seoul, over the 7 years. Some species showed significant correlation between pollen counts and sensitization rates over the 6 years.

540

Exposure and sensitization to dust mites in Peruvian cities

Calderón, O[1]; Uriarte, S[2]; Sastre, J[2]; Quirce, S[1]; Iraola, V[3]
[1]Allergy Department, Hospital Universitario La Paz Institute for Health Research (IdiPAZ), Madrid, Spain; [2]Allergy Department, Fundación Jiménez Díaz, Madrid, Spain; [3]LETI Laboratories, Madrid, Spain

Background: Knowledge of the prevalence of different species of dust mites according geographical areas is important to support the diagnostic and specific treatment of allergic diseases that dust mites trigger.
Method: Dust samples were collected from mattresses in peruvian cities grouped by regions; coast (Chiclayo, Trujillo, Chimbote, Lima, Tacna), sierra (Cajamarca, Cusco, Arequipa), Jungle (Puerto Maldonado), using an adapted vacuum cleaner. After collection samples were frozen for 48 h. Mites were extracted from 10 mg of each sample, under stereo microscope, by suspension method with saline solution. Mites were identified by optical microscope. We performed skin prick test (SPT) with standardized mites extracts (ALK-Abelló, Leti and Inmunotek Laboratories; Spain) to subjects who lived in the homes where dust samples were taken.
Results: Presence of dust mites was confirmed in almost 90% of samples collected. The predominant species was *Dermatophagoides pteronyssinus* in cities of peruvian coast, followed by *D. farinae*, *Euroglyphus maynei* and *Blomia tropicalis*. In the jungle, we identified 2 not previously cited species as *Malayoglyphus intermedius*

© 2015 The Authors

and *Tarsonemus* sp, being this last the predominant specie. Regarding SPT, most of the participants were positive to *D. pteronysssinus*, followed by *D. farinae*, *B. tropicalis*, *E. maynei* and *T. putrescentiae*, with different percentages by geographic areas.

Conclusions: To our knowledge this is the first study on mite count in dust and pattern of sensitization performed in Perú. The acarologic fauna and sensitization profile show different percentages depending on geographic areas. *D. pteronyssinus* is the dominant species in the coast, and *Tarsonemus* sp. in the jungle, though other species can be considered important. Further studies about mite species and sensitization profile (SPT, specific IgE) will elucidate the clinical importance of these findings.

541

Does the infant dietary pattern influence the development of allergy in early childhood?

Gandhi, M[1]; Bukuku, C[1]; Rotiroti, G[1]; Grimshaw, K[2]
[1]Child Health, Royal Free Hospital NHS Foundation Trust, London, United Kingdom; [2]Clinical and Experimental Sciences Academic Unit, University of Southampton Faculty of Medicine, Southampton, United Kingdom

Background: Worldwide allergic disease in children is rising exponentially and epidemiological studies indicate environmental changes, particularly those associated with affluence and western lifestyles, including diet, are increasingly important. Little information is available about the impact of the modern infant dietary patterns on early childhood allergies.

Emerging studies indicate the benefits of a traditional diet, high in fresh fruit, vegetables and home cooked foods. Additionally, increasing attention is being given to the way in which food is prepared and consumed, its impact on the gut micro biome and nutrient intake, all of which may influence allergy outcomes.

Method: Fifty- two children aged between 18 to 36 months, diagnosed with allergy ($n = 26$) or having no atopic disease ($n = 26$) were recruited from a tertiary level paediatric service. A screening questionnaire was used to collect patient demographics, details on allergic disease and potential confounding variables such as type and duration of milk feeding (exclusive breast, formula, mixed), age at weaning (<4 months, 4–6 months, >6 months), atopic risk and maternal smoking. Detailed infant data was collected using validated food frequency questionnaires for infant diet at 6 and 12 months of age.

Questionnaires were analysed and given an arbitrary score for pre-determined dietary pattern characteristics. These were processed, guidelines (fresh and home cooked foods), Mediterranean and fast food patterns.

Results: The diets of all the children included in the study were broadly similar for breast feeding initiation, duration, exclusivity and age at solid introduction. Dietary patterns were also similar although the predominant dietary pattern at each age and how the diets changed over time differed between cases and controls. There were however, no significant differences or risk associated with any particular feeding practice or dietary pattern in this group of children.

Conclusion: This data differs from other recent data on how dietary patterns may affect allergy development. In order to provide clear and consistent infant feeding advice, the impact of modern infant dietary patterns in addition to considering the age of solid introduction requires further study.

542

Levels of exposure to house dust mite (HDM) and presence of eczema affect HDM sensitization in early childhood

Shimojo, N[1]; Yamaide, F[1]; Saito, A[2]; Fukutomi, Y[2]; Nishioka, K[2]; Mashimo, Y[3]; Hata, A[3]; Suzuki, Y[4]; Sato, Y[5]; Yamamoto, T[1]; Inoue, Y[1]; Arima, T[1]; Nakano, T[1]; Kohno, Y[6]
[1]Pediatrics, Graduate School of Medicine Chiba University, Chiba, Japan; [2]Clinical Research Center for Allergy and Rheumatology, National Hospital Organization Sagamihara National Hospital, Sagamihara, Japan; [3]Department of Public Health, Graduate School of Medicine, Chiba University, Chiba, Japan; [4]Department of Education and Training, Genetic Epidemiology Research Support, Tohoku Medical Megabank Organization, Tohoku University, Sendai, Japan; [5]Clinical Research Center, Chiba University Hospital, Chiba, Japan; [6]Chiba Rosai Hospital, Chiba, Japan

Background and purpose: Allergic sensitization is one of strong risk factors for the development of allergic diseases. It was reported that certain levels of exposure to house dust mite (HDM) allergen is related to sensitization. Recent studies also suggest eczema may enhance allergen sensitization. We aimed to elucidate relationship among levels of HDM exposure, skin condition, and HDM sensitization in early infancy.

Subjects and Method: CHIBA (Chiba HIgh risk Birth cohort for Allergy) study is a longitudinal birth cohort study of 269 infants with family history of atopy in Chiba city, Japan to examine the relationship of genetic and environmental factors to the subsequent development of atopic/allergic diseases in childhood. HDM allergens and LPS in bedclothes were measured at 3 and 12 months of age and HDM-specific IgE (ImmunoCAP) was measured at 12 and 24 months. Eczema was evaluated by physical examination at 6 months of age.

Results: Rate of HDM sensitization (ImmnuoCAP class 2 or more) was 6.7% at 12 months of age and 25.0% at 24 months of age, respectively. Levels of Der p 1 and Der f 1 concentrations in bedclothes at 3 month of age but not that at 12 months of age was related with HDM sensitization at 12 and 24 months of age. Compared to infants without eczema at 6 months of age, those with eczema required lower HDM concentration to be sensitized with HDM at 1 year of age.

Conclusion: Sensitization to HDM is related to levels of its exposure and presence of eczema in early infancy. It remains that interventions to prevent eczema and to reduce exposure to HDM might reduce the sensitization to HDM and also prevent the development of allergic diseases.

543

Does maternal intake of egg and milk during the third trimester influence the allergy related outcome in the offspring? Long term follow up at 30 years of age

Flodström, C[1]; Kull, I[1,2,3]; Hallner, E[3,4]; Eriksson, M[1,2]; Stenius, F[1,2]; Lilja, G[1,2]
[1]Sachs' Childern and Youth Hospital, Stockholm, Sweden; [2]Department of Clinical Science and Education, Karolinska Institutet, Stockholm, Sweden; [3]Karolinska Institutet, Institute of Environmental Medicine, Stockholm, Sweden; [4]Centre of Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden

Background: The importance of exposure to food allergens during pregnancy and allergy related outcome in the offspring is an old question, but still highly topical. Reviews have not shown it beneficiary to recommend food alterations during pregnancy to prevent allergy development, yet new studies have shown results that may alter these recommendations.

Objectives: To investigate whether maternal intake of egg and milk during the third trimester affects the prevalence of allergy related symptoms and sensitization in the offspring at 30 years of age.

Method: A study population of 234 individuals has been followed prospectively from birth. In a follow-up of allergy related symptoms (eczema, asthma and rhinitis) at 27–30 years of age, 174 (75%) participated by responding to a web-based questionnaire, and 152 completed a clinical examination, including skin prick test (SPT) and IgE-analysis towards egg, milk, peanut, hazelnut, fish, cat, dog, horse, birch, timothy, *Dermatophagoides Pteronyssinus* and *Cladosporium*. SPT $\geq$ 3 mm and/or IgE> 0.35 kU/l was regarded as

© 2015 The Authors

positive. All mothers of the participating individuals were allergic. During the third trimester, 72 mothers were on a diet excluding egg and milk, 51 on a diet consisting of a high intake of milk and egg, and 51 mothers represented the control group, with an unrestricted intake of the two food allergens. In our analysis, we have combined the results from the groups of unrestricted intake and high intake of milk and egg.

Results: The prevalence of allergy related symptoms (asthma, eczema and rhinitis) at 30 years of age was 56% among the offspring of mothers in the non-egg and milk group, and 59% among the offspring of mothers in the groups exposed to egg and milk. IgE sensitization and the combination of sensitization and allergic symptoms were found in 62% and 43% respectively, among the offspring of the women avoiding egg and milk. The corresponding figures were 55% and 47% respectively, among the offspring of the women exposed to egg and milk. The differences between the study groups were not statistically significant, and the results did not change when performing a multiple regression model controlling for confounders (gender, breast feeding, smoking and exposure to animal dander).

Conclusion: Maternal intake of egg and milk during the third trimester does not seem to influence the outcome of allergy related symptoms and sensitization in the offspring at 30 years of age.

544
What impacts the development of an allergic sensitization? IgE profiles, allergen exposure and lifestyle of 501 Austrian pupils

Stemeseder, T[1]; Klinglmayr, E[1]; Schweidler, B[1]; Lueftenegger, L[2,3]; Moser, S[4]; Doppler, P[5]; Lang, R[3]; Himly, M[1]; Oostingh, GJ[2]; Bathke, A[5]; Zumbach, J[4]; Hawranek, T[3]; Gadermaier, G[1]
[1]Department of Molecular Biology, Division of Allergy and Immunology, University of Salzburg, Salzburg, Austria; [2]Salzburg University of Applied Sciences, Biomedical Sciences, Salzburg, Austria; [3]Department of Dermatology, Paracelsus Medical University Salzburg, Salzburg, Austria; [4]University of Salzburg, School of Education, Salzburg, Austria; [5]Department of Mathematics, University of Salzburg, Salzburg, Austria

Background: The number of patients suffering from allergic diseases is increasing around the world, especially in industrialized countries. Reasons for this development are still not fully elucidated. This study analyzed IgE sensitization profiles, allergen exposure and lifestyle of 501 Austrian pupils aged 13–21 to identify factors that might affect the development of an allergic sensitization.

Method: Capillary blood samples were obtained from a randomized cohort of 501 Austrian school children from different geographical regions (urban, rural and alpine areas) and analyzed for IgE sensitization to 112 single allergens using the ImmunoCAP ISAC. House dust samples were examined using a multiplex array to assess the exposure to indoor allergens from cats, dogs, house dust mites and molds. Demographic data, self-reported health status including allergies and other lifestyle conditions such as smoking and diet were surveyed in a detailed questionnaire.

Results: Fifty-seven percent of subjects declared to suffer from allergies including self-reported adverse reactions while 21% stated to have clinically confirmed allergies. IgE reactivity to any of the 112 molecules on the ISAC chip was observed in 53% of subjects with a slightly higher sensitization rate of 57% found in males (51% in females). Highest sensitizations to inhalant allergens were found to grass pollen (36% of subjects), tree pollen (28%), house dust mites (23%), weed pollen (21%) and animal hair (16%). No difference in overall IgE sensitization was observed between alpine, urban and rural living areas. Fel d 1 and Can f 1 were the most abundant allergens in the collected house dust samples while amounts of Alt a 1 were insignificant. The sensitization rate to house dust mite was significantly higher in urban regions while pupils in alpine regions were found to be less exposed to mite allergens which translated into lower IgE sensitization. Despite elevated levels of mite allergens found on farms, a decreased sensitization rate to Der p 2 was found in pupils living on farms compared to those living in flats. Additionally smoking was found to be a significant factor influencing the development of sensitizations.

Conclusion: Certain external triggers such as living environment, dwelling form or allergen exposure contribute to IgE development against distinct allergens and the onset of allergic diseases.

[Supported by Sparkling Science, Federal Ministry of Science, Research and Economy, Austria.]

545
Lifestyle modifies risk for sensitization, regardless of maternal heredity

Evers, K; Marell Hesla, H; Eriksson, M; Stenius, F; Wallén, C; Alm, J
Department of Clinical Science and Education, Karolinska Institutet, Södersjukhuset, Stockholm, Sweden

Background: Allergic diseases constitute a heterogeneous condition with multifactorial genesis. Both genetic, environmental and lifestyle factors are considered to influence the development of allergic disease. Heredity is known to be the most important single risk factor, even though most of the allergic children have no genetic predisposition. Maternal heredity is thought to be especially important. Anthroposophic lifestyle is associated with lower allergy risk in children. We aimed to investigate the association between maternal heredity and lifestyle as a risk factor for development of sensitization in children up to 5 years of age.

Method: IgE-analysis of allergic sensitization to a subset of allergens were performed in 474 children at 6, 12, 24 and 60 months of age. Maternal sensitization was tested at inclusion in the study. The population is a part of the Swedish birth cohort study ALADDIN. Sensitization was defined as IgE level of >0.35 kUA/l to at least one allergen (cow′s milk, hen′s egg, peanut, cat, dog, birch, timothy). Maternal heredity was defined as positive Phadiatop® (Phadia AB, Sweden). Children were divided into three lifestyle groups: anthroposophic, partly anthroposophic and non-anthroposophic. Generalized Estimating Equations (GEE) modelling was performed, taking into account possible dependence between time points within each child, with heredity and lifestyle as risk factors and sensitization as outcome. An interaction term was added to the model in order to investigate if the relationship between heredity and sensitization differed depending on lifestyle.

Results: Sensitization was significantly less prevalent in children of families with anthroposophic lifestyle in comparison to non-anthroposophic at both 6, 12, 24 and 60 months, 2.8%/18%; 4.2%/26.6%; 15.1%/23.8%; 20.6%/38.1%, respectively (OR (95% CI) 0.39 (0.23–0.69), $P = 0.001$). A significant difference was also seen between children with partly anthroposophic lifestyle and non-anthroposophic lifestyle (OR (95% CI) 0.42 (0.27–0.65) $P = 0.0001$). Adjusting for maternal heredity did not change the results markedly. There was no statistically significant interaction with maternal heredity ($P = 0.754$) thus signifying that the relationship between lifestyle and sensitization was independent of maternal heredity.

Conclusions: In this birth cohort lifestyle seems to be a more important risk factor for allergic sensitization than maternal sensitization. The association between lifestyle and sensitization of the child was similar regardless of maternal sensitization.

© 2015 The Authors

546

Environmental programming of respiratory allergy in childhood: the applicability of saliva

Langie, S[1]; Szarc vel Szic, K[2]; Van Der Plas, E[1]; Declerck, K[2]; Moisse, M[3]; Koppen, G[1]; Schepers, A[4]; Van Camp, G[4]; Lambrechts, D[3]; Schoeters, G[1,2]; Vanden Berghe, W[2]; De Boever, P[1,5]
[1]Environmental Risk and Health Unit, Flemish Institute of Technological Research (VITO), Mol, Belgium; [2]Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium; [3]KU Leuven, VIB Vesalius Research Center, Leuven, Belgium; [4]Laboratory of Cancer Research and Clinical Oncology, Center for Medical Genetics, University of Antwerp, Antwerp, Belgium; [5]Centre for Environmental Sciences, University of Hasselt, Hasselt, Belgium

Environmental exposures during fetal and early life may contribute to complex disease later in life such as allergy. The underlying mechanisms are still undiscovered, but changes in the epigenetic regulation of gene expression (such as DNA methylation) is a prominent candidate mechanism. Longitudinal birth cohorts are instrumental to study the relation between early-life environmental factors and the development of complex diseases, but investigations are hampered since blood sampling in children is kept minimal for practical and ethical reasons. Saliva is suggested as an alternative DNA source because the samples can be obtained in a decentralized manner with less constraints.

We hypothesize that prenatal exposures (environmental pollutants, mother's lifestyle) can alter fetal DNA methylation patterns, and thereby predispose the child to develop respiratory allergy (RA) later in life. Furthermore, we hypothesized that differential methylation regions (DMR) can be detected in saliva and blood of 11y old RA cases ($N = 20$) when compared to controls ($N = 20$).

R-based software was used for data normalization and Comb-p tool to identify DMR. DNA methylation analysis via Illumina Methylation 450K BeadChips showed that the methylation status was comparable between blood and saliva with ±11% of the probes having a differential methylation pattern (Padj< 0.05 and $|\Delta\beta| > 0.1$), indicating that saliva is a suitable matrix for DNA methylation studies. Comparing allergy cases vs. controls revealed 75 DMR in blood from 11y olds. An overlap with DMR detected in cord blood and saliva revealed 2 DMR in common. These 2 plus 4 other DMR were selected for further biological and technical validation with bisulfite pyrosequencing in 60 additional cases of the same birth cohort as well as in an independent birth cohort ($N = 79$).

This project will provide new levels of insight in the molecular mechanisms through which prenatal and early life environmental factors predispose children to RA. The use of saliva will simplify the assessment of the impact of environmental exposures on DNA methylation patterns in human biomonitoring studies, especially for children where blood collection is often cumbersome. The ultimate goal of the project is to contribute to the development of prevention strategies, particularly in children, thereby reducing the family and societal burden associated with RA.

© 2015 The Authors

Poster Discussion Session PDS 27

Gastrointestinal immunology and allergy

548
Histamine receptor expression in the gut is altered by local inflammatory activity

Smolinska, S[1,2]; Groeger, D[3]; Rodriguez, N[3]; Schiavi, E[3]; Jutel, M[1,2]; O'Mahony, L[3]
[1]Department of Clinical Immunology, Wroclaw Medical University, Wroclaw, Poland; [2]'ALL-MED' Medical Research Institute, Wroclaw, Poland; [3]Swiss Institute of Allergy and Asthma Research (SIAF), University of Zürich, Davos, Switzerland

Background: Histamine is a key immunoregulatory mediator in both immediate type hypersensitivity reactions and chronic inflammatory responses. Toll-like receptors (TLR) recognize bacterial ligands and histamine alters TLR signaling cascades, in particular via histamine receptor 2 (H2R). While the gastrointestinal mucosa is continuously exposed to bacterial ligands, the influence of histamine on inflammatory responses within the mucosa is poorly defined, particularly during active inflammatory or allergic responses. The overall goal of this project is to determine the immunoregulatory role for histamine within the gastrointestinal mucosa. The first specific aim of this project, addressed in this abstract, is to determine the gene expression levels of histamine receptors, histamine metabolic genes and key cytokine genes within inflamed and non-inflamed mucosa from Crohn's disease and ulcerative colitis patients.
Method: Biopsies were obtained from inflamed and non-inflamed gut mucosa from ulcerative colitis (n = 10 patients) and Crohn's disease patients (n = 10 patients). Both sites were biopsied at the same time from the same patient. Expression of histamine receptors (H1R, H2R and H4R), histamine metabolism-related enzymes (HDC, HNMT and DAO) as well as TNF-a, IFN-g, IL-12, IL-1b and IL-6 were investigated using REAL-TIME PCR.
Results: H2R and H4R gene expression were significantly elevated in inflamed versus non-inflamed mucosa from Crohn's disease patients, while H1R, H2R and H4R gene expression were significantly elevated in the inflamed mucosa of ulcerative colitis patients. No change in HDC, HNMT or DAO gene expression were observed between inflamed and non-inflamed regions. In inflamed biopsies from ulcerative colitis patients, H2R expression was positively correlated with TNF-a expression ($r^2 = 0.83$, $P = 0.0002$), while H4R expression was positively correlated with IFN-g gene expression ($r^2 = 0.58$, $P = 0.01$). No correlations between histamine receptor expression and cytokine expression were observed in biopsies from Crohn's disease patients.
Conclusion: Mucosal histamine receptor expression is significantly altered in the inflamed gastrointestinal tract. Histamine receptor expression correlates with certain proinflammatory cytokines suggesting that similar environmental or host factors may promote expression of these genes. The contribution of altered histamine receptor expression to gut inflammatory and allergic responses warrants further investigation.

549
Baicalein, a natural compound, attenuates food allergic responses

Shin, HS; Bae, M-J; Shon, D-H
[1]Korea Food Research Institute, Seoungnam-si, Korea

Background: The frequency of food allergy, or anaphylactic hypersensitivity, is increasing worldwide. The disorder is triggered by allergen permeation of the gastrointestinal tract and a Th2-mediated immune response. $CD4^+$ $Foxp3^+$ regulatory T (Treg) cells maintain immune tolerance, which is associated with food allergy. Changing intestinal permeability is a risk factor for the development of food allergy. We examined the effect of baicalein on Treg induction and intestinal barrier function via regulation of tight junctions (TJ).
Method: Naïve $CD4^+$ T cells isolated from BALB/c mice were evaluated for Treg induction and food allergy was induced in sensitized mice by 40-day oral challenge with OVA. The incidence of diarrhea and anaphylaxis were analyzed, and rectal temperature, serum cytokines, and T-cell populations in the mesenteric lymph nodes were evaluated. We also assessed the effect of baicalein on intestinal barrier function.
Results: Baicalein induced differentiation of $CD4^+$ $Foxp3^+$ Treg cells from naïve $CD4^+$ T cells, ameliorated the symptoms of food allergy (diarrhea, anaphylaxis, and rectal temperature), and attenuated serum IgE, mMCP-1, and Th1- (IFN-g and IL-12), Th2- (IL-4, IL-5, and IL-13), and Th17-(IL-17) cytokines. However, Treg-related cytokine TGF-b, transcriptional factor Foxp3, granzyme B, and aryl hydrocarbon receptor expression were induced by baicalein. Baicalein also enhanced intestinal barrier function via TJ regulation in a mouse model of food allergy.
Conclusion: Baicalein induces Treg differentiation and enhances barrier function; it is therefore a potential oral immune suppressor for the treatment of food allergy.

550
Combined exposure to beta-lactoglobulin-derived tolerogenic peptides and synbiotics alleviates food allergy response *in vivo*

Kostadinova, A[1,2]; van Esch, BC[1,2]; Garssen, J[1,2]; Willemsen, LEM[1]; Knippels, LMJ[1,2]
[1]Utrecht University, Utrecht, the Netherlands; [2]Nutricia Research, Utrecht, the Netherlands

Background: At-risk infants can be prevented from developing food allergy symptoms by feeding them hypoallergenic formulas containing cow's milk protein hydrolysates. This preventive effect might be a result of oral tolerance induction by immunogenic peptide fractions in the hydrolysates. Early exposure to tolerance-inducing peptides could prevent the development of allergic symptoms. It is hypothesised that synbiotics (pre- and probiotics) can further enhance tolerance induction.
Method: Three-week-old female C3H/HeOuJ mice (N = 6–8) were exposed orally to (1) PBS (positive control for allergy), to (2) whey protein (positive control for tolerance induction), or (3) a low dose mixture of beta-lactoglobulin-derived peptides (18 amino acids) with a synbiotics-enriched diet prior to sensitization.

Thereafter, the mice were fed a control cow's milk protein-free diet and sensitized to whey protein using cholera toxin as an adjuvant. Mice were intradermally challenged with whey protein, and clinical symptoms, such as acute allergic skin response and anaphylactic shock, were measured.
Results: The combination of the peptide intervention with a synbiotics-enriched diet resulted in a significant reduction in the

© 2015 The Authors

acute allergic skin response compared to the allergic positive control. Furthermore, the combined peptides-synbiotics exposure prevented from developing significantly higher anaphylactic shock symptoms when compared to the sham-sensitized controls.
Conclusion: Oral exposure to specific beta-lactoglobulin-derived peptides with synbiotics leads to protection against acute allergic responses. The beta-lactoglobulin-derived peptides were administered at a dosage 100-fold lower than previously described by Meulenbroek et al. [1], therefore a combined approach like this might reduce the peptide dose needed to prevent allergy. Future research is needed for unravelling the underlying mechanisms of the preventive effect of this combined exposure.

[1] Meulenbroek LA, van Esch BC, Hofman GA, et al. Oral treatment with beta-lactoglobulin peptides prevents clinical symptoms in a mouse model for cow's milk allergy. Pediatr Allergy Immunol. 2013; 24: 656–64.

551
Active eosinophilic esophagitis is characterized by epithelial barrier defects and eosinophil extracellular trap formation

Simon, D[1]; Radonjic-Hösli, S[2]; Straumann, A[3]; Yousefi, S[2]; Simon, H-U[2]
[1]Department of Dermatology, Bern University Hospital, Bern, Switzerland; [2]Institute of Pharmacology, Bern, Switzerland; [3]Swiss EoE Research Network, Olten, Switzerland

Background: Eosinophilic esophagitis (EoE) exhibits esophageal dysfunction owing to an eosinophil-predominant inflammation. Activated eosinophils generate eosinophil extracellular traps (EETs) able to kill bacteria. There is evidence of an impaired barrier function in EoE that might allow pathogens to invade the esophagus. This study aimed to investigate the presence and distribution of EETs in esophageal tissues from EoE patients and their association with possible epithelial barrier defects.
Method: Anonymized tissue samples from 18 patients with active EoE were analyzed. The presence of DNA nets associated with eosinophil granule proteins forming EETs and the expression of filaggrin, the protease inhibitor lympho-epithelial Kazal-type-related inhibitor (LEKTI), antimicrobial peptides, and cytokines were evaluated by confocal microscopy following immune fluorescence staining techniques.
Results: EET formation occurred frequently and was detected in all EoE samples correlating with the numbers of infiltrating eosinophils. While the expression of both filaggrin and LEKTI was reduced, epithelial antimicrobial peptides (human beta-defensins-2, -3, cathelicidin LL-37, psoriasin) and cytokines (TSLP, IL-25, IL-32, IL-33) were elevated in EoE as compared to normal esophageal tissues. There was a significant correlation between EET formation and TSLP expression ($P = 0.02$) as well as psoriasin expression ($P = 0.016$). On the other hand, a significant negative correlation was found between EET formation and LEKTI expression ($P = 0.016$).
Conclusion: Active EoE exhibits the presence of EETs. Indications of epithelial barrier defects in association with epithelial cytokines are also present which may have contributed to the activation of eosinophils. The formation of EETs could serve as a firewall against the invasion of pathogens.

552
Familial eosinophilic esophagitis (EoE) uncovers a new EoE-like syndrome without tissue eosinophilia

Straumann, A[1]; Blanchard, C[2]; Bussmann, C[3]; Hoesli, S[4]; Hruz, P[5]; Safroneeva, E[6]; Schoepfer, A[7]; Kozlowski, E[4]; Simon, D[8]; Simon, H-U[4]
[1]Swiss EoE Research Network, Olten, Switzerland; [2]Nestle Research Center, Lausanne, Switzerland; [3]Division of Pathology, Institute Viollier, Basel, Switzerland; [4]University of Bern, Division of Pharmacology, Bern, Switzerland; [5]University Hospital of Basel, Basel, Switzerland; [6]University of Bern, Institue of Social and Preventive Medicine, Bern, Switzerland; [7]University Hosptial of Lausanne, Lausanne, Switzerland; [8]Department of Dermatology, University of Bern, Bern, Switzerland

Background: Eosinophilic esophagitis (EoE) is a chronic-inflammatory disease of the esophagus characterized clinically by symptoms of esophageal dysfunction and pathologically by an eosinophil-predominant infiltration. EoE has a strong genetic component. We identified in four EoE-families totally five members with an EoE-like syndrome, presenting with typical symptoms of EoE but without tissue eosinophilia. The purpose of this study was to investigate this intriguing syndrome of "EoE without eosinophilia", in order to improve the understanding of this inflammatory condition.
Method: The five patients suffering from EoE-like syndrome were evaluated by laboratory analyses, endoscopy, histologic and quantitative immuno-histologic examinations and genome-wide association analysis. In addition, we searched in all 46 members of these EoE-families for EoE-associated molecular abnormalities.
Results: Using immunohistochemistry we detected in the esophagus of patients with EoE-like syndrome a chronic, Th2 type inflammation, but definitely a lack of eosinophils. Some gene known to be dysregulated in conventional EoE patients were found dysregulated in EoE-like syndrome. In addition, we found the EoE-risk allele *TSLP rs3806932* in their genome. First generation offspring of EoE-like syndrome patients had on average a 40% risk of being affected by conventional EoE.
Conclusion: These five members of EoE families suffering from "EoE without eosinophilia" do formally not fulfill the diagnostic criteria of EoE. However, clinical manifestation, symptom-response to corticosteroid-treatment, the finding of a Th2 type inflammation and the bequeath of conventional EoE to their offspring suggests a uniform underlying pathogenesis. Conventional EoE with the predominant eosinophilia might therefore be only one phenotype of this dysphagia syndrome and the role of the eosinophils must be reconsidered.

553
Management and follow up of paediatric eosinophilic esophagitis

Villafana, L; Perez, N; Negrin, Y; Camarero, C; De la Hoz, B; Terrados, S; Álvarez-Cuesta, E
Hospital Ramón y Cajal, Madrid, Spain

Background: Eosinophilic Esophagitis (EoE) is a chronic, immune/antigen mediated oesophageal disease characterized clinically by symptoms related to oesophageal dysfunction and histologically by eosinophil-predominant inflammation.
Method: Eosinophilic Esophagitis Pediatric patients diagnosed in our service from 2004 to 2014, were prospectively recruited in our protocol based in allergy studies (Skin prick test, Patch test, serum total Ig E, specific IgE were performed). Based on the result, we prescribe avoidance diet or elemental diet and sequential introduction of food. Clinical, Endoscopic and histological evaluation were performed to assess response. We continued clinic periodic evaluation in all patients. Remission is the presence of a normal biopsy after an accurate EoE diagnosis.
Results: Thirty three patients, with a median age of 9.6 years old were included. Most of them were male (81.8%) and atopic (90.9%). 9 patients had celiac disease (27%). The most common symptoms at diagnosis were impaction (54.5%), abdominal pain (39.4%), vomiting (36.4%), dysphagia (27%) and chest pain (12%).

Remission occurred in 30 patients: all patients (6) treated with elemental diet, 21 with avoidance diet and 3 with swallowed corticoids. Offending food was found in 24 patients being the milk the most frequent.

© 2015 The Authors

Other implicated foods were: legumes, eggs and gluten. Median time of follow up was 4.3 years (from 0.5 to 9 years). In the follow up a clinical and histological relapses of EoE were assessed in 5 patients (16%) without changes in their treatments.
Conclusion: The majority of our children got a permanent remission with dietary therapies based on an allergological intervention. Milk was the most frequent offending food. There was a small subgroup of patients in whom we failed to find the offending food or when cured returned to have symptoms despite diet.

554
The prevalence of environmental and food sensitization in patients with eosinophilic esophagitis

Pham, H[1]; Chad, Z[2]; Sussman, G[3,4]; Hébert, J[5]; Frankish, C[1]; Olynych, T[1]; Cheema, A[6]; Del Carpio, J[7]; Harrison, R[8]; Santucci, S[8]; Yang, WH[1,8]
[1]University of Ottawa Medical School, Ottawa, Canada; [2]University of Ottawa, Pediatrics, Ottawa, Canada; [3]University of Toronto, Toronto, Canada; [4]Gordon Sussman Clinical Research, Toronto, Canada; [5]Centre de Recherche Appliquée en Allergie de Québec, Québec, Canada; [6]Trillium Health Partners, Mississauga, Canada; [7]McGill University, Montreal, Canada; [8]Ottawa Allergy Research Corporation, Ottawa, Canada

Background: Eosinophilic esophagitis (EoE) is a chronic inflammatory disease of the esophagus histopathologically characterized by eosinophilic infiltration into the squamous epithelium and clinically results in symptoms of esophageal dysfunction. The pathogenesis of EoE is poorly understood. EoE has been associated with food allergy, allergic rhinitis, and asthma. The use of allergy testing to identify foods that may be contributing to EoE is controversial, especially in adults. We present data relating to EoE experience in tertiary allergy clinics in four Canadian cities.
Method: We conducted a retrospective chart review from multiple private allergy clinics in Montreal, Ottawa, Quebec City, and Toronto, Canada for patients diagnosed with EoE. Demographics (age, sex), atopic history (food and environmental allergy), skin prick tests (SPT), specific IgE antibody testing, and treatment data were collected and reviewed.
Results: A total of 190 patients (male:female ratio of 2:1, P -value< 0.01; 34 ± 17 years) were diagnosed with EoE. 82% of patients had documented biopsy confirmation.

Food sensitization was identified on SPTs in 51% of patients (nuts = 36%, milk = 13%, soy = 12%, seafood = 11%, egg = 10%, wheat = 6%).

Positive SPTs to environmental allergens were detected in 87% of patients (tree = 68%, grass = 63%, ragweed = 61%, cat = 55%, mould = 34%, dog = 22%, cockroach = 16%).

Most patients had both environmental and food sensitization (47%) or environmental sensitization only (40%). Few had food sensitization only (4%) and some were negative to both food and environmental allergens (9%).

The proportion of EoE patients with environmental sensitization was significantly higher than those with food sensitization (P -value< 0.01).

In terms of treatment, information was collected for 66% of patients. Most patients were on an inhaled (swallowed) corticosteroid (78%) or PPI (78%). Some patients were on other treatment (allergen immunotherapy = 23%, montelukast = 17%, antihistamines = 6%, omalizumab = 2%, ranitidine = 1%).
Conclusion: There is a weak correlation between food sensitization and EoE in adult patients. Environmental allergies had a significantly higher prevalence than food sensitization among EoE patients. This data strengthens the association between atopy and biopsy proven EoE. The pathogenesis of EoE remains poorly defined and further prospective studies are necessary.

555
Eosinophilic esophagitis: clinical and allergological descriptive study

Valbuena, T; Reche, M; Padial, MA; Nuñez, B
Hospital Infanta Sofia, Allergy, S. Sebastian de Los Reyes, Spain

Background: Eosinophilic esophagitis (EoE) is a chronic inflammatory disease characterized by a dense eosinophilic infiltrate limited to the esophagous with immunological aetiology. The objective of this study is to report the baseline clinical and allergological characteristics of a sample of adult patients with EoE.
Method: Adult patients older than 16 years diagnosed with EoE between 2008 and 2013 referred to our outpatient clinic. Skin prick tests (SPT) with a set of foods and inhalant allergens were performed. Total IgE level, eosinophilic cationic protein (ECP) level and eosinophil count were determined.
Results: Thirty-two patients were included (26 male-6 female). Mean age: 38 years (range 16–68). Twenty-two were atopic (69%), allergic rhinitis was the most frequently reported atopic disease followed by food allergy and asthma. The median duration of symptoms before endoscopy was 7 years (range: 3 months-20 years). Dysphagia (81%) was the most prevalent digestive symptom referred by the patients, followed by impactation (59%) and choking (53%). Mean number of eosinophils/hpf in the biopsy was 82 (range: 20–300). 75% of the patients had positive SPT to aeroallergens, of which 78% were sensitized to pollens. Nineteen patients (60%) had positive SPT to food allergens (34% tree nuts, 32% fruits, 18% cereals and 18% legumes). Peripheral eosinophilia was detected in 60% of the patients (range: 100–1400 eos/mm3). Total serum IgE mean was 429 KU/l (range: 6–3524). ECP was increased (>18 mcg/l) in 26 patients. All patients underwent treatment with fluticasone propionate 500 mcg twice daily over 3 months and 66% of them improved their symptoms.
Conclusion: These findings agree with previous reports in the demographic and clinical data of patients with EoE, which affect mostly middle-age atopic males and manifest with dysphagia and choking.

Further studies should be conducted in order to confirm the potencial role of the sensitizations found to pollen and food plant allergens, in EoE development.

556
IgE sensitization to cow's milk in infants with allergic proctocolitis

Kontogiorgaki, I; Chytiroglou, E; Sandilos, C; Angelides, X; Makris, M; Chliva, C
Dermatology and Venerology, Attikon University Hospital, Athens, Greece

Background: Allergic proctocolitis (AP) is a non-IgE mediated food allergy, manifesting with blood-streaked stools in otherwise healthy appearing infants. Skin prick tests (SPTs) and/or serum specific IgE (sIgE) levels to cow's milk (CM) proteins, are typically negative.
Method: Infants with a definite history of allergic proctocolitis were evaluated for IgE sensitization to CM proteins, via SPTs and/or sIgE quantification (ImmunoCAP), firstly at the initial consultation in the Allergy Unit and subsequently at the time before the introduction of CM. Open food challenges (OFC) with CM were performed under physician supervision, in sensitive to CM proteins individuals.
Results: Among 88 infants with AP (54.5% ♀; mean age at AP diagnosis 1.91 ± 1.14 mo; 63.6% breastfed), 11 (12.5%) revealed IgE-sensitization to CM. In 9 out of 11 subjects, IgE-sensitization to CM demonstrated during the baseline assessment (at the age of 8.45 ± 4.06 mo), whereas 2/11 children were sIgE-negative to CM initially and developed sIgE to CM proteins, during the monitoring, after 2 and 5 mo, respectively. All infants were under CM avoidance at the initial evalua-

© 2015 The Authors

tion. Notably, 6/11 children experienced an anaphylactic reaction due to accidental exposure or due to an OFC with CM (mean age 18.91 ± 14.5 mo), 3/11 passed an OFC with CM at the age of 16 ± 5.29mo, whereas in 2/11 subjects an OFC is to become. Not statistical significant correlation was shown between the appearance of atopic dermatitis and IgE-sensitization to CM in the study population (Fischer exact P value > 0.05).

Conclusion: Whereas IgE sensitization to CM is typically not present in allergic proctocolitis, our data suggests that some children might develop an IgE-mediated phenotype and eventually transition to acute reactions. Whether a period of strict avoidance of CM proteins might result in occurrence of acute allergic reactions has still to be revealed. Evaluation of sensitization to CM at the first visit and detailed follow-up, especially at the time of CM introduction, is required in order to prevent an anaphylactic reaction, in children with allergic proctocolitis.

557

Almost half cow's milk-induced food protein induced enterocolitis syndrome (FPIES) require amino acid feeding

Blanc, S[1]; De Boissieu, D[1]; Kalach, N[2]; Soulaines, P[1]; Campeotto, F[1]; Malka, C[1]; Cordier-Collet, M-P[1]; Montaudié-Dumas, I[3]; Giovannini-Chami, L[3]; Bourrier, T[3]; Dupont, C[1]

[1]Paediatric Gastroenterology and Allergology Department, Necker-Enfant Malades Hospital, Assistance Publique - Hôpitaux de Paris (AP-HP), Paris, France; [2]Department of Saint Vincent de Paul Hospital, Groupe Hospitalier de l'institut Catholique de Lille, Lille, France; [3]Paediatric Allergology Department, Hopitaux Pédiatriques de Nice CHU-Lenval, Nice, France

Background: FPIES, a non-IgE mediated food allergy (FA) which seems to expand, is mainly related to cow's milk and manifests as a chronic digestive disease or in its acute form with potentially life-threatening vomiting/diarrhea/dehydration. The objective of this study is to characterize the clinical features of cow's milk-induced FPIES in children.

Method: A cohort of patients with FPIES was constituted in French Children's Hospitals (Necker, Paris - Lenval, Nice). Children were recruited from a cohort registered within the French Ministry of Health (DC-2009–955). Data were collected from medical records including all patients referred for an acute episode of FPIES, and divided into two groups according to their tolerance of extensively hydrolysed formula (eHF) or their need to be fed an amino-acid formula (AAF).

Results: Forty two children were enrolled, 24 male and 18 female, with a personal history of allergy in 9/36 (25%) and a familial history of allergy in 22/34 (65%). Infants had been breastfed in 31/39 cases (79%), for a median duration of 92 days, with FPIES during breastfeeding in 4. Chronic symptoms (diarrhea, reflux, crying, failure to thrive, and/or blood in stool) had occurred in 24/40 (60%), after a median period of 10 days following introduction of milk-based formula. In the whole group, the acute episode occurred at a median age of 4 months, after a median delay from ingestion of milk of 2 h. Median age at diagnosis was 11 months for FPIES and 3 months for FA. Clinical features of acute form were vomiting 33/37 (89%), hypotonia 24/37 (65%), diarrhea 18/37 (49%), pallor 11/37 (30%), lethargy 5/37 (16%). Emergency treatment consisted of intravenous rehydration in 16/26 (62%), corticosteroids in 5/26 (19%), epinephrine in 4/26 (15%), antibiotics in 2/26 (8%). Allergy testing was rarely positive: patch test 17/30, skin prick tests 3/24, specific IgE 8/31. Recovery was observed in 18/31 (58%) at a median age of 31 months. The eHF group comprised 22 (52%) infants and the AAF one 20 (48%). They exhibited the following significant or trend towards significance differences: median duration of chronic symptoms 15 days vs 46 *($P = 0.05$)*, number of hospitalizations before diagnosis 8 vs 26 *($P = 0.06$)*, associated FA 2/10 vs 8/9 *($P = 0.0008$)*.

Conclusion: Half infants with milk-induced FPIES do not tolerate eHF, and need to be fed with an AAF, a condition associated with a delayed diagnosis.

558

Retrospective study of oral food challenges performed in children with history of FPIES in a tertiary hospital

Kostoudi, S; Psomiadou, M; Tzeli, K; Tziotou, M; Karantoumanis, D; Manolaraki, I; Michopoulou, C; Georgountzou, A; Maggina, P; Kyriakakou, M; Zisaki, V; Georgiou, A; Kapoula, A; Roumpedaki, E; Douladiris, N; Manousakis, E; Xepapadaki, P; Papadopoulos, NG

National & Kapodistrian University of Athens, 2nd Pediatric Clinic, Allergy Unit, Athens, Greece

Background: Food protein-induced enterocolitis syndrome (FPIES) is a non-IgE mediated allergic reaction, usually underdiagnosed, but not as rare as it was thought to be in the past. We describe our experience of oral food challenges (OFCs) in children either to exclude diagnosis FPIES (Group A) or to assess tolerance in already diagnosed children with FPIES (Group B).

Method: Data on OFCs in children with symptoms suggestive of FPIES, were recorded between 2010 and 2014.

Results: Forty-one children in total (mean aged 3 ± 2.6 years old, 61% males) were included in the study. In those challenges (7 children had 2 OFCs) were performed, out of which 23 resulted positive. Culprit foods were milk, fish, rice, egg and chicken (46.3%, 39.3%, 19.5%, 12.2% and 4.9% respectively), while 12.2% had more than one food implicated. In 3 out of 4 children (Group A) the diagnosis of FPIES was excluded, while in group B children tolerated milk by 42.1%, fish by 18.2%, rice by 40%, egg by 50% and chicken by 100%. Time at re-introduction was different according to food implicated. Concurrent IgE sensitization to the offending food was recorded in 26.3% of the children before re-introduction. Management of children with positive OFCs, included administration of iv fluids and iv ondansetron in 47.4% and 12.2% respectively.

Conclusion: OFCs remain the gold standard as in cases of atypical symptoms suggestive of FPIES excluded the disease thus avoiding unnecessary restriction diet. During the 4 years follow-up less than half of children outgrew FPIES, most commonly to milk, chicken and egg. A significant proportion of children had concurrent IgE sensitization and that should be taken into account at the re-introduction of the offending food.

559

Celiac disease: peripheral blood cell-mediated response to antigen-specific stimulation

Losa, F[1,2]; Galleri, G[2]; Fadda, R[2]; Biggio, ML[2]; Secchi, G[2]; Mura, MN[1]; Firinu, D[1]; Manconi, PE[1]; Del Giacco, SR[1]; Manetti, R[2]

[1]Department of Medical Sciences 'M. Aresu', University of Cagliari, Monserrato (Cagliari), Italy; [2]Department of Clinical and Experimental Medicine, University of Sassari, Sassari, Italy

Background: Celiac disease is an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals and characterized by a wide range of clinical manifestation. Nowadays, the diagnosis is based on presence of celiac disease specific antibodies (i.e. anti-tTG2) and typical changes in small intestinal mucosa, like intraepithelial lymphocytosis, crypt hyperplasia and villous atrophy. Despite of the validity of this diagnostic approach, part of the subjects with suggestive symptoms do not fully meet the criteria prescribed by official guidelines, while getting benefit from the intake of gluten free diet. The aim of our study is to identify a new diagnostic approach for this kind of patients. We investigated the adaptive immune response, through *in vitro* antigen-specific stimulation of peripheral T cells, with gliadin, the trigger of celiac disease.

© 2015 The Authors

Method: We studied twenty patients (range 12–48 years) with celiac disease, diagnosed by ESPGHAN criteria; 11 patients were in gluten-free diet for more than 3 months and 9 patients didn't follow any dietary restriction. The control group consisted of 11 healthy donors (same age range), with no signs and serological markers of celiac disease. Peripheral Blood Mononuclear Cells (PBMC), isolated from heparinized blood, were stimulated with Pepsin-Trypsin digested Gliadin (PTG) in the presence of anti-CD28/CD49d antibodies, IL-2 or IL-15 and brefeldine A (BFA). T-cell response was evaluated by flow cytometry. In particular we studied the expression of CD69 and the production of IFN-γ on CD4 and CD8 T cells.

Results: We found a statistically significant difference ($P < 0.01$) in either CD69 and IFN-γ frequency of expression, with higher expression in patients with celiac disease out of dietary restrictions compared to healthy controls, especially considering CD4 T cells.

Conclusion: Our model intends to identify new markers of celiac disease, connected to antigen-specific response, like CD69 and IFN-γ, that reflect the immune pathogenesis of the disease, to recognize also patients who don't meet the guidelines criteria.

560
Description of 63 pediatric patients diagnosed with food induced gastroenterocolitis syndrome in our hospital

Perez, N; Villafana, L; Terrados, S; De la Hoz, B; Alvarez-Cuesta, E
Hospital Ramon Y Cajal, Alergologia, Madrid, Spain

Background: FPIES is a non IgE mediated gastrointestinal food hypersensitivity whose clinical features are severe vomiting, diarrhea and dehydration within a few hours of ingesting food. The foods most frequently implicated in published series are cow's milk, soy, rice, fish.

Method: A retrospective study of 63 patients, diagnosed by history and/or oral challenge test between 2006 and 2014.

Results: Sixty three patients, 40 boys and 23 girls (mean age at diagnosis: 12.18 months) were included. The offending foods were fish (32 patients), milk (15), egg (9), beef (3), chicken (1), lentil (1), peanut (1) and chickpeas (1). The most frequent symptoms were vomiting (59 patients), letargy (20), diarrhea (16) and dehydratation (14). 23 patients have other atopic diseases, Skin prick tests y/or specific IgE against implicated food were positive in only one patient. In 12 patients, patch tests were conducted and were positive in 2 cases. 45 oral challenges test were performed in 33 patients 12 or more month after diagnosis We found that no patient outgrew FPIES by fish by 2 years of age, 58.3% by 3 years, 75% by 6 years and 91.6% by 8 years of age. By contrast 80% of FPIES by milk recovered by the age of 24 months and 100% by 3 years old. In case of egg, all patients recovered by 4 years old.

Conclusion: This is one of the largest series of FPIES. There seems to be an increase in prevalence of this syndrome. The most frequent culprit food is fish and the age of achieved tolerance was significantly higher than other foods.

560-A
A case of neonate and infant gastrointestinal allergy that turned into IgE-mediated food allergy

Sato, K[1,2]; Yagi, H[2]; Kaneko, M[2]; Nishida, Y[2]; Koyama, H[2]; Takizawa, T[2]; Arakawa, H[2]
[1]Takasaki General Medical Center, Pediatrics, Takasaki, Japan; [2]Gunma University Graduate School of Medicine, Pediatrics, Maebashi, Japan

Background: Neonate and Infant Gastrointestinal Allergy (NIGIA) is a non-IgE mediated immune reaction in gastrointestinal system to one or more specific food, commonly characterized by profuse vomiting, diarrhea, bloody stool and poor weight gain like Food Protein Induced Enterocolitis syndrome (FPIEs). Many patients with NIGIA have a positive result of allergen-specific lymphocyte stimulation test (ALST) and oral food challenge (OFC). We describe a case of typical NIGIA that turned into IgE-mediated immediate type food allergy after diagnostic OFC.

Method: A 3-month-old boy was referred for OFC. He had vomiting, diarrhea, bloody stool and poor weight gain from the age of 8 days, which subsided after using casein hydrolysated milk. A specific IgE antibody against milk was negative and ALST was positive for milk before OFC. We started OFC by giving him 10 ml/day of artificial milk and increased a dose by 10 ml each every day.

Result: On the 8th day, he presented with generalized urticaria, nasal discharge and sneezing, 1 h after he was fed with 80 ml of milk. A specific IgE antibody against milk turned positive, suggesting that cell-mediated immune reaction turned into IgE-mediated immune reaction within 8 days of provocation.

Conclusion: This case suggests that this sensitization is established in a short term only 8 days. Since it is difficult to predict the reaction of OFC, OFC needs to be carefully performed accounting into consideration the possibility that it might cause sensitization and IgE-mediated immediate reaction.

© 2015 The Authors

Poster Discussion Session PDS 28

Air pollution and pollens. New evidence

561
Relationship between atmospheric concentrations of *Olea europaea* L. pollen and aeroallergen Ole e 1

Plaza, MP; Alcázar, P; Galan, C
Universidad de Córdoba, Departamento de Botánica, Ecología y Fisiología Vegetal, Córdoba, Spain

Background: *Olea europaea* L. pollen is the second cause of pollinosis in the South of the Iberian Peninsula. Airborne pollen monitoring networks are of great importance to analyze the pollen evolution and dynamics in a determined study area. However, recently, it has been demonstrated that the airborne pollen not always represents the real exposure to aeroallergens, and even not coincides with the symptoms of sensitive patients to specific pollen grains. The direct measurement of the concentration of aeroallergens has lately been introduced as an important advance in public health information systems. To evaluate the correlation between airborne pollen and Ole e 1 aeroallergen concentrations in Córdoba to explain if airborne pollen can be precise information to understand the behavior of pollinosis symptoms.
Method: The Hirst volumetric spore trap was used for airborne pollen detection during 2012 and 2013. Counting and dada management has been carried out following the protocol proposes by the Spanish Aerobiology Network (REA). The Burkard Cyclone Sampler was use for aeroallergen detection. The major olive pollen allergen Ole e 1 was measured by ELISA following the protocol proposed by Arilla (2002).
Results: The trend of Olive pollen during 2012 y 2013 was characterized by a marked seasonality. In both years pollen season begins in late April to mid-June. The peak pollen was detected on 16 May 2012 with 3433 grains/m3 and in 2013 with 4886 grains/m3 on 13 May. Airborne pollen and aeroallergen follow similar trends. The peak concentration of allergens differs with the peak concentration of pollen in 1 day in 2012 and 4 days in 2013. However, the daily variations observed in the amount of aeroallergen generally match the presence of pollen in the air, with some differences where the concentration of allergens is slightly more than the amount of pollen.
Conclusion: The results of this study show that there is a close relationship between the amount of aeroallergen Ole e 1 and olive pollen during pollen season in our city, except for some specific days, before and after the pollen season. This difference is due to a possible release of allergenic proteins from pollen grains under certain weather conditions. These proteins can sometimes remain in the air for a long time. Therefore although the content of pollen in the air can be considered as useful information, these studies should be completed with an analysis of airborne allergens.

562
Purification and identification of major allergen in *Alnus orientalis* Decne. Pollen

Alan, S[1]; Tekin, IO[2]; Atalay, F[3]; Pınar, NM[4]; Kocer, F[1]; Sahin, S[1]; Sarisahin, T[1]
[1]Bulent Ecevit University, Biology, Zonguldak, Turkey; [2]Bulent Ecevit University, Immunology, Zonguldak, Turkey; [3]Department of Chest Diseases, Bulent Ecevit University, Zonguldak, Turkey; [4]Ankara University, Biology, Ankara, Turkey

Background: The pollens of other Betulaceae family members such as *Alnus*, *Corylus* causes pollinosis in temperature regions. Although allergens from Betulaceae family are well documented so far, this allergens mainly isolated from species native to Europe and North America. The species native to Asia such as *Alnus orientalis* hasn't draw enough attention. On the other hand, considering that Turkey is one of the largest hazelnut manufacturers of world, Betulaceae family pollen induced pollinosis is also important for Turkey. In this study, the major allergen has been isolated and identified from *A. orientalis* which is distributed south part of Turkey, Cyprus, Syria, Lebanon and Israel.
Method: Pollens have been collected from Denizli province of Turkey, where situated at southwest part of Turkey. Pollen proteins were extracted by 0.1 M Phosphate Buffered Saline (PBS) and transferred to PVDF membrane after separated by 14% SDS-PAGE.

Twenty Betulaceae pollen-allergic patients, and 20 nonallergic individuals as a negative control were selected on the basis of positive skin prick test. The sera were used for detection of the major allergen using by immunoblotting.

Combination of ammonium sulphate precipitation and 2D-electrophoresis were used for purification of the allergen. Pollen proteins were precipitated at gradually increasing salt concentrations. Only allergen contained fractions were used for further purification step. The precipitated proteins were separated by 2D electrophoresis using 17 cm, pH 4–7 IPG strip, after remove the salt by ultrafiltration.

After tryptic digestion of protein bands of purified allergen, the resulting peptides were analyzed by MALDI-TOF. Sequence coverage was obtained from Mascot Search.
Results: Only 7 of Betulaceae pollen-allergic patients showed a positive reaction to single protein band. It has been found that the most of allergenic protein was precipitated at 70% and 80% concentration. In 2D gel, target protein observed in the range of 17–18 kDa like the major allergens of Betulaceae. According to MASCOT search, the purified protein shows 59% sequence coverage with Aln g I.
Conclusion: Identified protein shows high similarities to PR-10 proteins. Two *Alnus* species are distributed in Turkey shows high sequence coverage, even their distribution areas doesn't cover each other. But there is a no matching between identified protein and Aln g I at sites of 136 - 147 in which most protein sequence differences seen with Bet v I.

563
Aerobiologic study of allergenic pollens during the first monitoring season in Bucharest

Leru, PM[1,2]; Eftimie, A-M[3]; Thibaudon, M[4]
[1]Colentina Clinical Hospital, Bucharest, Romania; [2]Carol Davila University of Medicine and Pharmacy, Faculty of Medicine, Bucharest, Romania; [3]Colentina Clinical Hospital, Allergology, Bucharest, Romania; [4]Reseau National de Surveillance Aerobiologique, Lyon, France

Background: Respiratory allergies induced by allergenic pollens, mainly allergic rhinitis and asthma have an increasing prevalence in most of European countries and have an important individual and socio-economic impact. Aerobiologic study of allergenic pollens is performed in many countries on regular basis and correlated with health data from allergists in the

© 2015 The Authors

frame of national aerobiology networks. Romania has no aerobiology network and pollen measurements have been done during the last 15 years in the West region only. No pollen measurement data are available for other regions, including the capital Bucharest. The aim of our study was to present the results of the first monitoring season of allergenic pollens from Bucharest.

Method: We used a pollen collector type Burkard placed on the roof of the four levels building of the Research -Development Pavilion (CDPC) from Colentina Clinical Hospital, based on the volumetric method. The analysis was done weekly and results expressed in pollen grains per air cube meter.

Results: The monthly results during the first pollen season, between March- October 2014, have been sent for validation to laboratory of Reseau National de Surveillance Aerobiologique (RNSA) from Lyon, France. Our attention was focused on grasses and Ambrosia artemisiifolia pollens. We found high amount of grass pollen during May-June 2014 and surprisingly in September also. The most abundant tree pollens were populus in March and moraceae in April. Regarding weeds pollen, Ambrosia was very abundant from August and increased significantly during September 2014. The results of this first pollen monitoring season in Bucharest show significant amounts of allergenic pollens, mainly Ambrosia artemisiifolia, which was previously considered to be more prevalent in rural areas. Our data have to be correlated with field observations from different plant specialists and health informations from allergists.

Conclusion: There is an urgent need to establish a national aerobiology network, to translate and implement the patient hayfever diary (PHD) according to European Aerobiology Society requirements and to develop research projects with heath and environment authorities.

564
Sensitization to ragweed pollen in two region parts of Romania

Bocsan, IC[1,2]; Muntean, A[3]; Barbinta, C[2]; Tallian, K[4]; Deleanu, D[3]; Buzoianu, AD[3]
[1]University of Medicine and Pharmacy, Cluj Napoca, Romania; [2]Almedo Clinic, Cluj Napoca, Romania; [3]University of Medicine and Pharmacy 'I Hatieganu' Cluj, Cluj Napoca, Romania; [4]Satu Mare County Hospital, Satu Mare, Romania

Background: Ragweed is a major source of allergen, rarely observed in Romania until now. In this study we evaluated the symptoms and associated factors in patients with allergic rhinitis to ragweed pollen in two distinct regions of Romania and the evolution of this in time.

Method: We evaluated the patients with allergic rhinitis induced by ragweed pollen in two allergological centers from North-West (NW) and Central part of Romania between 2009–2010 and 2012–2013. The patients were clinically evaluated regarding duration, presence and severity of the allergic rhinitis symptoms and the association with other allergic manifestations.

Results: In the first period of evaluation, 455 patients from NW center and 706 from Central one with allergic rhinitis were included. The sensitization to ragweed was significantly higher in the NW part compared to Central part (16.2% vs 4.1%, $P = 0.03$). Most of the patients had polisensitization (73% in NE center and 79.3% in Central one). Patients with monosensitization to ragweed pollen presented more severe forms of rhinitis (86% vs 54.8%, $P = 0.003$) in NW part compared to polisensitized patients. The total symptoms score was significantly higher in patients from Central part compared to NW part (9.21 ± 3.01 vs 5.76 ± 2.96, $P = 0.0001$). Bronchial asthma was similarly associated to allergic rhinitis in both centers. In the second phase of evaluation we observed an increased number of patients sensitized to ragweed pollen in both regions (35% vs. 16.2% in NW part and 9.37% vs. 4.1% in Central part). The pattern of allergic rhinitis to ragweed was similar in both phases of evaluation. The association of asthma decreased in both regions in the second phase of evaluation (28.57% vs. 32.3% in NW and 37.93% vs. 21.3% in the central part).

Conclusion: Allergic rhinitis to ragweed pollen is more frequent in NW part. The severe forms of rhinits is observed in Central part of Romania and in monosensitized patients in NW. The prevalence of ragweed sensitization significantly increased in a period of 2 years in both regions.

565
Criteria to determine a pollen season - the French example

Thibaudon, M; Oliver, G
RNSA, Brussieu, France

Background: Some clinical trials related to pollen allergy (e.g. allergen immunotherapy) are based on the start and/or the peak of a pollen season. To determine these periods, the pollen exposure is measured with Hirst-type volumetric spore traps allowing to obtain concentrations in pollens/m^3 of air/day.

But some recommendations are required in study protocols, especially to have a clear definition of the pollen counts which has to be reached to define an evaluation period.

Method: Currently, there are different criteria in each country to define a pollen season. A task force of the EAACI (European Academy of Allergy and Clinical Immunology) in collaboration with aerobiologists synthesized the definitions used in each country to homogenize these values to European level and to use them in the future protocols, even if at local level the definitions could be adapted because of a particular pollination.

Results: The criteria to determine a pollen season for Betulaceae and Cupressaceae in the case of France:

1. start of the season: 1st of 5 days (out of 7 consecutive days) with a minimum of 10 grains/m^3/24 h and a sum greater than 100 grains
2. end of the season: last of 5 days (out of 7 consecutive days) with a minimum of 10 grains/m^3/24 h and a sum greater than 100 grains
3. high pollen season: start the 1st day with at least 100 grains/m^3/24 h and end the 1st of 3 consecutive days with less than 100 grains/m^3/24 h

The criteria to determine a pollen season for grass and ragweed in the case of France:

1. start of the season: 1st of 5 days (out of 7 consecutive days) with a minimum of 3 grains/m^3/24 h and a sum greater than 30 grains
2. end of the season: last of 5 days (out of 7 consecutive days) with a minimum of 3 grains/m^3/24 h and a sum greater than 30 grains
3. high pollen season: start the 1st day with at least 50 grains/m^3/24 h and end the 1st of 3 consecutive days with less than 50 grains/m^3/24 h

566
Birch pollen: health indicator of climate change

Thibaudon, M; Monnier, S
RNSA, Brussieu, France

Background: Phenological observations (study of the occurrence of periodic events, annual most often in the living world, determined by seasonal climate variations) are used to evaluate the influence of climate change on vegetation and biodiversity. These observations, if carried out on allergenic plant species, enable to assess the influence of climate change on human health.

In 2012, ONERC (Observatoire National sur les Effets du Rechauffement

© 2015 The Authors

Climatique) asked the RNSA (Réseau National de Surveillance Aérobiologique) to find indicators of climate change that affect health. Among the species of allergenic trees, birch was chosen firstly because he has high allergy potency throughout Europe, it pollinates in March-April and releases large quantities of pollen in the air. These pollens are a real problem for allergy sufferers. Therefore, the birch, a tree very sensitive to long-term climatic events, was selected as an indicator of climate change.

Method: Quantities of birch pollen that are released in March-April depend on temperatures and weather that occurred before from July of the previous year. The temperatures used represent phenological year of birch i.e. from July to June of the following year. Six cities with different climates and types of vegetation and reliable pollens data (RNSA) were chosen; it's Lyon, Montluçon, Strasbourg, Paris, Toulouse and Amiens. To limit the effects of interannual variations related to simple weather, a moving average over 4 years (the current year and the previous 3 years) of these six cities was calculated to have the quantity of birch pollen and temperature for each year.

Results: The annual quantity of birch pollen in the air has a tendency to increase since the late 80s. An upward trend and a meaningful relationship are observable on the results for the quantity of atmospheric birch pollen. The results indicate an average increase of pollination by 20% in 20 years which is not negligible.

Conclusion: This work allowed the construction of an indicator in Health and Society Chapter of the catalogue of indicators of climate change ONERC, but more research needs to be done.

567
Lack of cross-reactivity between Pooideae sub-family and Chloridoideae and Arundinoideae sub-families of grasses

López-Matas, MA[1]; Gaig, P[2]; Viñas, M[3]; García-Moral, A[4]; Malet, A[5]; Labrador, M[6]; Alcoceba, E[7]; Carnés, J[1]
[1]Laboratorios Leti, R&D, Tres Cantos, Spain; [2]Hospital Universitari Joan XXIII, Allergy Unit, Tarragona, Spain; [3]Hospital de Terrassa, Allergy Unit, Terrassa, Spain; [4]Hospital Clinic de Barcelona, Allergy Unit, Barcelona, Spain; [5]Allergo Centre, Barcelona, Spain; [6]Hospital Vall d'Hebron, Allergy Unit, Barcelona, Spain; [7]Hospital Arnau de Vilanova, Allergy Unit, Lérida, Spain

Background: Gramineae pollens are the main cause of IgE-mediated respiratory allergy worldwide. The Poaceae family comprises more than 650 genera and around 12,000 species. This family includes the most allergenic grasses with a worldwide distribution.

In the Iberian Peninsula, *Phleum, Poa, Dactylis, Lolium* and *Festuca* genera are the most representative, but there are also grasses from other subfamilies including Chloridoideae (*Cynodon dactylon*) and Arundinoideae (*Phragmites communis*), responsible for a large number of sensitizations. The objectives were

1 to study the sensitization to *C. dactylon* and *P. communis* on a population from the North-East of Spain (Cataluña) sensitized to Pooideae grasses and
2 to investigate the degree cross-reactivity of these two species with Pooideae subfamily.

Method: Patients attending the Allergology services of six hospitals from the north-east of Spain were skin prick tested with a grass mixture containing *Phleum, Poa, Dactylis, Lolium* and *Festuca*. 31 patients were diagnosed as sensitized to grasses. Individual's sera were collected. Specific IgE to grass-mix (gx1), *C. dactylon*, *P. communis* and the individual allergens Cyn d 1 and Phl p 1 were measured by ImmunoCAP. A pool of sera was prepared with positive serum (gx1 values >1 kUA/l) and used for immunoblot assays. Cross-reactivity was studied by ELISA and immunoblot inhibition.

Results: A total of 30 patients had sIgE to gx1 (27.3 ± 34.7 kUA/l). 24 (80%) were positives to *C. dactylon* (7.4 ± 9.5 kUA/l), 27 (90%) to *P. communis* (13.6 ± 24.2 kUA/l), 22 (73.3%) to nCyn d 1 (14.9 ± 20.9 kUA/l). All sera tested were positive to rPhl p 1 (11.3 ± 20.8 kUA/l).

Bands were detected in the three extracts by immunoblot. No inhibition of the mixture of grasses was observed with *C. dactylon* and *P. communis* extracts by immunoblot inhibition with the pool of sera or by ELISA inhibition with individual sera.

Conclusion:

1 70% of the grass sensitised patients were also sensitised to *C. dactylon* and 90% to *P. communis*
2 sIgE to *C. dactylon* and *P. communis* is lower than to the mixture of grasses
3 Cross-reactivity between grasses and *C. dactylon* or *P. communis* extracts has not been observed
4 Patients sensitised to *C. dactylon* or *P. communis* seem to be co-sensitized

568
Impact of atopy status and smoking habits on airway inflammation and oxidative stress in compost workers exposed to bioaerosols

Hoffmeyer, F[1]; van Kampen, V[1]; Deckert, A[1]; Neumann, H-D[2]; Buxtrup, M[2]; Willer, E[3]; Felten, C[3]; Brüning, T[1]; Raulf, M[1]; Bünger, J[1]
[1]Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA), Bochum, Germany; [2]German Social Accident Insurance, Institution for the Public Sector in North Rhine-Westphalia, Düsseldorf, Germany; [3]German Social Accident Insurance, Institution for Transport and Traffic, Hamburg, Germany

Background: Respiratory diseases due to occupational exposure to bioaerosols were previously reported. We investigated the impact of atopy and smoking habits on inflammation and oxidative stress in compost workers. Exposure-response relationships were determined by means of non-invasive methods.

Method: Atopy was classified according to specific IgE concentrations to common inhalant allergens (sx1). Bioaerosol exposure was estimated according to job title, time daily worked under protection of filtered air supply and duration of employment. Concentrations of 8-iso-prostaglandin $F_{2\alpha}$ (8-isoprostane), prostaglandin E_2 (PGE_2) leukotriene B_4 (LTB_4), and acid-base balance (pH) in exhaled breath condensate (EBC) and exhaled nitric oxide (FeNO) were assessed in 59 never-smoking (NS) and 60 smoking (S) compost workers.

Results: Atopic subjects were equally distributed among NS and S ($n = 16$ each) with atopic smokers being younger ($P = 0.014$) and serving less years ($P = 0.006$). No associations could be revealed between exposure and biomarkers concerning compost workers in total but in atopic workers (duration of employment and FeNO: $r_s = 0.376$, $P = 0.041$; filtered air supply and FeNO: $r_s = -0.335$, $P = 0.071$). Smokers had significantly lower pH values compared to NS (non atopic, $P = 0.041$; atopic $P = 0.050$).

Conclusion: FeNO might be useful for monitoring of inflammation due to bioaerosol exposure, especially in atopic subjects. Besides smoking also atopy should be considered when investigating airway inflammation.

© 2015 The Authors

569
Allergen quantification by use of electrostatic dust collectors (EDCs) - influence of deployment time, extraction buffer and storage conditions on the results

Sander, I; Lotz, A; Fleischer, C; Zahradnik, E; Flagge, A; Brüning, T; Raulf, M
Institute for Prevention and Occupational Medicine, German Social Accident Insurance, Ruhr University Bochum (IPA), Bochum, Germany

Background: Sampling of endotoxin, beta-glucan or allergens on EDCs is a convenient method for exposure assessment. However, especially for allergens few experiments on validation of this method concerning deployment time or storage and extraction procedure have been performed. The aim of study was to optimize the EDC procedure for sampling of allergens in an indoor environment.
Method: EDCs with four cloths were placed in households or day care centers and after extraction, allergens were quantified by six immunoassays detecting mite antigens (Domestic mites DM, *Dermatophagoides pteronyssinus* Dp, *Tyrophagus putrescentiae* Tp) or the main allergens from cat (Fel d 1), dog (Can f 1) and mouse (Mus m 1). For 20 EDC holders, deployment times of cloths were varied between 7, 14, 21 and 28 days; for 36 EDCs sampled for 14 days three cloths were extracted with the same conditions to test reproducibility, and for 28 EDCs, extraction buffers were varied (with or without 0.05% Tween 20). In addition, the influence of storage length of cloths (2–629 days) at room temperature or extracts (7–639 days) at −80°C, and variation of extract storage temperature (−20°C and −80°C) for long time storage (1.5 years) on the outcome of allergen quantification were tested for about 150 EDCs.
Results: The allergens on EDC cloths increased with deployment time, and allergen loads on parallel sampled tissues were significantly correlated ($P < 0.0001$, Pearson DM r = 0.93; Dp r = 0.87; TP r = 0.81; Fel d 1 r = 0.94; Can f 1 r = 0.88; Mus m 1 = 0.98). Extraction without Tween reduced all results ($P < 0.0001$, -51% DM, -84% Dp, -60% Tp, -99% Fel d 1, -86% Can f 1, -52% Mus m 1). Storage of cloths significantly decreased Can f 1 amount ($P < 0.0001$, -4.8% loss per 30 days), whereas storage of extracts decreased DM results ($P < 0.0001$, -1.2% loss per 30 days). Extracts stored at −20°C gave higher DM and Fel d 1 results than extracts stored at −80°C.
Conclusion: Allergen exposure assessment is strongly influenced by the extraction procedure; the use of detergent Tween 20 is recommended. Furthermore, storage duration of cloths and extracts should be minimized because for some allergens small but significant reductions of the outcome were observed. Allergen concentrations on the cloths increased with deployment time as expected.

570
Marked disparity in environment, infections and prevalence of asthma and atopy between rural and urban children in China

Yang, Z[1]; Feng, M[1]; Pan, L[1]; Lai, X[2]; Xian, M[1]; Huang, X[1]; Chen, Y[3]; Schröder, PC[4]; Roponen, M[5]; Schaub, B[4]; Wong, GW[6]; Li, J[1]
[1]Guangzhou Medical University, Guangzhou Institute of Respiratory Disease, Guangzhou, China; [2]ALK A/S, Research Asia Pacific, Hong Kong, China; [3]Department of Internal Medicine, Guangdong Pharmaceutical University, Guangzhou, China; [4]Department of Pulmonary and Allergy, LMU Munich, University Children's Hospital Munich, Munich, Germany; [5]Department of Environmental Science, University of Eastern Finland, Kuopio, Finland; [6]Department of Paediatrics, Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China

Background: Many epidemiology studies have shown a marked difference in prevalence of asthma between urban and rural regions. We aimed to assess whether endotoxin, allergen exposure, previous infections and sensitization might be relevant to differential prevalence of asthma between urban and rural children in Guangdong, China.
Method: A questionnaire survey was performed in 7164 children from urban Guangzhou and 6087 from rural Conghua. A subsample of 854 children (419 from Guangzhou, 435 from Conghua) was recruited for further studies including pulmonary function, histamine bronchial provocation test, allergen skin prick test, and the presence of serum antibodies as markers of previous infections. House dust samples from 156 families (76 from Guangzhou, 80 from Conghua) were obtained to analyze concentrations of endotoxin, house dust mite (HDM) and cockroach allergens.
Results: The prevalence of doctor-diagnosed-asthma was lower in children from Conghua (3.4%) than Guangzhou (6.9%, $P < 0.001$) in the screening survey. In the subsequent nested case-control study, a lower percentage for confirmed asthma (3.0% vs 28.9%, $P < 0.001$) and sensitization (13.3% vs 50.6%, $P < 0.001$) was found in rural than in urban subjects. Indoor concentrations of endotoxin and cockroach were significantly higher in rural families, whereas *Dermatophagoides farinae* (*Der-f*) 1 was significantly lower. Exposure to *Der-f* 1 and *Der-f* sensitization was positively, whereas exposure to endotoxin negatively associated with asthma. No association between microbial antibodies and asthma was found.
Conclusion: Rural children from an agricultural background showed a reduced risk of asthma and atopy. Exposure to microbes *Der-f* 1, and *Der-f* sensitization, might contribute to the disparity in prevalence of asthma between rural and urban children in China.

Supported by the Natural Science Foundation of China (81261130023).

572
Correlation between skin prick test wheal sizes to 7 different pollen extracts in polysensitized patients in Spain

Subiza, J[1]; Fernández-Cáldas, E[2]; Pola, J[3]; Moral, Á[4]; Feo, F[5]
[1]Subiza Asthma and Allergy Centre, Madrid, Spain; [2]Inmunotek S.L, Madrid, Spain; [3]Allergy Unit, Sagasta Policlinic, Zaragoza, Spain; [4]Virgen del Valle Hospital, Allergy Unit, Toledo, Spain; [5]General Hospital, Allergy Unit, Ciudad Real, Spain

Background: Sensitization to various pollen species has practical implications in the management of allergic patients, especially in the diagnosis and treatment. The aim of this study was to detect correlations between the wheal sizes of the most important pollens implicated in allergic respiratory diseases in Spain.
Method: Thirteen allergy clinics from 13 different Spanish cities participated in this study. 1536 pollinosis patients were included (48% male and 52% female). All were born and still living in, or around, each study site. Mean age was 32 years (range 8 to 81 years). All patients were skin tested with a standardized, commercially-available, battery of aeroallergens at 50 HEP, which included *Cupressus arizonica, Platanus hispanica*, *Trisetum paniceum, Dactylis glomerata, Olea europaea, Chenopodium album* and *Parietaria judaica*. Steel lancets were used (one lancet per antigen, to avoid cross-contamination). Histamine chlorhydrate at 10 mg/ml was used as a positive control and 50% glycerol-saline as a negative control. All skin-test sites were evaluated after 20 min. The area of each wheal was measured by planimetry using an automated system. Skin prick test results were expressed in mm^2. A positive reaction was defined as a wheal of at least half the size of the histamine wheal in the absence of a reaction to the negative control. Spearman rank correlation coefficient was used to correlate the wheal area of different pollens.
Results: The Spearman rank correlation coefficient (r_s) between the wheal areas of *Trisetum* and *Dactylis,* grasses with a high degree of cross-reactivity was: 0.67, followed by *C. arizonica versus O. europaea*:

© 2015 The Authors

0.52; *P. hispanica versus P. judaica*: 0.43; *P. hispanica versus Ch. album*: 0.41; *Ch. Album versus P. hispanica: 0.37 and P. hispanica versus T. paniceum:* 0.37. All other correlations were below 0.3. The lowest correlations was obtained between *P. judaica versus O. europaea*: 0.03.

Conclusion: We have identified intriguing relationships between wheal sizes to different allergens in polysensitized patients in Spain. Some of these similarities may be due to cross-reactivity or to co-exposure to the offending allergens. This study may add valuable information to the identification of risk factors associated with polysensitization.

Acknowledgements to the whole study group.

573
Impact of climate and anthropogenic air pollutant exposure on pollen allergenicity and allergic sensitization

Beck, I[1,2]; Gilles-Stein, S[1,2]; Schneider, A[3]; Wolf, K[3]; Biernath, CJ[4]; Cyrys, J[3]; Klein, C[4]; Priesack, E[4]; Munch, JC[4]; Peters, A[3]; Traild-Hoffmann, C[1,2]
[1]Institute of Environmental Medicine, UNIKA-T, Technische Universität München, Munich, Germany; [2]CK Care, Christine Kühne Center for Allergy Research and Education, Davos, Switzerland; [3]Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology II, Neuherberg, Germany; [4]Helmholtz Zentrum München, Institute of Soil Ecology, Neuherberg, Germany

Background: Evidence is compelling for a positive correlation between urbanisation and increment of allergic sensitisation and diseases. The reason for this association is not clear to date. Some data point to a pro-allergic effect of anthropogenic factors on susceptible individuals. Data analysing the impact of environmental - natural and anthropogenic - factors on allergenicity of pollen grains are scarce. This study evaluates the effect of natural (e.g. climate) and anthropogenic (e.g. traffic pollutants) factors on birch pollen in a holistic approach. Moreover allergenicity of pollen will be placed in context to allergic sensitisation and symptoms of subjects (KORA-FF4) living in the surrounding of the respective trees.

Method: Therefore birch trees from urban and rural sites in the surrounding of KORA subjects in Augsburg were selected and sites were characterised for NO_2 and O_3 by passive samplers ($n = 40$). Temperature, NOx, PM2.5, PM10 and black smoke exposition of the trees were determined by temperature- and pollution models. Pollen was categorised according to maturation state and allergenicity was analysed by ELISA for Bet v 1, LTB_4 and PGE_2.

Results: Determing the Bet v 1 content for 2013 and 2014 a significant correlation of the Bet v 1 expression could be observed between the years. However while Bet v 1 concentrations of urban and rural trees do not differ significantly, analyses reveal that urban trees are exposed to significant higher NO_2- and lower ozone concentrations.

Conclusion: The results of this study will add to our understanding how urbanisation and climate change influence the allergenicity of birch pollen and how this will impact on allergic sensitisations and symptom severity.

© 2015 The Authors

Poster Discussion Session PDS 29

Drug allergy: From mechanisms to management

574

Patients with cutaneous non-immediate hypersensitivity reactions to drugs show a low expression of Tim3 during the acute phase of the reaction in skin and peripheral blood

Fernández, TD[1]; Palomares, F[1]; Ariza, A[1]; Doña, I[2]; Salas, M[2]; Ruiz, MD[2]; Blanca, M[2]; Torres, MJ[2]; Mayorga, C[1,2]
[1]IBIMA-Regional University Hospital of Malaga, Allergy Unit, Málaga, Spain; [2]IBIMA-Regional University Hospital of Malaga, Allergy Service, Málaga, Spain

Background: The involvement of Th1 lymphocytes in the development of cutaneous non-immediate hypersensitivity reactions to drugs has been proved, but the participation of other cell subtypes as Th17 is still unknown. The latter have shown effector activity in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis or psoriasis. In healthy conditions the interaction of Tim-3 with its ligand Gal9, can control the homeostasis of these cell subtypes inducing their apoptosis and the differentiation of regulatory T cells. The aim of this study is to assess the levels of Tim-3 in the skin and peripheral blood of patients with drug-induced-maculopapular exanthema (MPE) and -urticaria during the acute phase of the reaction.
Method: Skin biopsies from drug allergic patients with MPE ($N = 23$), urticaria ($N = 6$) and tolerant ($N = 16$) were obtained and processed for immunohistochemical staining, with CXCR3, Tim-3 and IL17 polyclonal antibodies. The presence of Th1, Th17 and T cells expressing Tim3 were assessed in peripheral blood from 18 patients (12 with MPE and 6 with urticaria) and 10 tolerant subjects by flow cytometry.
Results: We have observed a decrease of cells expressing Tim3 in MPE and urticarial compared to controls in the skin ($P = 0.000$ and $P = 0.017$ respectively) and in CD4 cells from peripheral blood ($P = 0.002$ and $P = 0.006$ respectively). This receptor was mainly decreased in CD4 Th1 cells, selected as CXCR3 + ($P = 0.002$ in MPE and $P = 0.006$ in urticaria). No changes were observed in the frequencies of Th17 cells in these patients compared with controls, but the presence of Tim3 was increased in these cells, although differences were not significant.
Conclusion: There is a decrease in the expression of Tim3 in the Th1 cells of patients with mild and moderate delayed hypersensitivity reactions to drugs. This could affect the homeostasis of this cell subtype in these patients. However, Th17 cells seem not to be involved in the development of these pathologies.

575

IgA and IgG antibodies directed against peptidoglycans must interfere in *in-vitro* assays for anti-penicillin specific IgE

de Boer, D[1]; Bons, JA[1]; Nieuwhof, CA[2]; Menheere, PP[1]
[1]Maastricht University Medical Center, Central Diagnostic Laboratory, Maastricht, the Netherlands; [2]Department of Internal Medicine, Immunology and Allergology, Maastricht University Medical Center, Maastricht, the Netherlands

Background: *In-vitro* assays for penicillin specific IgE (PEN IgE_{spec}) have been qualified as a poor choice or non-reliable. Moreover, the sensitivity of these assays is limited. Consequently *in-vivo* assays remain to be crucial in the diagnosis of penicillin allergy. Our hypothesis is that an endogenous interference is responsible for the non-optimal performance of PEN IgE_{spec} assays. Based on the mechanism, which explains the clinical effectiveness of penicillin, likely candidates to interfere are antibodies (Abs) against the dipeptide D-Ala-D-Ala. This dipeptide is a structural analogue of penicillin and a component of peptidoglycans, cell wall constituents of Gram-negative and -positive bacteria.
Method: Systematic literature review was performed by PubMed for the period of January 1, 1970 and December 1, 2014 using the search terms "peptidoglycan", "D-Ala-D-Ala", "immunoglobulin". Reference lists and citations were used to extend the number of hits. Inclusion criteria were the application of immunoassays based on peptides containing at least D-Ala-D-Ala or based on peptidoglycans in combination with inhibition using peptides containing at least D-Ala-D-Ala in order to detect antibodies with specificity towards D-Ala-D-Ala.
Results: Fourteen studies of interest were included directly, while indirectly three were included. The reports described heterogeneous profiles of Abs against oligopeptides containing the immunodominant C-terminal D-Ala-D-Ala. In healthy subjects these Abs were present in the range of < 5%, repeatedly even absent and occasionally up to 10% of the total Ab amount. The Ab isotypes were IgA and IgG and represented so-called "natural" Abs against Gram-negative and positive bacteria, probably from intestinal origin. Under pathological conditions (infections with Gram-positive bacteria) the percentage increased with outliers up to 20% for IgA of total IgA and 50% for IgG of total IgG ("pathological" Abs).
Conclusion: Abs against D-Ala-D-Ala must cross-react with Abs against penicillin. "Natural" as well as "pathological" IgA and IgG Abs against D-Ala-D-Ala have been reported and are directed against the peptidoglycans of Gram-negative and positive bacteria. Consequently, high concentrations of these Abs must interfere in PEN IgE_{spec} assays and might be the cause of poor qualifications and low sensitivity of those assays. Moreover, these Abs may play a role in the discrepancies between *in-vivo* and *in-vitro* assays for PEN IgE_{spec}.

576

Basophil activation test (BAT) in the diagnosis of immediate drug hypersensitivity to amoxicillin (AMX): comparison between children and adults

Barni, S[1]; Mori, F[1]; Sarti, L[1]; Valleriani, C[2]; Mangone, G[2]; Testi, S[3]; Saretta, F[4]; Azzari, C[2]; Novembre, E[1]
[1]Allergy Unit, A. Meyer Children's Hospital, Florence, Italy; [2]Department of Pediatric, A. Meyer Children's Hospital, Florence, Italy; [3]Allergy and Clinical Immunology Unit, San Giovanni di Dio's Hospital, Florence, Italy; [4]Department of Pediatric, A.A.S. 2 Bassa Friulana Palmanova's Hospital, Udine, Italy

Background: A new functional *in vitro* test, called basophil activation test (BAT), is going to be studied in the diagnostic management of immediate-type allergy to drugs and related compounds that are generally mediated by drug-specific IgE (sIgE) antibodies. The aim of this study was to study the BAT in children and adults with history of immediate reactions to AMX.
Method: Nine children (average age 8 years) and eight adults (avarage age 64 years), with clinical history of immediate reactions to AMX, were referred to Anna Meyer Children's Hospital and San

© 2015 The Authors

Giovanni di Dio Hospital, respectively. They underwent *in vivo* tests according to the European Network for Drug Allergy (ENDA) and the BAT was performed with the culprit drug, mostly 6 months from the reaction.

Results: In the pediatric group, six out of nine patients (66.6%) had both skin test and BAT negative, three out of nine patients (33.3%) had positive skin test and negative BAT. No one had positive BAT either those with skin test positive or negative. The concordance between skin test and BAT results was 66.6%. The specificity of BAT was 66.6%. Moreover the skin test positivity did not correlate with BAT results.

In the adult group, threeout of eight patients (37.5%) had both skin test and BAT positive, two out of eight patients (25%) had positive skin test and BAT negative and three out of eight patients (25%) had both skin test and BAT negative. The concordance between skin test and BAT results was 75%. The sensitivity and specificity were 100% and 24.4%, respectively.

Conclusion: In conclusion the BAT seems not to be improve the sensitivity of the allergy work-up in children otherwise in adults patients, with severe hypersensitivity reactions to AMX, seems to be useful, saving patients from *in vivo* tests. Our results should be confirmed in a greater number of patients.

577
Ex-vivo challenge tests with NSAIDs. Analysis of the role of basophil activation test

Lopez-Salgueiro, R[1]; Marti Garrido, J[2]; Pacheco Coronel, V[2]; Arévalos Prettte, J[2]; Durán López, B[3]; Hernández Fernández de Rojas, MD[2]
[1]Health Research Institute La Fe, Allergy, Valencia, Spain; [2]University Hopsital La Fe, Allergy, Valencia, Spain; [3]University of Valencia, Biotechnology College, Valencia, Spain

Background: In Spain, drug adverse reactions are the third leading cause of consultation to Allergy Departments. NSAIDs are the second group of drugs after beta-lactam antibiotics. The development of reliable and safe complementary tests is a highly demanded need at allergy diagnosis laboratories.

Objective: To study the usefulness of Basophil Activation Test (BAT) in the evaluation of NSAID induced immunological adverse reactions.

Method: Forty-three patients (both gender, no age restrictions) evaluated during 2013 and 2014 for suspicion of NSAID induced immunological adverse reactions were included. Intradermal tests were performed according to EAACI/ENDA recommendations with suspect culprit drug and a NSAID panel. BAT was performed with 2 concentrations of the culprit drug, using a commercial kit (BASOTEST®) following the manufacturer's protocol. Flow cytometric analysis was performed on a FACScan® flow cytometer and analyzed by CellQuest® software. The result was considered positive if basophil activation was > 5% or SI (stimulation index) >2.

Results: Female gender accounted for 58% of patients and 53% were over 15 years old. BAT was performed in all cases and intradermal skin tests were not performed to 17 patients. In the age group < 15 years old the culprit drug was ibuprofen in 16 patients (76%), dypirone in 4 (19%) and acetaminophen in 1 (5%). In the group of age >15 years old the culprit drug was dypirone in 10 patients (44%), ibuprofen in 7 (31%) and desketoprofen in 3 (13%). BAT was positive in 26 patients (60.46%) and negative in 17 (39.54%). BAT with ibuprofen was positive in 19 patients (82.60%) and negative in 5 (17.40%) while with dypirone was positive in 5 patients (38.43%) and negative in 9 (61.54%). Intradermal skin tests were positive in 10 (25%) patients and negative in 33 (75%). Ibuprofen skin test was positive in 2 (15.40%) patients and negative in 11 (84.60%). Dypirone skin test was positive in 4 patients (50%) and negative in 4 (50%).

Conclusion: The culprit drug varies with age: ibuprofen is the main culprit NSAID in people under 15 years and dypirone in people over 15 years. BAT showed a higher rate of positive results than intradermal tests, especially with ibuprofen. BAT seems to be a better diagnostic option than intradermal tests to confirm the causality of immunological adverse reactions due to ibuprofen.

578
The high impact of a suspicion of beta-lactam allergy in the clinical care of hospitalized patients: a case control study in a Dutch medical center

van Dijk, SM[1]; Gardarsdottir, H[2]; Wassenberg, MWM[3]; Oosterheert, JJ[4]; Röckmann, H[1]
[1]Department of Dermatology/Allergology, University Medical Center Utrecht, Utrecht, the Netherlands; [2]Department of Clinical Pharmacy, Division Laboratory and Pharmacy, University Medical Center Utrecht, Utrecht, the Netherlands; [3]Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, the Netherlands; [4]Department of Internal Medicine and Infectious Diseases, University Medical Center Utrecht, Utrecht, the Netherlands

Background: Suspected beta-lactam antibiotic allergy is a common cause for deviations of antibiotic treatment protocols and consumption of broad spectrum 'back-up antibiotics'. However, when evaluated, 80–90% of the patients is not true allergic.

Objective: To identify the prevalence of suspected beta-lactam antibiotic allergy in a large Dutch tertiary university medical hospital (UMC Utrecht) and its impact on antibiotic use in hospitalized patients.

Method: A retrospective case control study at the University Medical Center Utrecht. Between 1 July 2013 and 1 July 2014, patient reported and/or verified drug allergy was systematically registered of every admitted patient by means of a standardized questionnaire by a trained pharmacy assistant. Patients with a suspected beta-lactam antibiotic allergy registration were matched by sex, age and department of admission to three control subjects. Number and type of antibiotics prescribed, length of hospitalization, readmission within 12 weeks and mortality were compared between cases and control subjects.

Results: Of 17 959 admissions 1010 (5.6%) patients had a registration of beta-lactam antibiotic allergy. Of these, 997 were matched to 2939 control subjects (median age: 55 years; 67% female). Patients with a beta-lactam antibiotic allergy registration got significantly more different types of antibiotics prescribed (=1 antibiotic OR 1.28; 95% CI 1.09–1.49; =2 antibiotics OR 1.49, 95% CI 1.22–1.83) and received significantly less penicillins as control subjects (OR 0.45, 95% CI 0.34–0.60). Instead, cases were treated significantly more with macrolides and clindamycin (OR 3.32, 95% CI 2.49–4.43), quinolone antibacterials (OR 2.10, 95% CI 1.50–2.95) and tetracyclines (OR 2.21, 95% CI 1.55–6.66). Cases had a significant higher risk of readmission (OR 1.33, 95% CI 1.12–1.58) as compared to controls. There were no differences in length of hospitalization and mortality.

Conclusion: Hospitalized patients with suspected beta-lactam antibiotic allergy received more different types of antibiotics, especially 'back-up antibiotics' and had a higher risk of readmission compared to control subjects. These findings emphasize the importance of a verified diagnosis of beta-lactam antibiotic allergy.

579
Use of a herbal preparation Yin Qiao San for treatment of fever in children with hypersensitivity to paracetamol and non-steroidal anti-inflammatory drugs

Loh, W[1]; Liew, WK[1]; Chiang, WC[1]; Goh, A[1]; Lim, HH[1]; Chay, OM[1]; Kidon, MI[2]
[1]KK Women's and Children's Hospital, Singapore, Singapore; [2]Sheba Medical Center, Tel Hashomer, Israel

Background: Paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) are used extensively among children as antipyretic agents. Children with a diagnosis of

© 2015 The Authors

cross reactive hypersensitivity to both antipyretics are extremely limited in their choice for the treatment of fever. Some parents have resorted to using herbal preparations with no adverse problems. The aim of this study is to evaluate the feasibility of using a Chinese Proprietary Medicine, Yin Qiao San (YQS) for fever relief.

Method: An open label, one arm clinical trial using YQS for fever relief in these children with an upper respiratory tract infection was studied. Children between 1–18 years of age with a clinical or drug provocation test proven diagnosis of paracetamol and ibuprofen cross-reactive hypersensitivity were recruited. All eligible participants underwent an oral provocation test with YQS in the outpatient clinic. Those who had an adverse reaction during the oral provocation test or during the study course were withdrawn from the study. Children who passed the oral provocation test were instructed to take a prescribed dose of YQS when the temperature was $\geq$ 38.0°C and continued till the fever settled. Each episode of illness was recorded in a diary card. Time taken for fever resolution and any adverse events were collected.

Results: 21 children with a mean age of 10.7 years (Range: 2.1–16.2 years) were recruited. All had a clinical or drug provocation test proven diagnosis of paracetamol and ibuprofen cross-reactive hypersensitivity. In addition, 2 had a clinical diagnosis of diclofenac hypersensitivity and 1 failed an etoricoxib provocation test. All except one patient successfully tolerated an outpatient oral challenge of YQS. There were a total of 30 febrile episodes in the 1 year period. YQS was used safely, and found to be effective in reducing fever in 51% of fever epiosdes. There were 2 reports of urticaria after YQS use which were attributed to flare of recurrent spontaneous urticaria during the illness.

Conclusion: YQS was well-tolerated in patients with paracetamol and ibuprofen hypersensitivity with no reported severe adverse reactions. It was also found to be moderately effective in reducing fever during an upper respiratory tract infection.

580
Comparison of immediate adverse reactions induced by low osmolar nonionic radiocontrast media in computed tomography

Kim, DH
[1]Department of Internal Medicine, Yonsei University College of Medicine/Severance Medical Center, Seoul, Korea

Purpose: Immediate adverse reactions (IARs) by iodide radiocontrast media (RCM) are not uncommon, and different RCMs have been used in practice. The differences in the chemical structures of each RCM may have a role in the development of IARs. We attempted to compare the incidences of IARs caused by different low osmolar nonionic RCM used for computed tomography (CT).

Method: We reviewed 252,029 of 290,746 CT cases performed in a general hospital between January 2006 and December 2010. Incidences of IARs induced by iobitridol, iohexol, iopamidol, and iopromide were analyzed, and other risk factors, such as consecutive CT examination, age, volume, and concentration of iodide were evaluated.

Results: Subjects with consecutive multiple CT examinations with RCM (1.15%) showed a higher incidence of adverse reactions than the single RCM-CT group (0.62%) ($P < 0.001$). Concentrations of each RCM did not influence the incidences of IARs significantly. The incidence of IARs was greater in subjects older than 40 years old (1.05%) than in the younger-than-40-years group (0.51%; $P < 0.001$).

Use of iopromide resulted in the highest incidence of IAR cases (1.0%), followed by iopamidol (0.68%), iohexol (0.61%), and iobitridol (0.45%). The incidence of IARs by iopromide was significantly higher than those by the other RCM, and the use of iobitridol correlated with a significantly lower incidence of IARs than those correlated with the other RCM.

Conclusions: Incidences of IARs by each RCM may be different, and a simultaneous multiple RCM study increased the risk of IARs. Clinicians should choose the appropriate RCM based on the different risks of IARs caused by different RCMs.

Keyword: Adverse reaction, nonionic radiocontrast media

581
Turkish version of the drug hypersensitivity quality of life questionnaire: assessment of reliability and validity

Bavbek, S[1]; Kepil Ozdemir, S[2]; Doganay Erdogan, B[3]; Karaboga, I[4]; Buyukozturk, S[5]; Gelincik, A[5]; Yılmaz, I[6]; Goksel, O[7]; Dursun, AB[8]; Karakaya, G[9]; Kalyoncu, AF[9]; Ozseker, F[10]; Pasaoglu Karakis, G[11]; Oner Erkekol, F[12]; Koycu, G[12]; Keren, M[12]; Baiardini, I[13]; Romano, A[14]
[1]Department of Chest Diseases, Division of Allergy and Immunology, Ankara University School of Medicine, Ankara, Turkey; [2]Department of Internal Medicine, Division of Allergy and Immunology, Adnan Menderes University School of Medicine, Aydın, Turkey; [3]Department of Biostatistics, Ankara University School of Medicine, Ankara, Turkey; [4]Department of Psychiatry, Ankara University School of Medicine, Ankara, Turkey; [5]Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Allergy and Immunology, Istanbul University, Istanbul, Turkey; [6]Department of Chest Diseases, Division of Allergy and Immunology, Erciyes University School of Medicine, Kayseri, Turkey; [7]Department of Pulmonary Medicine, Asthma and Allergy Unit, Ege University School of Medicine, Izmir, Turkey; [8]Department of Internal Medicine, Division of Allergy and Immunology, Recep Tayyip Erdoğan University School of Medicine, Rize, Turkey; [9]Department of Chest Diseases, Division of Allergy and Immunology, Hacettepe University School of Medicine, Ankara, Turkey; [10]Department of Chest Diseases, Division of Allergy and Immunology, Sureyyapasa Chest Diseases and Thoracic Surgery Training and Research Hospital, Istanbul, Turkey; [11]Independent Doctor, Istanbul, Turkey; [12]Department of Chest Diseases, Division of Allergy and Immunology, Ataturk Chest Diseases and Thoracic Surgery Training and Research Hospital, Ankara, Turkey; [13]University of Genoa, Allergy & Respiratory Diseases Clinic - DIMI, IRCCS AOU San Martino-IST, Genoa, Italy; [14]Complesso Integrato Columbus, Allergy Unit, Rome, Italy

Background: The first disease-specific quality of life questionnaire in patients with drug hypersensitivity, Drug Hypersensitivity Quality of Life Questionnarie (DrHy-Q), was developed and validated recently. The aim of our study was to assess validity, reliability and responsiveness to interventions of the Turkish version of the DrHy-Q.

Method: The DrHy-Q was translated into Turkish using forward and back-translation procedures. The Turkish version of the DrHy-Q was administered to 736 patients with drug hypersensitivity from 10 allergy units. The psychometric properties of the questionnaire were evaluated by means of reliability and validity. To assess validity, all patients completed the validated Turkish version of Psychological General Well-Being Index (PGWBI). For test-retest reliability, 182 patients completed the DrHy-Q 1 week after the first questionnaire administration without any intervention. Responsiveness was assessed on 97 patients who had a DrHy-Q recorded at a follow-up visit after the intervention.

Results: A total of 736 patients with a reliable history of drug hypersensitivity were included in this study (73.6% female; median (minimum - maximum) age 42 (18–78)

© 2015 The Authors

years) and 711 patients completed the DrHy-Q. The internal consistency and test-retest reliability of the scale were adequate (Cronbach′s alpha=0.934, intra-class correlation coefficient=0.783). The uni-dimensional structure obtained for the original Italian version of the instrument was confirmed for the Turkish version of the DrHy-Q. The DrHy-Q scores showed weak negative correlations with the PGWBI total and domain scores ($r = -0.37$ to -0.254, $P < 0.001$). DrHy-Q was able to discriminate the patients with one drug hypersensitivity reaction from the patients with two and above two reactions ($P = 0.012$ and $P < 0.001$, respectively), and the patients who experienced a respiratory reaction from the patients who did not ($P = 0.018$). However, it did not discriminate the patients with comorbid disease including psychiatric comorbidity ($P > 0.05$). The baseline DrHy-Q scores were significantly higher than the post-intervention scores ($P = 0.008$).

Conclusion: The Turkish version of DrHy-Q is reliable and valid for evaluating quality of life in patients with drug hypersensitivity and it appeared responsive to interventions. This tool can be used in patients with drug hypersensitivity for both determining the subjective experiences and response to interventions.

582
Hypersensitivity to chemotherapy and atopy: are they connected?

Ornelas, C; Ferreira, R; Fernandes, N; Mendes, A; Caiado, J; Barbosa, MP
Santa Maria Hospital, North Lisbon Hospital Center, Lisbon, Portugal

Background: Patients who have hypersensitivity reactions (HSRs) to chemotherapeutic agents are believed to have a higher prevalence of atopy when compared to the general population. Platins and taxanes are among the most common chemotherapeutic agents currently in use. HSRs to platins are usually IgE-mediated while reactions to taxanes are caused by direct nonspecific degranulation of mast cells or basophils. We aimed at characterizing the atopic/allergic background of a group of cancer patients with HSRs to platins and taxanes that underwent drug desensitization.

Method: Data from 76 patients with HSRs to platins (carboplatin-19; oxaliplatin-23) and taxanes (docetaxel-21; paclitaxel-13) who were desensitized in the Immunoallergology Department were analyzed. The clinical history of atopy/allergy was assessed. Total serum IgE and inhalant Phadiatop® were obtained before the first desensitization. Statistical analysis was performed using the Mann-Whitney U test.

Results: The majority of the patients was female ($n = 67$, 88.16%) with a mean age of 55.9 years with no differences between the two groups. Only a few patients had positive clinical history of atopy/allergy, namely rhinitis, asthma, skin, food or drug allergy (platins $n = 4$, 9.52%; taxanes $n = 4$, 11.76%), with an overall prevalence of atopy of 10.52%, with no significant differences between both groups. Inhalant Phadiatop® was positive in 9 out of the 42 platin-sensitized patients (21.4%) as opposed with 4 out of 34 taxane-sensitized patients (11.8%). The median total serum IgE was above the reference value in use (< 87 mg/dL) for the platins group (90.05 mg/dl) and clearly higher than the one of the taxanes group (26 mg/dL) ($P < 0.0001$).

Conclusion: Unlike recent findings, in our cohort we did not find a higher prevalence of clinical allergy in patients with HSRs to chemotherapy when compared to the general population. Despite the low prevalence of clinical history of atopy, the results of the Phadiatop® suggest that patients reacting to platins are more likely to be sensitized to inhalant allergens. The high levels of total IgE found in the platinum group could be explained not only by a positive atopic background (shown by the Phadiatop®) but also by HSR to the platin itself. Following the IgE-mediated to the drug, patients present detectable platin-specific IgE (documented by different authors) which may lead to an increase of the total IgE. Larger studies will be needed to support these findings.

583
Evaluation of our drug allergy cases

Kont Ozhan, A[1]; Altintas, DU[1]; Dogruel Karagoz, D[2]; Bingol Karakoc, G[1]; Aycin Duyuler, G[1]; Yilmaz, M[1]
[1]Cukurova University Faculty of Medicine, Pediatric Allergy & Immunology, Adana, Turkey; [2]Baskent University Faculty of Medicine, Pediatric Allergy & Immunology, Adana, Turkey

Background: Drug adverse effects can be seen in % 5–15 of the patients treated with drugs. This proportion may increase up to %30 in hospitalized subjects. %5–10 of these adverse effects are allergic reactions which can be seen either as systemic anaphylactic reactions or restricted to a single organ. The aim of this study is to present the profile of 70 children who admitted to Cukurova University, School of Medicine, Pediatric Allergy&Immunology outpatient clinic between January 2010 and December 2014 with drug allergy.

Method: A total of 70 children (25 girls and 45 boys) who admitted to our outpatient clinic with drug allegy were enrolled to the study. Detailed anamnesis, physical examination findings, skin prick tests, allergen specific Ig E levels and provocation tests were used for the diagnosis of drug allergy.

Results: The subjects were aged between 9 months and 17 years. Penicillin/amoxicillin group antibiotics were the most accused drugs with % 37 ($n = 52$) followed by NSAIDs (%18, $n = 25$), sefalosporins (%15, $n = 21$), paracetamol (%8, $n = 12$), metamizol (%4, $n = 6$), klaritromisin (%3, n:4) and local anesthetics (%1, $n = 1$). Besides, various types and multi drugs were responsible from the %13 ($n = 18$) and %44 ($n = 31$) drug allergy respectively. Urticaria was the most seen finding with %55 ($n = 39$), followed by urticaria + angioedema (%20, $n = 14$), angioedema without skin rash (%10, $n = 7$), anaphylaxis (%8, $n = 6$), Steven Johnsons Syndrome (%3, $n = 2$) and urticarial vasculitis (%3, $n = 2$). While 4 of 42 subjects had drug specific IgE positivity, 8 subjects showed skin prick test positiviy with suspicious drugs. Provocation tests were performed to the 21 patients which had multi drug use as suspicious drugs were not detected with anemnesis and laboratory findings. Provocations were done with paracetamol ($n = 6$), NSAIDs ($n = 7$), amoxicillin ($n = 3$), clarithromycin ($n = 6$), ampicillin ($n = 1$) and penicillin ($n = 2$), ciprofloxacin ($n = 1$) and rifampicin ($n = 1$). Two subjects with NSAID showed urticarial rash, another one showed angioedema. 3 subjects provacated by clarithromycin and 1 subject provacated by paracetamol also showed urticarial rash.

Conclusion: Even though anamnesis, physical examination, skin prick test and *in vitro* tests hold important place in the diagnosis of drug allergy, drug provocation tests in order to show hypersensitivity to certain components should always be kept in mind as the gold standart in selected patients when necessary.

584
Betalactam allergy in children

Tziotou, M; Karantoumanis, D; Psomiadou, M; Tzeli, K; Kostoudi, S; Manolaraki, I; Roumpedaki, E; Douladiris, N; Manousakis, E; Xepapadaki, P; Papadopoulos, NG
National & Kapodistrian University of Athens, 2nd Pediatric Clinic, Allergy Unit, Athens, Greece

Background: Betalactams are often implicated in drug allergy in children, however in most cases allergy is not confirmed after extensive work-up. Recently, the need to perform *in vitro* and *in vivo* tests in children with suspected allergy to betalactams has been doubted. Our purpose was to describe the characteristics and evaluate the potential utility of skin testing prior to

© 2015 The Authors

challenge in betalactam allergy in a pediatric population.

Method: We retrospectively reviewed the medical records of children who underwent oral drug challenges to betalactams in our unit in 2014. Patients' and reactions' characteristics were recorded. *In vivo* tests in PPL, MDM, amoxicillin and cefuroxime were performed following the ENDA/EAACI guidelines.

Results: We included 29 children, 12 boys, mean age 4.5 y (range 1–12 year). 47 possible reactions were referred. The culprit drug was amoxicillin (AX) in 12 cases, amoxicillin/clavulanic acid (AX/CLV) in 18 cases, penicillin in 1 case, cefuroxime (CEF) in 5 cases, cefaclor in 5 cases, cefprozil in 3 cases and other cephalosporins in 3 cases.

Nine reactions were immediate and 38 non-immediate. Immediate reactions included 3 reactions with urticaria and dyspnea, 1 with urticaria and vomiting, 1 with urticaria and hoarseness, 3 with urticaria alone and 1 with maculopapular exanthema. Non-immediate reactions included 16 with maculopapular exanthema, 14 with urticaria and 2 with serum sickness like syndrome. In 7 reactions the type of exanthema was not defined.

In vivo tests were performed in the majority of children (25/29 children). One child with undefined rush on 7^{th} day of treatment with AX/CLV acid presented a positive ID test in AX at 48 h. All other SPTs/IDs were negative.

Thirty six drug challenges were carried out. In cases where according to medical history drug allergy was considered unlikely, challenge was carried out to the culprit drug (22 challenges). Otherwise, one of the tested drugs was given as an alternative. Two challenges were positive, not predicted by skin tests. The first an immediate type reaction in CEF and the second a serum sickness like syndrome in AX/CLV. The child with the positive ID test in AX tolerated CEF.

Conclusion: Betalactam allergy is overestimated in children, as it is not so frequent as referred by parents and pediatricians. We have confirmed the conclusions of recent studies that *in vivo* tests are not always helpful and may not be necessary. Drug challenge remains the gold standard.

© 2015 The Authors

Poster Discussion Session PDS 30

Hereditary angioedema

585

Characterising the safety and use of C1 inhibitor in routine clinical practice: interim results from a European Registry study

Aygören-Pürsün, E[1]; Magerl, M[2]; Martinez-Saguer, I[3]; Longhurst, H[4]; Caballero, T[5]; Straßen, U[6]; Staubach, P[7]; Martin, L[8]; Maurer, M[2]; Hamdani, M[9]; Andresen, I[10]; for the Cinryze Registry Study Group
[1]Department for Children and Adolescents, Angioedema Centre, University Hospital Frankfurt, Frankfurt, Germany; [2]Department of Dermatology and Allergy, Charité-Universitätsmedizin, Berlin, Germany; [3]Hemophilia Centre Rhine Main GmbH, Mörfelden-Walldorf, Germany; [4]Department of Immunology, Barts Health NHS Trust, London, United Kingdom; [5]Hospital La Paz Institute for Health Research (IdiPAZ), Biomedical Research Network on Rare Diseases (CIBERER, U 754), Madrid, Spain; [6]Technische Universität München, Hals-Nasen-Ohrenklinik und Poliklinik, München, Germany; [7]Department of Dermatology, University Medical Center, Mainz, Germany; [8]CREAK, French 'Centre de Référence des Angioedèmes à Kinines', Angers, France; [9]Shire, Lexington, United States; [10]Shire, Zug, Switzerland

Background: Human, plasma-derived C1 inhibitor (C1 INH) is approved for routine prophylaxis against angioedema attacks in adolescent and adult patients with hereditary angioedema (HAE) with C1 inhibitor deficiency. Data characterising the real world use of C1 INH in these patients are limited. Here we report interim data from an ongoing European registry of patients treated with C1 INH between May 2012 and October 2014.
Method: This is a European multi-centre, post-authorisation, observational study designed to characterise the safety and use of C1-INH in routine clinical practice during a 12 month follow-up period (NCT01541423).
Results: Forty-five patients (all ≥ 18 years of age at enrolment) from centres in Germany (60.0%), France (24.4%), Spain (11.1%) and the United Kingdom (4.4%) comprised the interim analysis population. 24 patients (53.3%) completed the study. Patients had a mean (SD) age of 42 (13.7) years and were predominantly women (66.7%). Indication for C1 INH use at enrolment included routine prevention (73.3%) and acute treatment of angioedema attack (26.7%). A total of 2603 doses of C1 INH were administered for routine prevention; 2269 (87.2%) were given at home. 1738 (66.8%) were self-administered, 642 (24.7%) were administered by health care professionals, and 223 (8.6%) by trained non-health care professionals. Of the 41 patients who received prescribed regimens for routine prevention, 19 (46.3%) unique patients received the approved dose-frequency of twice per week, every 3 or 4 days. Other prescribed regimens included less frequent and more frequent use of C1-INH for routine prevention. Eight patients (19.5%) received more than 1 prescribed regimen. A total of 3 adverse drug reactions were reported in 2 of 45 patients; all were rated mild in severity and included rash ($n = 1$, probably related) and abnormal liver function tests and hyperlipidemia (both in same patient, both possibly related). Two patients received C1 INH throughout pregnancy, each resulting in an uncomplicated singleton birth, no other serious adverse events were reported.
Conclusion: In this ongoing study in a real world setting, C1 INH administration was well tolerated, most patients self-administered therapy, and a variety of dose regimens were being used.

586

The effect of body mass index on icatibant treatment outcomes in patients with hereditary angioedema with C1 inhibitor deficiency: findings from the Icatibant outcome survey

Caballero, T[1]; Zanichelli, A[2]; Aberer, W[3]; Maurer, M[4]; Longhurst, H[5]; Bouillet, L[6]; Fabien, V[7]; Andresen, I[7]; for the IOS Study Group
[1]Hospital La Paz Institute for Health Research (IdiPaz), Madrid, Spain; [2]Università di Milano, Ospedale Luigi Sacco, Milano, Italy; [3]Medical University of Graz, Graz, Austria; [4]Charité - Universitätsmedizin Berlin, Berlin, Germany; [5]Barts Health NHS Trust, London, United Kingdom; [6]Grenoble University Hospital, Grenoble, France; [7]Shire, Zug, Switzerland

Background: The approved dose of icatibant is 30 mg and is not adjusted by patient body weight. However, the relationship between body mass index (BMI) and icatibant treatment outcome have not previously been evaluated in the real-world setting.
Method: The Icatibant Outcome Survey (IOS; NCT01034969) is an international, multicenter, prospective, observational study monitoring the safety and effectiveness of long-term treatment with icatibant. Data from icatibant-treated patients with normal BMI (NW; BMI < 25 kg/m^2) and overweight BMI (OW; BMI ≥25 kg/m^2) obtained between July 2009 and September 2014 were compared retrospectively using descriptive statistics.
Results: Data from 136 NW (23.5% male) and 133 OW (49.6% male) icatibant-treated patients from 11 countries were analyzed; BMI data were missing for 87 (49.4% male) patients. Median (min, max) BMI was 22.3 (16.7, 25.0) in NW patients, 28.1 (25.0, 46.7) in OW patients, and 24.9 (16.7, 46.7) overall. The number of attacks was comparable between the two groups (mean [median] attacks per patient was 5.2 [2.0] for NW and 6.1 [3.0] for OW patients). There were more abdominal attacks in NW patients (67.7% vs 49.1% of attacks in OW patients) but fewer skin (34.8% vs 45.3%) and laryngeal (4.5% vs 10.6%) attacks. Icatibant use was similar between the two groups; the mean (median) number of injections required per attack was 1.13 (1.00) for NW patients vs 1.10 (1.00) for OW patients. C1 inhibitor concentrate was used as a rescue medication in 14.8% and 5.4% of icatibant-treated attacks in OW and NW patients, respectively. Of note, OW patients had a shorter duration of attacks (mean 12.9 vs 18.2 h, $P = 0.0003$), time to resolution (mean 9.6 vs 12.4 h, $P = 0.023$), and time between attack onset and first injection (mean 3.3 vs 5.8 h, $P = 0.0001$). Adverse events (AEs) were reported by 40 NW (19.1%) and 31 OW (15.6%) patients. Of these, 11 NW patients (5.3%) and 4 OW patients (2.0%) reported icatibant-related AEs, 11 NW (5.3%) and 14 OW (7.0%) patients reported serious AEs, and 1 patient in the OW group reported 2 serious AEs related to icatibant (gastritis and reflux oesophagitis).
Conclusion: The frequency of attacks was similar between NW and OW patients. OW patients were not more likely than NW patients to require multiple injections of icatibant to treat an attack, and they had better treatment outcomes, possibly because they treated attacks earlier. Icatibant was well tolerated in OW patients.

© 2015 The Authors

587
Evaluation of adherence to treatment in patients with hereditary angioedema due to C1 inhibitor deficiency: a comparison of three alternative strategies

Squeglia, V[1]; Barbarino, A[2]; Gravante, C[1]; Bova, M[1]; Petraroli, A[1]; Di Paola, N[3]; Spanò, R[3]; Triggiani, M[4]; Marone, G[1]
[1]Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy; [2]Department of Public Health, University of Naples Federico II, Naples, Italy; [3]Department of Economics, Management, Institutions, University of Naples Federico II, Naples, Italy; [4]Department of Medicine, University of Salerno, Salerno, Italy

Background: Hereditary Angioedema with C1 inhibitor deficiency (C1-INH-HAE) is a rare autosomal genetic disorder presenting with recurrent episodes of swelling of the skin, gastrointestinal tract and upper airways. International guidelines suggest to treat all the attacks regardless of location and severity, and to administer therapy as soon as possible. However we noticed in our patients a variable adherence to therapy.

The aim of this study is to evaluate the compliance to the different therapeutic strategy used in our center.

Method: Sixty-one of 67 HAE patients followed in our department were enrolled in our study (7 patients were dropped out). The patients were divided in three groups:

1 24 patients in home therapy with pdC1INH;
2 11 patients self-administering icatibant;
3 19 patients treated with pdC1INH in healthcare setting.

The patients were interviewed monthly from August to October 2014. The interviews allowed to collect data about the total number of attacks, the number of treated attacks, as well as the time to infusion in the three groups. The compliance was evaluated by means of the percentage of treated attacks and the time from symptom onset to treatment administration.

Results: During the observation period there were 504 attacks, of which 288 were treated (57%). The analysis reveals that the adherence to therapy of the patients (group 1 + 2) that chose self-administration strategy was significantly higher than the patients (group 3) that performed health professional strategy (61% vs 23%; $P = 0.005$). In particular, in the home therapy group, the compliance was higher in patients that administered pdC1INH (66%) than the patients using icatibant (37%). The average time to start therapy was 1.9 h in first group, 1.5 h in the second group and 18.6 h in the last group.

Conclusion: Our data demonstrated a significantly better adherence to therapy in patients that choose self-administration than those performing health professional strategy, both for the percentage of treated attacks and for the time to infusion. In conclusion home therapy improves the efficiency of treatment of C1-INH-HAE, and in our opinion this strategy has to be implemented in all the centers treating C1-INH-HAE patients.

588
Subcutaneous human C1-inhibitor (C1-INH) with recombinant human hyaluronidase (rHuPH20) for the prevention of angioedema attacks in patients with hereditary angioedema (HAE): health-related quality of life (HRQoL) results from a randomized, double-blind, dose-ranging, crossover study

Weller, K[1]; Maurer, M[1]; Magerl, M[1]; Fridman, M[2]; Supina, D[3]; Schranz, J[3]
[1]Department of Dermatology and Allergy, Charité-Universitätsmedizin, Berlin, Germany; [2]AMF Consulting Inc., Los Angeles, CA, United States; [3]Shire, Wayne, PA, United States

Background: To estimate changes in HRQoL in patients with HAE with C1-INH deficiency who received subcutaneous human C1-INH with rHuPH20 for the prevention of angioedema attacks in a randomized, double-blind, dose-ranging, cross-over study.

Method: Forty-seven patients with a confirmed diagnosis of HAE were randomly assigned to receive subcutaneous injections of 1000U C1-INH with 24,000U rHuPH20 or 2000U C1-INH with 48,000U rHuPH20 every 3 or 4 days for 8 weeks and then crossed over to the other treatment for another 8-week period after a washout period of ≥7 but ≤30 days. The study was terminated early as a precaution related to non-neutralizing antibodies to rHuPH20. The Angioedema QoL Questionnaire (AE-QoL) (secondary study endpoint) was administered at the beginning and at week 5 of each of the two treatment periods and 1 week after the last dose of the second treatment period to measure HAE-related impairment. Changes in AE-QoL scores were calculated over the entire study (both treatment periods) and within each treatment period for patients with 4 or more weeks of treatment duration. The between-treatment difference in mean AE-QoL change was estimated using a repeated measures analysis of covariance model for end of period scores adjusted for beginning of period scores, age, gender, and treatment sequence.

Results: Forty-six patients had evaluable AE-QoL data on 67 treatment periods, and 22 patients completed treatment. Forty-three percent of patients were receiving prophylactic intravenous C1-INH at screening. AE-QoL scores are shown in the table. Adjusted between-treatment differences (1000U vs 2000U) were not statistically significant.

AE-QoL Score	Baseline ($n = 46$)	End of Study ($n = 42$)	Change from baseline to end of study ($n = 41$)	Mean (95% CI) Treatment Effect*
Functioning	34.38 (23.26)	19.94 (21.10)	−12.8 (26.37)	6.45 (−4.8, 17.7)
Fatigue/Mood	32.83 (23.47)	30.12 (25.46)	−1.95 (21.93)	−1.69 (−12.3, 8.9)
Fears/Shame	47.83 (26.52)	34.52 (28.62)	−10.26 (22.23)	−1.98 (−11.3, 7.3)
Nutrition	25.54 (26.21)	16.37 (21.11)	−7.62 (18.51)	5.34 (−3.0, 13.6)
Total	37.63 (21.52)	27.66 (21.14)	−8.11 (18.34)	0.96 (−7.2, 9.2)

[Mean (SD) AE-QoL scores at baseline and study end]
*Treatment effect = adjusted difference in AE-QoL score between 1000U and 2000U. Positive difference indicates advantage for 2000U. $P > 0.05$ for comparison of 1000U and 2000U doses.

Conclusion: Despite early study termination and pre-study use of prophylactic intravenous C1-INH by nearly half of patients, improved AE-QoL scores were observed after 8 or 16 weeks of prophylaxis treatment with subcutaneous C1-INH and rHuPH20. A non-significant dose-response relation was observed for the functioning and nutrition sub-scale scores.

589
BIOBRAD: study for acute bradykinin and histaminic-mediated angioedema attacks biomarkers

Deroux, A[1]; Dumestre-Perard, C[2]; Vilgrain, I[3]; Boccon-Gibod, I[1]; Bouillet, L[1]
[1]CHU de Grenoble, Internal Medicine, 38700, France; [2]CHU Grenoble, Immunology, Grenoble, France; [3]UMR1036 INSERM- CEA, Grenoble, France

Background: Angioedema (AE) is characterized by localized swelling of subcutaneous tissues or mucosa. There are two distinct subtypes of angioedema, caused by different pathological processes: histamin or bradykinin-mediated AE. In an emer-

© 2015 The Authors

gency department, it is essential that acute angioedema was identified as quickly as possible to resolve potentially life-threatening swelling. We carried out a prospective study evaluating the diagnostic value of different biomarkers in the AE crisis.

Method: The inclusion was made prospectively and patients were divided into 3 groups. All had bradykinin-mediated AE attack confirmed in a consultation in the French National Reference Center for AE (group 1), histamin-mediated AE (group 2), or abdominal pain of known etiology (control group or group 3). Clinical parameters (intensity of the crisis, ongoing treatment…) and laboratory tests were collected at baseline (H0) and at each sampling (3, 6, 12, 24, 48 h after the beginning of the attack). Another blood sample was performed 7 days later, without any symptoms. Markers explored were complement components (C1 inhibitor -or C1INH-activity and weight, C4 fraction), D-dimers, and serum VE-cadherin, a marker of vascular stress.

Results: Thirty one patients were included, 10 in group 1 and 3, 11 in group 2. For the group 1, D-dimer level was significantly higher during the crisis compared to the value of inclusion ($P < 10^{-3}$) and D7 ($P < 10^{-3}$); the level during a crisis was higher than in control patients ($P = 0.04$), but not than group 2. There were no changes in the rate of C1INH, C4 and C1INH activity. Morevover, the study of the kinetics of the VE-cadherin showed a significantly increased level during attacks in group 1 ($P = 0.002$), with higher values than group 2 ($P = 0.03$) and group 3 ($P = 0.01$). For the group 2 (histamin-mediated AE), D-dimer level was significantly higher during the crisis compared to D7 ($P = 0.04$); as expected, exploration of complement component was in normal value. Study of VE-cadherin rate was unremarkable for this group.

Conclusion: These results showed that D-Dimer level could be a biomarker for bradykinin-mediated AE attack. As expected, fraction of complement does not vary during the crisis. Finally, a marker of endothelial stress, VE-cadherin, could be considered as a biomarker for bradykinin-mediated AE attack, especially in abdominal crisis, which diagnosis is often difficult. These data need to be validated in largers cohorts of patients.

590

A new volume-reduced formulation of a plasma-derived C1 inhibitor concentrate reduces the time to treatment of acute hereditary angioedema attacks

Andritschke, K[1]; Martinez-Saguer, I[1]; Machnig, T[2]; Waldhauser, H[2]; Pfeifer, T[2]
[1]HZRM Hemophilia Center Rhine Main, Mörfelden-Walldorf, Germany; [2]CSL Behring GmbH, Marburg, Germany

Background: Plasma-derived C1 inhibitor (C1-INH) concentrate is a first-line treatment for acute attacks of hereditary angioedema (HAE) at any body location. Since HAE attacks can be life-threatening, timely and easy administration is an important aspect of treatment in an emergency situation. In the case of an acute attack, several vials of the current formulation of lyophilized C1-INH concentrate have to be prepared and injected for body-weight adjusted dosing. A new 10-fold volume-reduced formulation of the human plasma-derived, pasteurized and nanofiltered C1 inhibitor (pnfC1-INH, CSL Behring, Marburg, Germany) has been developed to reduce the time of preparation and administration (time to treatment). The objective of this investigation is to assess time to treatment with the volume-reduced formulation versus the current formulation of pnfC1-INH concentrate.

Method: A professional nurse simulated the setting and treatment administration in an emergency room. Three runs of preparation and administration were performed for each formulation (volume-reduced versus current pnfC1-INH concentrate) in accordance with respective product labelling. Using a 75-kg adult as an example, a total dose of 1500 IU was prepared and the respective dose of physiologic saline solution was administered to a healthy volunteer. This corresponds to 1 vial (1500 IU in 3 ml) of the volume-reduced versus 3 vials (500 IU in 10 ml each) of the current formulation. Institutional guidelines for aseptic technique, cannulation insertion, and intravenous administration were adopted as appropriate. The number and duration of all steps were monitored and recorded.

Results: The process of preparation and administration required fewer steps with the volume-reduced formulation than with the current formulation and time to treatment was reduced with the volume-reduced (mean 5:13 min) versus the current formulation (mean 7:55 min). With strict adherence to the labelled infusion times, differences were even more pronounced (mean 5:13 versus 12:00 min).

Conclusion: With the new volume-reduced pnfC1-INH concentrate formulation, not only the number of infusions is minimized but also time to treatment of acute, potentially life-threatening HAE attacks can be reduced. This is due to reduced complexity of having to reconstitute several vials and reduced intravenous infusion times because of a much lower infusion volume.

591

Hereditary angioedema: clinical characteristics and response to attack treatment with Berinert® in the French Cohort COBRA

Bouillet, L; COBRA Register
[1]Grenoble University Hospital, French National Reference Center for Angioedema, Grenoble, France

Background: The Cobra registry reports clinical data and treatment response with Berinert® in the French population of patients with Bradykinin mediated Angioedema (AE): Hereditary AEwith and without C1Inh deficiency, idiopathic non histaminergic AE, drug induced AE.

Method: We conducted an analysis of patients with HAE included in the National Angioedema Reference Centre from 2007 to December 2014. Data were obtained from the established *Cohort Berinert Angiædème* (COBRA) registry study, which aims to collate information on all Berinert-treated patients with HAE throughout France. The analysis included retrospective data extracted from patients' medical record and prospective data directly recorded in the electronic registry. Data on treatment response was only available for documented attacks after COBRA enrollment.

Results: Of 202 patients with C1Inh deficiency HAE ($n = 115$), normal C1Inh HAE ($n = 30$), drug induced AE ($n = 14$), idiopathic non histaminergic AE ($n = 43$) are today included in the registry. Among them 154 patients (76.2%) presented at least an attack treated with Berinert: 80.7% in C1Inh deficiency HAE, 70.0% in normal C1Inh HAE- 92.8% in drug induced AE and 60.5% in idiopathic non histaminergic AE. These patients presented 408 attacks: abdominal (44.7%), acial (34.1%), laryngeal (34.8%), and peripheral (18.9%). Intensity was severe in 60.4% in all AE: 52.9% in C1Inh deficiency HAE, 72.9% in normal C1Inh HAE, 84.6% in drug induced AE and 68.9% in idiopathic non histaminergic AE. On available data, symptoms began to be improved in less than 1 h in 45.7% (respectively 33.3%, 53.3%, 50.0%, 71.4%) and disappeared in less than 24 h in 64.2% (respectively 61.5%, 63.6%, 80.0%, 72.2%). Patients' satisfaction rates was 92.0% (respectively 93.3%, 82.1%, 100.0%, 95.8%). Data are

© 2015 The Authors

also available in the registry concerning on prophylaxis.

Conclusion: COBRA registry affords the opportunity to systematically describe all kind of bradykinin mediated AE patients treated with Berinert® and to monitor its efficacy in attack treatment or in prophylaxis.

592
Hereditary angioedema with normal C1 inhibitor: an Italian case series

Bova, M[1]; Bafunno, V[2]; Barca, MP[3]; Firinu, D[3]; Margaglione, M[2]; Marone, G[1]; Montinaro, V[4]; Suffritti, C[5]; Triggiani, M[6]; Zanichelli, A[5]; Zoli, A[7]; Cicardi, M[5]
[1]Allergy and Clinical Immunology, University of Naples 'Federico II', Naples, Italy; [2]Medical Genetics, University of Foggia, Foggia, Italy; [3]Unit of Internal Medicine, Allergy and Clinical Immunology, University di Cagliari, Cagliari, Italy; [4]Division of Nephrology, University Aldo Moro, Bari, Italy; [5]Department of Biomedical and Clinical Sciences L. Sacco, University of Milan, Milan, Italy; [6]Medicine, University of Salerno, Salerno, Italy; [7]SOD Immunologia Clinica e Tipizzazione Tessutale, Ospedali Riuniti Ancona, Ancona, Italy

Background: Hereditary Angioedema with normal C1-inhibitor (nC1-INH-HAE) with and without factor XII mutations (FXII-HAE and U-HAE respectively) were recently described as familial disorders. The clinical symptoms include recurrent, localized subcutaneous or submucosal edema lasting 2–5 days and involving the skin, tongue, upper airways and gastrointestinal tract. Patients do not respond to treatment with corticosteroides and anthistamines. In this study, we present the genetic and clinical features of a cohort of patients with nC1-INH-HAE followed up in centers of the Italian network for angioedema (ITACA).

Method: Fifty-nine patients with personal or family history of angioedema and normal plasma levels of C1 inhibitor were studied. Direct DNA sequencing for *F12* gene was performed in all patients. C1-INH function was measured by chromogenic assay; C1-INH and C4 antigen were measured by immunologic assay.

Results: Fifty-two patients had angioedema symptoms. Of these patients, 17 females (median age 41 years, range 10–76), belonging to seven unrelated families, had the same mutation in F12 gene, leading to the most common disease-causing aminoacid substitution, p.Thr309Lys. These patients were diagnosed with FXII-HAE. Seven subjects (5 males) in three FXII-HAE families were asymptomatic carriers of the same mutation. Thirty-five patients (57% F; median age 40 years, range 10–80) had history of angioedema in their 23 families and normal F12 gene sequence. These patients were diagnosed as having U-HAE. Sequencing analysis revealed the presence of different SNPs that have been previously described as not affecting protein activity or function. In both U- and FXII-HAE groups the median age at onset of clinical manifestations was 23 years. A majority of FXII-HAE patients experienced laryngeal (56%) and abdominal (81%) attacks and symptoms of skin swelling, involving arms (70%) or the face (88%). In the U-HAE group the percentages of attack location were similar except for the abdominal site, that was reported in 59% of patients. In both groups about 35% of patients reported tongue edema.

Conclusion: These data suggest that in Italy U-HAE is more frequent than FXII-HAE. Italian patients with FXII-HAE appear to come from the same common ancestor as those from other countries. We also confirm that males with *F12* gene mutation do not present angioedema symptoms. Clinical data are consistent with previously reported case series except for the lower percentage of patients reporting tongue swelling.

593
Hereditary angioedema with normal C1 inhibitor: prevalence, clinical features and treatment of patients in a University Hospital in Brazil

Aun, MV; Longen, E; Portilho, NC; Agondi, RC; Kalil, J; Motta, AA; Giavina-Bianchi, P
Clinical Immunology and Allergy Division, University of Sao Paulo School of Medicine, São Paulo, Brazil

Background: Hereditary angioedema with normal C1 inhibitor function (HAE-nC1INH), also known as HAE type III, is a familial condition which causes angioedema episodes of the skin, gastrointestinal tract, and airway. It is believed that HAE-nC1INH is much less prevalent than HAE due to C1 inhibitor deficiency, but the true prevalence is unknown. Being a rare condition, there are few groups of HAE-nC1INH patients described in the literature. We present a HAE-nC1INH sample from a University Hospital in Sao Paulo, Brazil.

Method: This is a cohort of HAE patients followed in our allergy and immunology outpatient clinic. Medical records of patients who had a confirmed diagnosis of HAE based on international guidelines were reviewed and analyzed. We described prevalence, clinical features and management of patients with HAE-nC1INH.

Results: We studied 110 patients with hereditary angioedema, of which 18 (16.4%) had diagnostic criteria for HAE type III. The mean age was 32.9 years (17–57) and only one patient was male. The first symptoms appeared, on average, at 20.3 years of age (13–34) and the average time between onset of symptoms and diagnosis was 7 years (0.5–12). Seventeen patients (94.4%) had already had subcutaneous edema, 10 (55.5%) presented abdominal pain and 6 (33.3%) reported laryngeal attacks. Only one patient had been intubated, but 3 had been admitted to the Intensive Care Unit due to the risk of asphyxia. The most cited trigger of angioedema was exogenous estrogen (contraceptives) in 74.5% of women, followed by spontaneous angioedema (61.1%) and local injury (44.4%). Eight patients (47.0%) became asymptomatic with no long-term prophylaxis only after the suspension of exogenous estrogen and six patients (35.3%) prevented attacks with progestins. The male patient and one female controlled the symptoms with danazol and two patients achieved control with tranexamic acid. Only one patient was treated with icatibant during four different attacks before starting long-term prophylaxis.

Conclusion: HAE-nC1INH seems to be more prevalent than previously believed. The clinical profile is similar to HAE with C1-INH deficiency, perhaps with less involvement of the upper airways. However, it is clear that estrogen play an important role in the pathogenesis of the disease, given that over 70% of our patients had attacks in the presence of exogenous estrogen and 82.3% became asymptomatic after the suspension or inhibition of estrogen with progestins.

594
Urticaria and angioedema as possible misleading skin symptoms in families with hereditary angioedema

Firinu, D[1]; Bafunno, V[2]; Barca, MP[1]; Meleddu, R[1]; Pisanu, M[1]; Matolo, A[1]; Serra, P[1]; Manconi, PE[1]; Margaglione, M[2]; Del Giacco, SR[1]
[1]Department of Medical Sciences, University of Cagliari, Monserrato, Italy; [2]Medical Genetics, Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

Background: Hereditary Angioedema (HAE) is a genetic disease, so far related to mutations in the genes encoding for the C1-esterase inhibitor protein (C1-INH) or for Factor-XII. Both the C1-INH-HAE and FXII-HAE share a phenotype of recurrent bouts of cutaneous and mucosal swellings lasting between 1 to 5 days, involving the skin, the gut and the respiratory tract. Bradykinin is involved as key mediator. Hence, urticaria is absent in these conditions, in which erythema marginatum (C1-INH-HAE) or skin brusing (FXII-HAE) may be found. Patients with a history of urticaria or allergic dis-

© 2015 The Authors

eases concomitant to the angioedema (AE) are usually excluded from further investigations for HAE. Histaminergic AE without wheals may show similar clinical features to non-histaminergic AE and the differential diagnosis could be challenging.

Method: During the workup of families with normal C1-INH AE and the index patient positive for a *F12* gene mutation, we recorded the symptoms, clinical and laboratory data of available family members.

Results: In two out four families identified with FXII-HAE, a total of 26 subjects were studied. Ten patients had the typical intermittent skin, mucosal and visceral swellings lasting 1–5 days, typical triggers and unresponsive to anti-histamines. Most attacks were associated with skin bruising in the sites of AE. Within these two families five subjects (including one with an history and symptoms strongly suggestive for HAE) presented in their history recurrent urticaria and/or AE. In four patients, the F12 gene resulted wild-type and they were diagnosed as having Chronic Spontaneous Urticaria (two patients), Chronic Urticaria due to to peach LTP (one patient), Idiopathic histaminergic angioedema (one patient). A single female asthmatic patient had the coexistence of clear-cut severe FXII-HAE symptoms since adolescence, and mild recurrent urticaria (1 episode/year) started at the age of 30. This patient carried the F12 mutation p.Thr328Lys; accordingly she is managed with prophylaxis using oral tranexamic acid and icatibant for acute attacks. On demand courses with antihistamines have been given for urticaria.

Conclusion: AE recognizes different etiologies and should be classified according to clinical and laboratory data. We highlight the role of a proper screening to identify patients with both histaminergic and non-histaminergic AE, even within HAE families. The screening of F12 gene is useful in these kindreds, in which urticaria can be a misleading symptom.

595
Difficulties encountered in emergency department in patients with hereditary angioedema

Ucar, R[1]; Arslan, S[1]; Baran, M[2]; Caliskaner, AZ[1]
[1]Department of Immunology and Allergic Disease, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey; [2]Department of Internal Medicine, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

Background: Hereditary angioedema (HAE) is a disease caused by quantitative or qualitative deficiency of complement one inhibitor (C1inh). The clinical course is episodic angioedema on the skin and mucous membranes and abdominal pain. Clinical features usually interfere with surgical emergencies and level of awareness is estimated to be low within doctors. In this study, difficulties encountered in emergency department (ED) of HAE patients were evaluated.

Method: Clinical features and difficulties encountered in the ED of 34 HAE patients were identified, those are being followed-up in Immunology and Allergy Clinic, Meram Faculty of Medicine, Turkey.

Results: The mean age at diagnosis of 34 patients (25 female, 9 male) was 31.8 ± 11.3 years, and delay in diagnosis was found to be 17.2 ± 9.7 years. In 58.8% of patients predominant attack was peripheral angioedema and in 23.5% of them outstanding symptom was abdominal attack. Thirteen of the patients (38.2%) reported that they were admitted to ED at least once a month. Abdominal attacks were as; abdominal pain only in 38.2%, abdominal pain and vomiting in 23.5%, and abdominal pain, vomiting and diarrhea in 29.4%. About 23.5% of the patients had been exposed to appendectomy, and 5.9% of them to exploratory laparotomy because of abdominal attacks. In addition, 38.2% of patients had a family history of death due to HAE attack.

Appropriate attack treatment was given in only 11.8% of patients in ED. About 52.9% of patients were directed to proper clinic after an attack. Although patients declared the diagnosis of HAE and keep C1-inh concentrate with them, 87.5% of them encounter resistance from ED personnel for administering C1-inh concentrate, on the pretext of 'We do not know this drug'. Further difficulties encountered were as follows; application process of C1-inh concentrate was not known (32.4%), HAE as a disease was not known (17.6%), absence of C1-inh concentrate in the referred hospital (8.8%), application of another ineffective treatments (8.8%), keep waiting much patients with head and neck edema although that can be fatal (5.9%). In addition, 5.9% of patients complained with the decrease in the interest of ED personnel due to their frequent ED administration (especially abdominal attacks).

Conclusion: At present, HAE has no curative therapy and some HAE patients are still succumbed because of fatal attacks. Our results suggest that the level of awareness about HAE and its treatment is not enough in ED physicians.

596
C1 esterase inhibitor concentrate for short-term prophylaxis in hereditary angioedema: findings from a large international registry

Craig, T[1]; Frank, MM[2]; Lumry, W[3]; Shapiro, R[4]; Feuersenger, H[5]; Rojavin, M[6]
[1]Asthma & Immunology, Penn State Hershey Allergy, Hershey, United States; [2]Duke University Medical Center, Durham, United States; [3]Allergy Specialists, Dallas, United States; [4]Midwest Immunology Clinic, Plymouth, United States; [5]CSL Behring Biotherapies for Life, Marburg, Germany; [6]CSL Behring, King of Prussia, United States

Rationale: The plasma-derived, pasteurized, nanofiltered C1-Inhibitor concentrate (pnfC1-INH; Berinert®/CSL Behring) is approved worldwide as an on demand treatment for hereditary angioedema (HAE) attacks and in Europe, also as pre-procedural short-term prophylaxis (STP). The use of pnfC1-INH as STP has not been as extensively studied as has on demand administration, and clinical practice data are needed to further evaluate this indication. An international patient registry was recently conducted to gather data on the safety, efficacy and prescribing patterns of pnfC1-INH for any indication, including STP.

Methods: The registry (34 US and 7 European sites) collected prospective and retrospective data between 2009 and 2014 on 318 HAE patients treated with a total of 15 000 infusions of pnfC1-INH for any indication. For patients using pnfC1-INH as STP, the analysis included assessments of dosing patterns and efficacy outcomes.

Results: Within the registry, there were data on 79 HAE patients [75% female; mean (SD) age 42.4 (17.6) years; range, 8–76 years] who used pnfC1-INH for STP, for a total of 149 STP infusions and procedures. The median dose per infusion was 1000 IU (range, 500–3500 IU), or 14.6 IU/kg (range, 3.6–33.9 IU/kg). Out of 79 subjects using pnfC1-INH for STP, 27 experienced 52 breakthrough attacks during the 7-day period following dosing. This corresponded to breakthrough rates of 0.66 attacks per subject and 0.35 attacks per STP infusion. The rate of breakthrough attacks through the first day following STP administration of pnfC1-INH was 0.04 per infusion, which increased to cumulative rates of 0.06 and 0.11 attacks per infusion at 2 and 3 days post-infusion, respectively. The median time between pnfC1-INH STP administration and breakthrough attack occurrence was 84.8 h (range 10.0–165.0 h). As measured over 7 days post-STP infusion of pnfC1-INH, the rates of breakthrough attacks per infusion by dose category were 0.53 (<15 IU/kg), 0.24 (15 to <20 IU/kg), 0.17 (20 to <25 IU/kg), and 0.07 (≥25 IU/kg).

© 2015 The Authors

Conclusions: Data from this international registry suggest that pnfC1-INH provides effective STP in HAE patients. A possible dose-response effect of pnfC1-INH prophylaxis was identified with regard to breakthrough attack rate, suggesting a dose dependent lowering of attack frequency, a finding which deserves further clinical investigation.

597

Current manifestations, diagnosis and treatment of hereditary angioedema (HAE): survey data from ninety-four physicians in Japan

Ohsawa, I; Honda, D; Hisada, A; Shimamoto, M; Inoshita, H; Mano, S; Tomino, Y
Division of Nephrology, Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan

Background: Hereditary angioedema (HAE) is a rare and potentially life-threatening condition resulting from mutations in C1-inhibitor (C1-INH). Awareness of HAE among physicians in Japan is growing, but real-world data are lacking. The objective of the present study was to evaluate the current state of clinical manifestations, diagnosis, quality of life (QOL), and treatment of HAE patients in Japan.
Method: A 14-point survey was developed and sent to 387 physicians in Japan (March–May, 2014) to gather clinical data on their HAE patients' family history, severity and frequency of attacks, QOL, and therapy usage.
Results: Responses were received from 94 of 387 (24.3%) physicians and data were collected for 171 (Male:Female = 54:117) HAE patients. The mean age was 43.6 years. Treatment for HAE had been supported by governmental co-paying in 108 cases (63.2%), while 44 cases (25.7%) had not involved governmental financial support. HAE type I occurred in 99 cases (57.9%), type II in nine cases (5.3%), HAE with normal C1-INH in three cases (1.8%), and a further 60 cases were unclassified. Of these patients, 76.6% had a family history of angioedema (AE) and 11.7% had experienced a death in the family due to an AE attack. Mean time from initial symptoms to the diagnosis was 13.8 years. Suffocation and abdominal surgery with uncertain diagnosis were observed in 9.5% and 2.9% of cases, respectively. In the past year, 21.0% of patients presented with >10 attacks, 21.1% were admitted to hospital for >1 day, and 28.7% were absent from work or school. In on-demand therapy, infusion of C1-INH concentrate was used in 81 patients (47.4%). Tranexamic acid (TA) was used in 50 patients (29.2%). Four patients (2.3%) were treated with androgens. In prophylaxis, TA was used in 39.2% among 171 patients. A combination of TA with other drugs, including Chinese herbs, and anti-histamines, was used in 9.4% of cases. Androgens alone were used in 6.4% of cases and the combination of androgen and TA in 4.1% of patients. Androgens were also used with anti-histamines in 0.6% of cases and 4.1% were treated with Chinese herbs or anti-histamines.
Conclusion: As the same as other Western countries, HAE is also characterized by recurrent AE attacks and the diagnosis of HAE patients have been delayed in Japan. To reduce the burden of the disease, we should keep on up-dating treatment options and educating not only the physicians but also medical staff.

598

Adverse events in patients treated with attenuated androgens for the long term prophylaxis of hereditary angioedema

Lleonart, R[1]; Andrés, B[1]; Vallano, A[2]; Girbau, A[3]; Corominas, M[1]
[1]Allergology, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Spain; [2]Pharmacology, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Spain; [3]Hepatology, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Spain

Background: Long-term prophylaxis (LTP) is indicated in patients with hereditary angioedema (HAE) that have more than one severe, acute episode per month. Attenuated androgens (danazol and stanozolol) have proved useful in reducing the frequency or severity of attacks. However, their use as maintenance treatment may be accompanied by side effects.
Method: We reviewed the patients diagnosed with HAE that have been treated with androgens for LTP with regular follow-ups in the Allergy Unit of our hospital, in order to evaluate any potential side effects of this treatment.
Results: The reported population consisted of three females and three males (27–69 years old, mean age 40.5 years). In three of the patients there have been serious conditions that could be due to the treatment with androgens. Two patients, a 66 year old man and a 34 year old woman, developed elevated liver enzymes (predominantly parameters of cytolysis) while receiving low doses of stanozolol (1–2 mg/day). Other causes of liver enzyme elevation were excluded in both cases and the transaminase levels returned to normal after discontinuing the androgens. The 66 year old patient had tolerated the administration of stanozolol 1 mg/day during 10 years without any alteration in the enzyme levels during monitoring. In the female patient AST increased three times and ALT increased eight times their normal value after 3 weeks of treatment with stanozolol. The relation between the transaminitis and the androgens is definitive because she experienced again the hepatotoxicity when she was re-exposed to stanozolol a few years later. A 37 year old woman with essential thrombocythemia (JAK 2 negative) and HAE, was diagnosed with a deep portal thrombosis and Budd–Chiari syndrome while she was taking danazol 600–200 mg/day for more than 20 years. She was treated with fibrinolysis and indefinite anticoagulant treatment was recommended, danazol was discontinued. The other three patients treated with androgens (stanozolol 3 mg/day, danazol 50–100 mg/day) tolerated the treatment without any significant serious side effect.
Conclusion: A significantly high percentage of our HAE patients treated with androgens had a serious adverse effect. When prescribing androgens for LTP in HAE patients, we have to consider these effects associated with these treatments, mainly in women and patients with co-morbidities. In all patients we should use the lowest effective dose and carefully monitor them for potential side effects.

© 2015 The Authors

Poster Discussion Session PDS 31

Safety and efficacy of AIT

599

Clinical relevance of the SQ HDM SLIT-tablet in patients with moderate-severe house dust mite allergic rhinitis

Demoly, P[1]; Mosbech, H[2]; Hansen, B[3]; Rehm, D[3]; Krčmová, I[4]; Kleine-Tebbe, J[5]
[1]Division of Allergy, Department of Pulmonology, Hôpital Arnaud de Villeneuve, University Hospital of Montpellier, Paris, France; [2]Allergy Clinic, Copenhagen University Hospital Gentofte, Gentofte, Denmark; [3]ALK, Hoersholm, Denmark; [4]Institute of Clin. Immunology/ Allergology, Faculty Hospital (Charles University) Hradec, Kralove, Czech Republic; [5]Allergy & Asthma Center Westend, Outpatient Clinic & Research Center, Berlin, Germany

Background: The MT06 trial (EudraCT2011-002277-38) confirmed efficacy of the SQ HDM SLIT-tablet in adult subjects with moderate-severe house dust mite (HDM) allergic rhinitis (AR). The treatment effect was above the pre-specified criterion for clinical relevance (difference to placebo in TCRS >1) and was statistically significant from 14 weeks of treatment and onwards. This abstract presents findings from the trial, further illustrating the clinical relevance of the observed efficacy.
Method: The trial was a randomised, double-blind, placebo-controlled phase III trial including 992 adults with moderate-severe HDM AR despite having received symptomatic treatment. Subjects were randomised 1:1:1 to 1 year of daily treatment with placebo, 6 or 12 SQ-HDM sublingual tablets (1:1 mixture of allergen extracts from *D. pteronyssinus* and *D. farina*). The primary endpoint was the total combined rhinitis score (TCRS). Key secondary endpoints supporting clinical relevance were rhinitis quality of life questionnaire (RQLQ) and AR symptoms. Two post-hoc endpoints concerning days with a rhinitis exacerbation and mild days were calculated. A rhinitis exacerbation was defined as a day with reoccurrence to the level of symptoms required for inclusion (at least two moderate or one severe symptom). A mild day was defined as a day where no single AR symptom scored higher than 1 (mild) and with no need for oral antihistamine for symptom relief.
Results: After 1-year treatment the difference to placebo was statistically significant for 12 SQ-HDM for the overall RQLQ and for four of the seven RQLQ domains: nasal symptoms, non-nose/non-eye symptoms, practical problems and sleep impairment. In addition all four rhinitis symptoms were statistically significantly improved compared to placebo. The estimated probability of experiencing a rhinitis exacerbation was 11.1% for placebo and 5.3% with 12 SQ-HDM (OR: 0.45, $P < 0.05$). The estimated probability for experiencing a mild rhinitis day was 16.4% with placebo and 34.2% with 12 SQ-HDM (OR: 2.66, $P < 0.001$).
Conclusion: Treatment with 12 SQ-HDM significantly improved AR symptoms and RQLQ. Furthermore, the treatment halved the patient′s probability for having a rhinitis exacerbation day and doubled the probability for having a mild day with no more than minimal awareness of the patient′s HDM AR. Taken together these findings further illustrate the clinical relevance of the efficacy seen in the MT06 trial with the SQ HDM SLIT-tablet in moderate-severe HDM AR.

600

Safety of a 300IR house dust mite sublingual immunotherapy (SLIT) solution in patients with moderate, persistent asthma

Devillier, P[1]; Fadel, R[2]; de Beaumont, O[2]
[1]Foch Hospital, UPRES EA 220, Suresnes, France; [2]Stallergenes, Antony, France

Background: House dust mites (HDM) are the most prevalent inhaled allergen wolrdwide. Primary outcomes of a randomized, double-blind, placebo-controlled study of HDM SLIT, performed in China among adult patients with mild-to-moderate, persistent asthma have been reported previously. Here, we report safety results in more detail.
Method: Patients with mild or moderate, persistent, HDM-induced asthma for at least the previous 12 months ($n = 484$) were randomized 2:1 to 12 months of daily sublingual treatment with either a 300IR solution of Dermatophagoides pteronyssinus and Dermatophagoides farinae extracts or placebo. At each clinic visit, adverse events (AEs) were recorded and a physical examination, including vital signs, was performed. Laboratory safety tests were performed at the beginning and end of the study.
Results: The overall incidence of AEs was 87% in the 300IR group and 76% in the placebo group. Adverse reactions (i.e. treatment-related AEs) were reported in 79% of patients receiving SLIT, vs 48% of placebo recipients. The most frequent adverse reactions were oral (300IR: 69% vs placebo: 32%), principally oral pruritis (300IR: 24% vs placebo: 15%), tongue swelling (300IR: 27% vs placebo: 2%), glossitis (300IR: 20% vs placebo: 10.5%), cheilitis (300IR: 11% vs placebo: 6%) and oedema of mouth (300IR: 8% vs placebo: 0%), while abdominal pain (300IR: 31% vs placebo: 14%) was the most frequent non-oral adverse reaction. The incidences of most oral adverse reactions occured more frequently during the dose escalation phase (first 15 days of treatment) than in the dose maintenance phase (after 15 days). Most adverse reactions were mild or moderate in severity, the incidences of mild and moderate adverse reactions being 71% vs 43% and 30% vs 15% with 300IR vs placebo. Eight (2.5%) patients in the 300IR group and 1 (0.6%) patient in placebo group of patients stop the treatment due to AEs. Serious AEs, all unrelated to study drug, affected four patients in the 300IR SLIT group and one patient in the placebo group. No significant between-group differences were observed in laboratory tests, vital signs or physical examinations. No significant difference in the number of asthma exacerbation in the two groups and/or decrease in FEV1 (300IR: 16% vs placebo: 12%) was observed.
Conclusion: Treatment of adults asthmatic patients with a 300IR HDM SLIT solution was well tolerated and did not induce a risk of asthma exacerbations.

601

Treatment satisfaction during sublingual immunotherapy with a 5-grass pollen tablet for allergic rhinoconjunctivitis: a prospective, non-interventional study

Hadler, M; Karagiannis, E
Stallergenes GmbH, Kamp-Lintfort, Germany

Background: Sublingual immunotherapy (SLIT) is a safe and well-tolerated alternative to allergen injection immunotherapy for treating allergic rhinoconjunctivitis. As

© 2015 The Authors

for all long-term treatments, patient compliance is essential. Patient-related outcome measures such as treatment satisfaction are informative, as these are indicative of compliance.

Method: A prospective, open, non-interventional, post-authorization study was conducted over 1 year to assess patient satisfaction with pre- and co-seasonal SLIT with a 5-grass pollen tablet (Stallergenes, Antony, France) for allergic rhinoconjunctivitis. The satisfaction of patients was assessed with a QUARTIS questionnaire; QUARTIS is a self-reported questionnaire with 28 items dedicated to the management of patients treated with SLIT for allergic rhinoconjunctivitis. It assesses patients' expectations, satisfaction, adherence, persistence and attitude towards SLIT.

Results: Of the 327 patients included in the study, 253 had at least one evaluable item of the QUARTIS questionnaire and were included in the analysis of patient satisfaction. Between the reference and treatment seasons, significant improvements were documented in nasal and ocular symptoms, and in the effect of allergy in everyday life. At the end of the treatment season, patients' also had an improved opinion of the ease of SLIT intake and a significantly improved perception of SLIT. On completion of the first year of pre- and co-seasonal SLIT, satisfaction and motivation to continue SLIT the following year was high. The need for symptomatic medication was reduced throughout the study, and SLIT was well tolerated.

Conclusion: Effectiveness, tolerability and patient adherence are important factors in determining the success of a treatment. This study found a high level of patients' satisfaction with SLIT which, together with the effectiveness and tolerability previously documented in controlled clinical trials, will help to improve treatment adherence and success.

602

Safety of the 5-grass pollen sublingual tablet for the treatment of grass pollen-induced allergic rhinoconjunctivitis in subjects with and without asthma

Zeldin, RK; Cognet-Sicé, J; Abiteboul, K
Stallergenes S.A., Antony, France

Background: Allergic asthma is frequently associated with grass pollen-induced allergic rhinoconjunctivitis (ARC). Here we present the safety experience across the development program of the 5-grass pollen extract sublingual tablet by asthma status.

Method: Subjects (5–65 years) with medically confirmed grass pollen-induced ARC for at least 2 years were randomised in one of eight double-blind placebo-controlled trials of the 5-grass pollen tablet. Subjects with asthma requiring treatment with nothing more than a beta-2 agonist were eligible. Asthma status (presence/absence) was recorded at randomisation. Treatment was administered outside the pollen season in two studies (for 10 days and 4 months, respectively), 4 months pre-seasonally and co-seasonally in four studies and 2 months pre- and co-seasonally in one study. In one study, both the 2- and 4-month pre-seasonal and co-seasonal regimens were evaluated. Adverse events (AEs) were similarly monitored throughout the studies. Safety data were pooled and analysed descriptively. AE terms were screened for those suggestive of asthma (e.g. asthma, cough, dyspnoea, and wheeze). In subjects with asthma at randomisation, these AE reports were further reviewed to identify any potential worsening.

Results: A total of 2512 subjects (2200 adults and 312 children/adolescents) were included in the safety database. Of these, 425 (17%) had asthma at baseline (Active = 242; Placebo = 183) and 2085 did not (Active = 1270; Placebo = 815). Percentages of actively treated subjects reporting at least one AE during treatment were similar in those with and without asthma (81% and 76%, respectively) as were percentages of those experiencing at least one treatment-related AE (60% and 58%, respectively). AEs leading to discontinuation were also reported at a similar incidence in both subsets (5.8% and 5.0%, respectively) and were mostly application site reactions. Of the 425 subjects with asthma, 101 [Active 54/242 (22%); Placebo 47/183 (26%)] reported an AE of asthma or a related symptom during treatment. Three actively treated participants (1.2%) and 10 who received placebo (5.5%) were administered oral corticosteroids as treatment for an AE suggestive of asthma. There were no serious drug-related AEs in any subject with asthma who received the 5-grass pollen sublingual tablet.

Conclusion: Treatment with 5-grass pollen extract sublingual tablet had a similar safety and tolerability profile in subjects with and without intermittent asthma.

603

Efficacy of sublingual allergen immuntheraphy on ragweed allergic patients. Open, controlled, one year follow up clinical study

Balogh, K[1]; Mohácsi, E[2]
[1]Buda Allergy Center, Allergology, Budapest, Hungary; [2]St. John's Hospital, Allergology, Budapest, Hungary

Background: Data in literature confirm the spread of ragweed in many countries in Europe and in North America. The number of days with concentration levels causing symptoms is increasing due to growing spread and climate conditions. Ragweed causes serious inhalative symptoms among allergic patients, posing a formidable challenge to health care providers.

Method: We continued sublingual allergen immuntheraphy with standardized Staloral ragweed extract for 3 years with 86 patients suffering from allergic rhinitis from ragweed pollen. During the therapy we recorded the symptom score and the use of the rescue medication. Upon conclusion of the therapy, we followed the patient for a year to monitor the change in their symptoms and use of symptomic medicine. We compared results with that of a ragweed allergic control group of similar age and sex who did not receive SLIT. We also recorded ragweed pollen data during the therapy and the follow-up year.

Results: The symptom and medication scores have significantly decreased in the treatment group during the 3 years of the therapy (nasal symptoms: 2.55–0.30; eye symptoms 2.21–0.21; symptomic medicine use: systemic antihistamines: 78–15%, intranasalis steroid: 41–6%, antihistamin eye drops: 38–7%) and these low symptom scores kept low during the follow-up year (nasal symptoms: 0.30–0.47; eyes: 0.21–0.28). We recorded significant differences in the follow up year with the control group in terms of symptom score, medication score and days with symptoms (nasal symptoms: 0.47–2.67; eye symptoms 0.28–1.94, days with symptoms: 3.4–41.5).

Conclusion: Sublingual allergen immuntheraphy provided an effective and lasting reduction in the symptoms of patients with allergic rhinitis from ragweed regardless of high ragweed pollen concentration.

604

Safety of subcutaneous immunotherapy with inhalant allergen extracts: a single-center 30-year experience from Turkey

Kartal, O[1]; Gulec, M[1]; Caliskaner, Z[2]; Musabak, U[1]; Sener, O[1]
[1]Division of Immunology and Allergic Diseases, Gulhane Military Medical Academy and Medical School, Ankara, Turkey; [2]Division of Immunology and Allergy, Necmettin Erbakan University Meram Medical Faculty, Konya, Turkey

Background: Although subcutaneous allergen immunotherapy (SCIT) is effective in allergic rhinitis and asthma, it carries a risk of local and systemic adverse reactions. The aim of this study was to evaluate the rates and clinical characteristics of local and systemic reactions (LR-SR), and to identify their relation of demographic features, allergen extracts and diagnosis.

© 2015 The Authors

Method: This study analyzed the administration of SCIT from 1983 to 2013; involving 1816 patients (2114 immunotherapy protocols and approximately 262 300 injections) suffered from respiratory hypersensitivity. Systemic reactions were adapted and graded according to the World Allergy Organization (WAO) 2010 grading system. Local reactions were quantified by measuring the diameter of erythema and/or swelling diameter at the injection area; those with a mean diameter of ≥5 cm were considered as large local reactions (LLR) and from 5 mm to 5 cm were described as small local reactions (SLR).

Results: The rates of SR from SCIT were 0.078% per injection and 9% per patient. According to the WAO 2010 grading system, 91 grade 1 reactions (44%), 67 grade 2 reactions (32.3%), 33 grade 3 reactions (16%), and 16 grade 4 reactions (7.7%) were seen. There was no fatal outcome from any of the SRs. Risk factors for a SR included: aluminium adsorbed extract, pollen containing vaccines, LLR and recurrent (≥2) LRs.

The total LR rates were 0.062% per injection and 5.2% per patient; the SLR rates were 0.027% per injection and 2.3% per patient, and the LLR rate were 0.035% per injection and 2.9% per patient. Female gender, depot extracts, calcium phosphate adsorbed extract, and pollen vaccines were identified as risk factors for LR.

Conclusion: The analysis of our data over a 30-year period confirmed that SCIT with inhalant allergens conducted strictly according to the standard protocols and when administrated by experienced staff, is a safe method of treatment.

605
A double-blind, placebo-controlled study of allergen immunotherapy with depigmented-polymerised *Phleum pratense* pollen extract in allergic rhinoconjunctivitis, a placebo-based analysis

Sánchez Alonso, A[1]; Carretero Anibarro, P[2]; Mencía Bartolomé, J[3]; Cerecedo Carballo, I[4]; Miñano Oyarzábal, A[4]; Gómez Sánchez, MJ[4]; Levitch Merán, R[4]
[1]Allergy Department, Hospital Universitario Río Hortega, Valladolid, Spain; [2]Allergy Department, Hospital Universitario de Burgos, Burgos, Spain; [3]Allergy Department, Hospital El Bierzo, Ponferrada, Spain; [4]Laboratorios Leti, Tres Cantos, Spain

Background: Subcutaneous immunotherapy (SCIT) is a disease-modifying treatment for allergic diseases.

Our objective was to evaluate efficacy and safety of a depigmented-polymerized *Phleum pratense* pollen extract in the treatment of rhinoconjunctivitis with of without seasonal asthma of grass-pollen sensitized patients.

Method: A double-blind, placebo-controlled trial of 153 *Phleum pratense* pollen allergic adults with a median age of 33 years was performed in Spain and Hungary. SCIT consisted of four increasing doses at 7-day intervals, then maintenance injections every 4 weeks for 23 months. Although wWe have previously reported the primary outcome, we now present a placebo-based analysis as proposed and published by other authors. Combined symptom and medication score (SMS), symptom score (SS) and medication score (MS)) during two pollen seasons were calculated including all days for which the mean SS of the Placebo group was >2.

Results: No differences in gender or grass pollen specific IgE (15.5 kU/l vs 13.4 kU/l) between groups and there was a similar distribution of asthma at baseline, around 30% in both groups. For both seasons, the differences between the treatment groups were statistically significant (SMS median; first pollen season: 3.31 vs 4.55, second pollen season: 2.60 vs 5.31; ITT- set) with *P*-values of 0.04 and <0.0001 respectively in favor of Active group). Also the median SS and MS only were clearly lower in Active group compared to the Placebo group. SS median; first pollen season: 2.65 vs 3.65, second pollen season: 1.91 vs 3.23 respectively; ITT- set) with *P*-values of 0.05 and <0.0001 respectively. MS median; first pollen season: 0.70 vs 1.10, second pollen season: 0.44 vs 1.26; ITT- set) with *P*-values of 0.35 and <0.0001 respectively. Side-effects were minimal, 0.1% of the total number of injections were followed by local reaction (the majority of them were mild) and only three systemic reactions (all grade I) were registered in actively treated group.

Conclusion: Depigmented polymerized *Phleum pratense* pollen extract was clinically effective compared to placebo and well tolerated.

606
A prospective observational post-authorisation study of the safety, tolerability and effectiveness of Acarovac Plus: a modified, mite allergoid subcutaneous immunotherapy powered by MCT technology

Roger, A[1]; Depreux, N[1]; Jurgens, Y[1]; Heath, MD[2]; Mwange, JD[2]; Garcia, G[2]; Skinner, MA[2]
[1]Unitat d'Allèrgia, Hospital Universitari Germans Trias Pujol, Barcelona, Spain; [2]Allergy Therapeutics Plc, Worthing, United Kingdom

Background: Allergy to house dust mite is one of the most common causes of allergic rhinitis. A novel Micro Crystalline Tyrosine (MCT)-adsorbed, modified allergen product, Acarovac Plus, developed for the treatment of perennial mite allergy seeks to address the underlying cause of allergic rhinitis in this instance. We sought to compare the safety and tolerability of the up-dosing schedules, satisfaction and short/long term effectiveness of Acarovac Plus with *Dermatophagoides pteronyssinus* in adult patients (18–65 years) with allergic rhinitis and/or asthma.

Method: Patients were divided into two dosing groups: conventional (0.05, 0.1, 0.3, 0.5 ml. weekly) and cluster (0.05 + 0.1 ml, 0.1 + 0.3 1 week later, 0.5 ml 1 month later). The effectiveness of the extract was assessed by nasal provocation test (NPT) measuring peak nasal inspiratory flow (PNIF) and symptoms (SEAIC guidelines) and *in vitro* immunologic changes (IgE, IgG_4 and cytokines such as IL-4, IL-5, IL-10 and IL-13). These tests were performed three times: before the first administration of the vaccines (visit 1), at a 3–5 week visit with the first maintenance dose (visit 2) and at a 1 year visit (visit 3). The satisfaction was monitored with a validated questionnaire (TSQM) answered by the patients in visit 2 and 3.

Results: No adverse events were reported during the up-dosing schedules, in either the conventional or cluster regimens. In the NPT, symptoms decreased 18.6% and 54.9% in visits 2 and 3 respectively. A significant decrease in the drop of PNIF was also recorded in visits 2 (34.5%) and 3 (50.8%). A significant increase in IgG4-specific antibody titres was measured in visits 2 and 3. There were no significant changes in total or specific IgE during the study. A significant increase was observed in the IL-5 and IL-10 at 1 year visit compared to baseline visit. In the TSQM, patients declared themselves most satisfied in relation to 'Secondary effects' (96.4) and rated a high total score (76.2) in visit 3.

Conclusion: Cluster and conventional specific immunotherapy resulted in no reported adverse reactions and led to significant decreases in clinical symptoms, improvements in immunological parameters and a high degree of patient satisfaction.

607
Pollen season as a risk factor for systemic reactions due to allergen immunotherapy. Results of a 10 years long, real life, prospective study

Pitsios, C[1]; Dimitriou, A[2]; Lefousis, C[3]; Giannakopoulos, D[4]; Kouri, M[5]; Ergazakis, M[6]; Vartholomaios, S[5]
[1]Private Practice, Athens, Greece; [2]Private Practice, Chalkida, Greece; [3]Private Practice, Volos, Greece; [4]Private Practice, Piraeus, Greece; [5]Private Practice, Corfu, Greece; [6]Private Practice, Irakleion, Greece

Background: Allergen Immunotherapy (AIT) with allergen extracts absorbed onto adjuvants, may rarely cause allergic reac-

© 2015 The Authors

tions as a side effect. Although guidelines mention that the presence of pollen is not a risk factor, some allergologists practice a decrease of the maintenance dose during the pollen season. The objective of this study was to establish the potential effect of pollen season on AIT-related side effects.

Methods: After a pilot study, which showed no affect of pollen season on the rate of allergic reactions, a prospective study took place, during 2004–2014. Inclusion criteria included adult ages, sensitivity to only pollen allergens (study group) or only to mites (control group), allergy for more than 2 years before AIT and treatment using only one allergen per extract. No decrease of the maintenance dose during the pollen season was applied. Exclusion criteria were: past AIT, the use of mixtures of extracts and the existence of other allergen sensitizations. Decreasing the maintenance dose was considered a drop-out. The number of injections during maintenance dose, seasonal and off-season, were calculated and all types of allergic reactions were registered. Patients were monitored before each visit (once monthly) and were followed-up for at least 30 min after the shot.

Results: Seventy-four pollen allergic and 24 mites-allergic patients were treated during this decade. About 3790 injections with pollen extracts were administered and 12 allergic reactions were registered (risk rate: 0.31%); 4 were large local, while 8/12 were mild systemic reactions.

Injections of pollen extracts during pollen season/reactions: 1210/5

Injections of pollen extracts off-season/reactions: 1700/7

Totally 880 injections using mites′ extracts were administered with three reactions registered; two large local and one mild systemic reaction.

AIT to pollen has a similar risk with AIT to mites ($x^2 = 0.06$, $P = 0.8$). The risk rate of allergic reactions (any type) during the pollen season (0.41) was exactly the same to the respective off-season (OR = 1.0035, $P = 0.9$) and similar to the one of mites' control group (0.45).

Conclusion: The overall risk of allergic reaction due to AIT with pollen extracts is very low and similar to the one with mites' extracts. Pollen season is not a risk factor for allergic reactions due to AIT with pollen extracts. The maintenance dose should not be decreased during pollen season.

608
Allergic reactions during the maintenance dose of allergen immunotherapy: risk factors related to pollen extracts

Dimitriou, A[1]; Ergazakis, M[2]; Lefousis, C[3]; Vartholomaios, S[4]; Giannakopoulos, D[5]; Kouri, M[4]; Pitsios, C[6]
[1]Private Practice, Chalkida, Greece; [2]Private Practice, Irakleion, Greece; [3]Private Practice, Volos, Greece; [4]Private Practice, Corfu, Greece; [5]Private Practice, Piraeus, Greece; [6]Private Practice, Athens, Greece

Background: Although rare, allergic reactions during Allergen Immunotherapy (AIT) may happen; they are more often during the up-dosing, but are probable throughout the course of AIT. The aim of this study is to examine the effect of extracts′ type and of pollen allergens, on AIT-related side-effects.

Method: A large prospective study on AIT-related side effects taking place during the maintenance phase of pollen-treated patients, has been performed during a decade (2004–2014). As part of the study it was aimed to investigate extract-related risk factors:

extracts′ type (absorbed onto adjuvants vs allergoids) and: pollen allergens.

The pollen allergens used for AIT in our area are mainly Olive, Parietaria and Grasses.

Results: A total of 74 patients treated with absorbed extracts and 80 patients treated with allergoids were followed. The administered injections and side-effects were as follows:

AIT with absorbed extracts:

1) 1285 injections of Grass with eight registered side-effects; four off-season reactions and four during the pollen season,
2) 1188 injections of Parietaria with four reactions; all off-season,
3) 406 injections of Olive – no reactions.

AIT with allergoids:

1) 945 injections of Grass resulted in one off-season reaction,
2) 1423 Parietaria with two reactions during pollen season,
3) 333 injections of Olive – no reaction.

It results that allergoids are safer that absorbed extracts ($x^2 = 5.1$, $P < 0.05$), although all side-effects noticed were large local or mild systemic reactions. It is clear that Grasses are the most likely pollen extract to cause a side effect, however -due the rare incidence- there was no statistically significant difference notices among allergens.

Conclusion: AIT to pollen is safe with both types of extracts and only large local or mild systemic reactions were registered. However side-effects due to allergoids are more rare than due to absorbed extracts. Grass pollen was the allergen responsible for the most frequent AIT-related side-effects, while Olive has caused no reaction throughout the study.

609
Quantitative benefit and risk assessment of SQ HDM SLIT-tablet; results from a DBPC phase III trial in allergic asthma

Kleine-Tebbe, J[1]; de Blay, F[2]; Fernandez, FR[3]; Ljørring, C[4]; Peter, F[4]; Riis, B[4]; Durham, S[5]
[1]Allergy & Asthma Center Westend, Outpatient Clinic & Research Center, Berlin, Germany; [2]Chest Disease Department, University Hospital of Strasbourg, Strasbourg, France; [3]Allergy Service, Hospital Universitario Marqués de Valdecilla, Santander, Spain; [4]Global Clinical Development, ALK, Hørsholm, Denmark; [5]Imperial College London, London, United Kingdom

Background: The SQ house dust mite (HDM) SLIT-tablet has been shown to improve asthma control by a clinically significant risk reduction for a moderate or severe asthma exacerbation during ICS reduction, with a safety profile that was compatible with at-home administration (EudraCT 2010-018621-19). The numbers needed to treat (NNT) and needed to harm (NNH) are quantitative methods to characterise the relative benefit and risk based on the efficacy and safety of treatment. Lower NNT indicates more effective treatment, and higher NNH indicates safer treatment. Both NNT and NNH depend on the definition of clinical response used.

Method: The trial was a European, randomised, double-blind, placebo-controlled trial, including 834 subjects with HDM allergic asthma, not well-controlled by inhaled corticosteroid (ICS). The primary endpoint was time to first moderate or severe asthma exacerbation during ICS reduction. NNT was estimated based on the time-to-event data and calculated for multiple time points within the ICS reduction period (based on a published method for trials where the primary outcome is time-to-event[1]). NNH was estimated based on three definitions of harm: epinephrine use, treatment-related systemic allergic reactions, and treatment-related severe adverse events (AEs).

Results: For 12 SQ-HDM, the NNT was 14 after 3 months on 50% reduced ICS, and 10 after additionally 3 months without ICS at the end of trial. There were no epinephrine uses or physician-reported, treatment-related systemic allergic reactions [CI 95% for proportion with event: (0; 1.3%)]. For severe AEs the proportion with placebo was 1.1%, CI 95%: (0.2%; 3.1%) and with 12 SQ-HDM 2.1%, CI 95%: (0.8%; 4.6%). Accordingly NNH was 96 for 12 SQ-HDM based on severe AEs.

© 2015 The Authors

Conclusion: Protection against asthma exacerbations even during reduced ICS treatment is considered a benefit of direct relevance to the patient as periods with sub-optimal controller treatment are known to be prevalent among asthma patients[2]. With NNH being 7–10 times higher than NNT, the HDM SLIT-tablet is considered to have a favourable benefit-risk profile.

References: [1]Altman DG, Andersen PK. Calculating the number needed to treat for trials where the outcome is time to an event. BMJ 1999; 319 (7223): 1492–1495

[2]From the Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA) 2014. Available from http://ginasthma.org. 2014

610

Does specific IgE (sIgE) or the skin prick test (SPT) predict the response of birch pollen allergic patients exposed to birch pollen (*Betula pendula*) in an environmental challenge chamber (ECC)?

Homolla, V[1]; Akboga, Y[1]; Karjalainen, M[1]; Narkus, A[1]; Häfner, D[2]

[1]Allergopharma GmbH & Co. KG, Reinbek, Germany; [2]Clinical Development, Allergopharma GmbH & Co. KG, Reinbek, Germany

Background: The level of sIgE or the wheal size following SPT is often used to assess the disease severity of patients with allergy. We were interested whether these parameters can predict the symptom response of birch pollen allergic patients exposed to pollen from *Betula pendula* in an ECC.

Method: We evaluated the wheal size (longest diameter) following birch SPT and sIgE data (assessed by ImmunoCap®) for birch pollen allergens from the screening phase of a clinical trial in which patients with allergic rhinitis/rhinoconjunctivitis ± bronchial asthma ($n = 129$) were exposed to pollen from *Betula pendula* during the ECC visit. The total nasal symptom score (TNSS) was determined once prior to the challenge and six times every 20 min during allergen exposure. TNSS measured four symptoms: nasal congestion, rhinorrhea, nasal itching, and sneezing. For comparisons, the pre-exposition adjusted area under the curve (AUC) of the 2-h exposure was calculated. For correlation assessment, Pearson's correlation coefficient was calculated.

Results: The patients had sIgE levels of 27.2 ± 33.0 kU/l (mean ± SD) and wheal sizes of 9.7 ± 4.7 mm. The AUC of TNSS after the 2-h exposure was 20.8 ± 11.2. Neither for the wheal size following SPT ($r = -0.07$) nor for the sIgE level ($r = -0.01$), a meaningful correlation with the AUC of TNSS was shown. However, for the sIgE level and the wheal size following SPT, a modest but statistically significant correlation was found ($r = 0.31$).

Conclusion: For birch pollen allergic patients, neither the level of sIgE nor the wheal size following SPT predict the response of birch pollen allergic patients in an ECC. Clinical symptoms do not correlate with either sIgE levels or SPT results. The severity of allergy can only be assessed by clinical symptoms.

611

Significance of extending the diagnostic algorithm for sIgE to rApi m1, rVes V5 and CCD (MUXF3) in patients with hymenoptera sting allergy: importance for selecting insect venom species for allergen-specific immunotherapy

Blagojevic, G[1]; Vuckovic, O[1]; Arsenijevic, J[1]; Bonaci-Nikolic, B[2]

[1]Institute for Virology, Sera and Vaccines, Torlak, Allergology and Immunology Diagnostic Laboratory, Belgrade, Serbia; [2]Institute for Allergology and Clinical Immunology, Clinical Center of Serbia, Belgrade, Serbia

Background: Resolving a clinician's dilemma on the composition of SIT in patients with positive IgE tests for complete bee and wasp venom extracts (i1 and i3) is not possible without additional diagnostics. Detecting sIgE for species specific recombinant major venom allergens and CCD allows for differentiating between true double sensibilization and cross reactivity.

Method: In total, 41 sera from patients with systemic anaphylaxis on hymenoptera sting have been analyzed. Out of these, four sera were IgE positive only for bee venom, three for wasp venom, while 34 patients showed double positive results for both venoms. There were 12 non-atopic controls with negative history on hymenoptera sting. In both patients and controls the IgE i1, i3, CCD and recombinant major venom allergens IgE were determined using ImmunoCAP. All results over ≥0.1 kU/l were considered positive.

Results: Out of nine patients whose reaction was triggered by a bee, rApi m1 IgE were present in 7, while out of 12 patients whose reaction was set off by the wasp, rVes V5 IgE were present in 11 patients. Out of 13 patients where both bee and wasp set off the reaction, rApi m1 IgE were present in eight and rVes V5 IgE were present in 11 patients. Sensitivity within anamnestic groups is 78% for rApi m1 IgE and 92% for rVes V5 IgE. In the group of patients classified according to the history of reaction on bee venom (6) and wasp venom (10), as well as the presence of double sensibility on the complete venom, rVes V5 IgE was present in three patients, thus the specificity (SP) was 50%, while rApi m1 IgE was present in six patients, resulting in SP 40%. In the control group, one serum was positive for rApi m1 IgE (SP 91%), and two sera were positive for rVes V5 IgE (SP 83%). PPV for rAPi m1 is 58% and NPV is 93%. PPV for rVes V5 is 86% and NPV is 87%. CCD IgE were positive in 18 sera positive for both venoms (52%). Among patients with double positivity for both venoms with negative CCD IgE there were 7 (44%) with true double sensibilization.

Conclusion: Recombinant allergen-based IgE tests rApi m1 and rVes V5 show a considerable level of specificity and sensitivity and can significantly improve the diagnostic procedure for differentiating between true double sensibilization and cross reactivity in patients with double IgE positivity for i1 and i3. SIT for both venoms is applied to few patients with true double sensibilization, thus avoiding unnecessary use of SIT for both venoms in monosensitized patients.

612

Safety and efficacy of 3-injection *immunotherapy* protocol for treating grass pollen-induced *rhinoconjunctivitis in American adolescents/young adults*

Patterson, A[1]; Bonney, A[2]; Shiels, W II[3]; Erwin, E[1]

[1]Allergy/Immunology, Nationwide Children's Hospital/ The Ohio State University, Columbus, United States; [2]Adolescent Medicine, Nationwide Children's Hospital/ The Ohio State University, Columbus, United States; [3]Diagnostic Radiology, Nationwide Children's Hospital/ The Ohio State University, Columbus, United States

Background: Allergen-specific immunotherapy by three, ultrasound-guided, lymph node injections [intralymphatic immunotherapy (ILIT)] given before pollen season has shown safety and efficacy in adult, European trials. We hypothesized that ILIT for adolescents/young adults with grass-pollen induced rhinoconjunctivitis could be done safely and effectively using extract commercially available in the United States.

Method: We recruited 15 adolescents/young adults with grass-pollen induced rhinoconjunctivitis for a randomized, double-blind, placebo-controlled pilot trial of ILIT with Center-Al Phleum pretense 500 PNU/ml (ALK). Three patients with mild, intermittent asthma were included. The sum of safety scores for each injection (based on examination for erythema, edema, pruritus, or other adverse reaction) gave a total safety score (TSS) for each participant. Efficacy was evaluated by symptom/medication diaries. The combined symptom + medication score (total

© 2015 The Authors

combined score = TCS) was recorded by participants daily in online diaries during summer grass pollen season.

Results: Extract (0.1, 0.2 and 0.5 ml) was administered into a right, inguinal lymph node with a 1.5 inch, 25 gauge needle, by ultrasound-guidance at three visits, each at least 4 weeks apart. Treatment dose was 50 PNU for the first injection followed by 100 PNU and 250 PNU for second and third injections, respectively. Participants were monitored for 2 h post-injections. All participants completed 100% of injections. There was no difference in TSS between active treatment and placebo groups ($P = 0.779$). Mean TSS for the two groups were 0.6 and 0.4 (maximum score 9). Though median TCS of 12.1 (placebo) and 9.3 (active treatment) were not significantly different between, the effect size was 0.83.

Conclusion: Intralymphatic immunotherapy with commercially available extract was a well-tolerated and acceptable therapy in American adolescents/young adults. Safety scores were remarkably low overall. During peak grass pollen season, participants receiving active ILIT treatment had different total combined allergy symptom and medication use scores than those on placebo, and the effect size suggests this difference is more likely to be clinically relevant.

613
Safety and quality of ultrasound-guided intralymphatic allergen specific immunotherapy

Skaarup, SH[1]; Schmid, JM[1]; Graumann, O[2]; Skjold, T[1]; Hoffmann, HJ[1]
[1]Pulmonary Medicine, Aarhus University Hospital, Aarhus C, Denmark; [2]Radiology, Aarhus University Hospital, Aarhus, Denmark

Background: Delivery of allergen specific immune therapy (AIT) is the only causal treatment of allergy. The treatment regime is time consuming and expensive. A new way to administer AIT is direct ultrasound-guided delivery of allergen into a lymph node (ILIT). This reduces treatment duration from 3 years to 2 months. It is very important that allergen is delivered precisely into the lymph nodes. We investigate the clinical effects and immunological changes in a double-blinded randomized placebo controlled trial that is still ongoing. We here report on quality of ultrasound-guided injections and the number of adverse effect.

Method: We treated 36 patients with grass allergy with ultrasound-guided intralymphatic injections with ALK phleum pratense. They were randomized to either three allergen injections (24 patients) or three placebo injections (12 patients). We recorded adverse events 1 h after each injection and at a visit 1 week later. We graded the quality of the ultrasound-guided injection with a 5-point scale in video recordings of the injection.

Results: 79% of the injections had a good quality with a score at 4–5. There was an increase in the quality of injection with operator experience. The success was 69% for the first injection, 75% for the second, and 94% for the third. Of the 108 injections, 12 patients had adverse event. One patient had a mild acute allergic reaction. Seven patients had a localized reaction with redness, edema, and itching. Four patients had mild late reaction with headache and muscle tenderness.

Conclusion: Intralymphatic ultrasound-guided delivery of AIT is a safe treatment. Quality of injections improves with experience of the operator.

© 2015 The Authors

Poster Discussion Session PDS 32

Food allergy: epidemiology

614
Two different Gaussian distributions of specific IgG for milk in the Italian population: IgG levels can reflect nutritional profiles

Speciani, AF[1]; Piuri, G[1]; Soriano, J[2]; Ferrazzi, E[3]
[1]Food Intolerances Department, GEK Srl, Milan, Italy; [2]Department of Statistical Science, Duke University, Durham, United States; [3]Department of Woman, Mother and Neonate – Buzzi Children's Hospital, Biomedical Sciences School of Medicine, University of Milan, Milan, Italy

Background: As discussed by Ligaarden, the level of specific IgG for food depends on the personal nutritional profile and the different distribution pattern of IgG for food can be important to understand immune response and symptoms of patients with reaction to food. Finkelman's work describes the alternative pathway of anaphylaxis, avoiding to focus only on IgE-mediated reactions and giving more importance to IgG than in the past. At the same time, IgG can modulate both tolerance and immune reactions to foods. The aim of this study is to investigate the production pattern of IgG for milk in the Italian population.
Method: The level of specific IgG for milk was measured in plasma of 20 135 healthy Italian patients (74.1% female, mean age 44.5 years ± 15.5 as standard deviation) referring their symptoms to the intake of food and in particular to milk. The IgG antibodies were measured using the ELISA technique.
Results: Interestingly, the distribution of the levels of IgG for milk in this population is bimodal. The smaller component has median 7.45 U/ml and 95% bootstrap CI from 7.31 to 7.58 U/ml, while the larger component has median 37.09 U/ml and 95% bootstrap CI from 36.73 to 37.44 U/ml.
Conclusion: The concentration of IgG for food reflects nutritional habits, so these results show the statistical evidence of two different nutritional profiles for milk. Probably, the profile identified by the first Gaussian represents the part of population eating or drinking only occasionally milk and dairy products, while the second Gaussian reflects the part of Italians drinking milk every day. Although the use of dairy products is very frequent in Italy, in adulthood a large part of the Italian population does not consume large amounts of milk, due to lactose intolerance, influencing the profile of consumption of milk and dairy products in their diet. In conclusion, the analysis of specific IgG for food can be useful for a better understanding of non-IgE-mediated reactions to food. This area of investigation on food allergy and food reactivity opens a new epidemiological perspective and allows researchers to also evaluate the connection between IgG and inflammatory markers of anaphylactic reactions.

615
Relationship between ethnicity, migration, early life environment and childhood food allergy

Koplin, J[1,2]; Peters, R[1,2]; Tang, M[1,2,3]; Matheson, M[2]; Dharmage, S[1,2]; Allen, K[1,2,3]; for the HealthNuts and SchoolNuts Study Groups[1]
[1]Murdoch Childrens Research Institute, Parkville, Australia; [2]University of Melbourne, Parkville, Australia; [3]Royal Children's Hospital, Parkville, Australia

Background: We recently reported that challenge-confirmed food allergy was more common in Australian-born infants with East Asian parents compared to infants of Australian (Caucasian) parents in the HealthNuts cohort (OR 3.4, 95% CI 2.2–5.1). Using data from two population-based studies in Melbourne, Australia (HealthNuts and SchoolNuts), we aimed to validate our findings in the SchoolNuts cohort and explore the hypothesis that the risk of food allergy in children of Asian migrants is higher in families adopting a 'Westernised' lifestyle in HealthNuts.
Method: In SchoolNuts, parents of children aged 10–14 years reported on their child's history of allergic disease, ethnicity and timing of migration (n = 4730). In HealthNuts, skin prick tests to egg, peanut and sesame were conducted at age 1 year (n = 5300); those sensitised underwent food challenges and parents also reported dietary changes after migration. We investigated the effect of increased consumption of processed foods after migration (as a marker of adopting a more 'Westernised' lifestyle) on risk of food allergy.
Results: SchoolNuts cohort: Compared to children born in Australia to Caucasian parents (n = 2963), those born in Australia to Asian parents (n = 383) had an increased risk of food allergy (OR 1.9, 95% CI 1.5–2.4), while those born in Asia to Asian parents (n = 217, mean age at migration to Australia 6.5 years) were less likely to have food allergy (OR 0.6, 95% CI 0.4–0.9).

HealthNuts cohort: East Asian families (n = 204) who reported consuming more processed foods after moving to Australia were more likely to have infants with food allergy (OR 2.3, 95% CI 1.1–4.8). An association between more processed foods post migration and food allergy was not present among infants of families migrating from other countries (OR 0.7, 95% CI 0.3–1.5).
Conclusion: An increased risk of food allergy in children born in Australia to East Asian parents is reproducible in independent cohorts. This may be related to the adoption of a more 'Westernised' lifestyle following migration to Australia, which if present prenatally or in early infancy, acts in conjunction with genetic factors in the Asian population to result in a high risk of food allergy. Early life exposure to an Asian environment appears to be protective against food allergy even with subsequent migration to a high risk country. Studying this phenomenon has the potential to shed light on factors driving an increasing prevalence of food allergy.

616
The natural history of peanut allergy over the first 10 years of life

Venter, C[1,2]; Patil, V[2,3]; Grundy, J[2]; Glasbey, G[2]; Arshad, HS[2,3]; Dean, T[1,2]
[1]SHSSW, University of Portsmouth, Portsmouth, United Kingdom; [2]The David Hide Asthma and Allergy Research Centre, Newport, United Kingdom; [3]University of Southampton Faculty of Medicine, Southampton, United Kingdom

Background: Prevalence, incidence and natural history of peanut allergy and its trends in older children are unclear.
Method: A birth cohort born on the Isle of Wight (UK) between 2001 and 2002 was followed up prospectively. Children were clinically examined and skin prick tested to peanut allergens (ALK abello) at set times and invited for food challenges when indicated. Peanut allergy was defined as a posi-

© 2015 The Authors

tive a food challenge or a positive SPT and a good clinical history.

Results: A total of 969 children were recruited and 92.9%, 88.5%, 91.6% and 85% were assessed at one, two, three and 10 years of age. Prevalence of sensitisation to foods was 1.9%, 3.8%, 4.5% and 2.7% at these ages. Prevalence of sensitisation to peanut at these ages was 0.4%, 2.0%, 2.0% and 2.4%. The COT advice (UK) which recommended the avoidance of peanut till 3 years in high risk families, was still relevant at the time. Children were therefore first challenged to peanut at 3 years of age and 11/891 (1.2%) children were diagnosed with peanut allergy. At 10 years of age, 12/828 (1.5%) children were diagnosed with a peanut allergy. One child (8%) outgrew her peanut allergy between 3 and 10 years of age and two children (15%) presented with a new onset peanut allergy. Over the course of the first 10 years of life, 13/969 (1.3%) children were diagnosed with a peanut allergy. At 10 year of age, lupin SPT was performed as well. Four children were lupin sensitised. They were all sensitised to peanut and none of them clinically lupin allergic.

Conclusion: Peanut allergy appears to be stable over the first 10 years of life with about 10% of children outgrowing their peanut allergy and about 20% developing new onset peanut allergy. Lupin cross-sensitisation does not necessarily indicate clinical allergy to lupin.

617
Epidemiology and clinical features of legume allergy in adults

Somoza, ML[1]; Blanca-López, N[1]; Pérez-Alzate, D[1]; Ruano, FJ[1]; Vázquez de la Torre, M[1]; Garcimartín, MI[1]; Canto, G[1]
[1]Allergy, Infanta Leonor – University Hospital, Madrid, Spain

Background: The 7% of Food Allergy in Spain is caused by Legumes and its incidence is increasing. The aim of this study was to describe the allergy to Legumes in adults in our area.

Method: We studied 1007 adults (>14 years old) who were attended at our Allergy Unit over a period of 3 years (2012–2014) referring food allergy. The allergological evaluation included: clinical history, skin prick test and detection of specific IgE levels both, to most common pneumoallergens and food allergens in our area.

Results: A total of 73 (7.2%) patients reported symptoms after the intake of Legumes: 42 only to Legumes and 31 to Peanut and Legumes. We studied only the first group.

The range of age was 15–62 years old (mean age: 32 year-old); 60% were Female and 90% Spanish; 12.5% suffered from Atopic Dermatitis and 62.5% from Rhinoconjunctivitis and/or Asthma caused by pollen allergy.

	Anaphylaxis (16 episodes) (%)	Urticaria/ Angioedema (9 episodes) (%)	Oral Allergy Syndrome (14 episodes) (%)	Eosinophilic Esophagitis (12 episodes) (%)	Asthma (2 episodes) (%)	Gastro intestinal symptoms (19 episodes) (%)
Lentil (21 episodes)	25	44.4	14.3	33.3		36.8
Chickpea (17 episodes)	25	11.1	35.7	25		21
Bean (14 episodes)	12.5	22.2	14.3	16.6	50	26
Pea (8 episodes)	12.5	22.2		16.6		11
Soy Bean (8 episodes)	12.5		28.6		50	5.2
Green Bean (4 episodes)	12.5		7.10	8.5		

[Clinical Manifestations.]

In all these patients, skin prick tests were positive to: Chickpea: 54%; Pea, 50%; Bean, 44%; Peanut and Green Bean, 37%; Lentil, 15%; and 5% to SoyBean.

And the specific IgE levels were positive to: Green Bean and Pea: 72%; Peanut, 70%; Lentil and Soy Bean, 67%; Chickpea, 55%; and Bean 42%.

Conclusion: In our study, most patients who were reported to have allergy to Legumes in adults were Spanish, Female and suffered from pollen RC and/or Asthma. The most common clinical manifestations were Anaphylaxis followed by OAS and EoE. Lentil was the Legume most frequently involved and responsible for most of the systemic reactions, followed by Chickpea. Soy Bean and Chickpea were responsible for most OAS episodes.

618
Environmental analysis of specific peanut allergens in house dust indicates high prevalence of Ara h 2 and Ara h 6

Kuklinska-Pijanka, A[1]; Fuller, N-J[1]; Custovic, A[2]; Chapman, MD[1]; Hindley, JP[1]
[1]INDOOR Biotechnologies Ltd., Cardiff, United Kingdom; [2]Institute of Inflammation and Repair, University of Manchester, Manchester, United Kingdom

Background: Allergy to peanut is considered a major public health concern due to its prevalence, persistency and potential severity. At present, it is assumed that peanut allergic individuals become sensitised by ingestion. However, environmental exposure to peanut may also be relevant. Many peanut allergic individuals are sensitised to specific peanut allergens, Ara h 1, Ara h 2 and Ara h 6. The goal of this study was to determine the levels of these three major peanut allergens in the home environment.

Methods: More than 350 settled dust samples from the Manchester Asthma and Allergy Study (MAAS) cohort were screened using a newly developed multiplex array for Ara h 1, Ara h 2 and Ara h 6, which measures each allergen simultaneously. Representative positive samples were re-assayed using calibrated, quantitative ELISA for each allergen, with lower limits of detection of 31.5 ng/ml (Ara h 1), 2.0 ng/ml (Ara h 2) and 0.8 ng/ml (Ara h 6).

Results: A high proportion of the dust samples contained peanut allergens, especially Ara h 6 and Ara h 2. Approximately 89% of samples contained Ara h 6; 66% of samples contained Ara h 2; and 2% of samples contained Ara h 1. The levels of

© 2015 The Authors

Ara h 1, Ara h 2 and Ara h 6 were quantified to be as high as 0.6, 1.86 and 1.14 μg/g of dust, respectively. The apparently low prevalence of Ara h 1 in dust samples may be explained by the relatively lower level of sensitivity of the Ara h 1 ELISA and the multiplex assay.

Conclusions: These findings indicate that there is a high prevalence of Ara h 2 and Ara h 6 in the household environment, at levels which might be expected to induce allergic sensitization in atopic individuals. Measurement of specific peanut allergens in the environment provides the opportunity to compare allergen exposures with sensitization and immune responses to specific peanut proteins. Further research is needed to confirm the observations in this study and to investigate associations between environmental exposures, allergic sensitisation and clinical severity.

619
A novel approach to food challenge documentation and evaluation

Reich, A[1]; Grabenhenrich, LB[1]; Bellach, J[2]; Trendelenburg, V[2]; Xepapadaki, P[3]; Sigurdardottir, ST[4]; Kowalski, ML[5]; Jedrzejczak-Czechowicz, M[5]; Dubakiene, R[6]; Sprikkelman, AB[7]; Quirce, S[8]; Roberts, G[9]; Keil, T[1]; Beyer, K[2]
[1]Institute for Social Medicine, Epidemiology and Health Economics, Charité – Universitätsmedizin Berlin, Berlin, Germany; [2]Department of Paediatric Pneumology and Immunology, Charité – Universitätsmedizin Berlin, Berlin, Germany; [3]Allergy Department, 2nd Pediatric Clinic, University of Athens, Athens, Greece; [4]Department of Immunology, Landspitali University Hospital, Reykjavik, Iceland; [5]Department of Clinical Immunology & Allergy, Medical University of Łódź, Łódź, Poland; [6]JCS Perspektyvos Allergy Clinic, Vilnius, Lithuania; [7]Department of Paediatric Respiratory Medicine and Allergy, Emma Children's Hospital AMC, Amsterdam, The Netherlands; [8]Department of Allergy, Hospital La Paz Institute for Health Research (IdiPAZ) and CIBER of Respiratory Diseases CIBERES, Madrid, Spain; [9]Paediatric Allergy and Respiratory Medicine, University of Southampton, Southampton, United Kingdom

Background: The prevalence of food allergies in children is not well known. The EuroPrevall project aimed to assess food allergies in nine European countries, using the gold standard, a double-blind placebo-controlled food provocation test [Keil et al., *Allergy* 2010 April; 65(4): 482–90.]. It was found that the centres differed in the way they documented symptoms in terms of number and symptom details. Evaluations of ambiguous or inconclusive food challenges were not sufficiently standardized.

Method: We aimed to improve the existing protocols by developing a novel protocol and documentation form, based on the PRACTALL consensus report on standardizing double-blind, placebo-controlled oral food challenges [Sampson et al., *J Allergy Clin Immunol* 2012 December; 130 (6): 1260–74]. We added a grading system and developed a formalized decision algorithm to handle ambiguous food challenges.

Results: As part of the iFAAM (Integrated Approaches to Food Allergen and Allergy Risk Management) project, we developed a novel food challenge protocol and documentation form within the clinical consortium, consisting of allergy experts in paediatric and adult care and piloted it in three clinics. This novel approach utilizes a 3-point grading system for symptoms in the following five domains: skin (four symptoms), respiratory (four symptoms), gastro-intestinal (four symptoms), cardiological (one symptom) and neurological (one symptom).

Conclusion: It adds a formalized decision algorithm to specifically handle ambiguous food challenges. Beyond the common categorization of food challenge outcomes into positive and negative, sometimes including recognition of placebo reactors or a broad category of inconclusive challenges, the novel algorithm accounts for different degrees of uncertainty, allowing different case definitions on the subjective-objective continuum. The new protocol is being used for the follow-up of the birth cohort initiated by EuroPrevall (now iFAAM, FP7). It can be used for food challenges in children as well as adults.

620
The effect of folic acid supplementation before conception on the onset of food allergies in infants

Abe, M
Human Biology, Oita University of Nursing and Health Sciences, Oita, Japan

Background: From 2000, efforts have been made in Japan to provide more information about folic acid supplementation for women of childbearing age and approximately 50% of pregnant women take a folic acid supplement. Intake during pregnancy of folic acid, which acts as a methyl donor, may increase the risk of asthma onset in infants. Therefore, folic acid intake in the late stages of pregnancy is not recommended. In this study, we observed the impact of the timing of folic acid supplementation for mothers on the onset of food allergies, which are the most common type of allergy during infancy.

Method: Questionnaires were conducted at the time of 18-month health check-ups. The questionnaires were collected between January and October 2013 and January and August 2014; the number of valid responses was 5763.

Results: The incidence of food allergies was 10.9% in 2602 infants born to mothers who had not taken folic acid either before conception or during pregnancy, 5.7% in 87 infants born to mothers who had taken folic acid only before conception (pFO), 13.2% in 2492 infants born to mothers who had taken folic acid only during pregnancy (dFO), and 14.3% in 582 infants born to mothers who had taken it before conception as well as during pregnancy (p + dFO). As infant gender and parental history of allergies affect food allergy onset in infants, we took these elements into account in our investigation. Results indicated that for female infants, if their fathers had a history of atopic dermatitis or food allergy or eczema, the incidence in 29 'p + dFO' infants and 109 'dFO' infants was 37.9% and 17.4%, respectively, demonstrating that the incidence in 'p + dFO' infants was significantly higher (odds ratio: 2.90, confidence interval: 1.18–7.11). The same tendency was confirmed for male infants if their parents had a history of allergies but no statistically significant difference was observed.

Conclusion: Results suggested that folic acid supplementation not only during pregnancy but also before conception might affect the onset of food allergy in infants.

621
Assessment of allergy to cow's milk, hen's egg, cod, and wheat in a population-based cohort of Swedish schoolchildren

Winberg, A[1]; West, CE[1]; Strinnholm, Å[2]; Nordström, L[1]; Hedman, L[2]; Rönmark, E[2]
[1]Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden; [2]Public Health and Clinical Medicine, the OLIN Unit, Umeå University, Umeå, Sweden

Background: There is a lack of knowledge about the prevalence of allergies to essential foods and the phenotypes underlying reported FHS in schoolchildren and adolescents. The aim of this study was to investigate the prevalence of allergies to milk, egg, cod, and wheat using reported data, clinical examinations, and double-blind placebo-controlled food challenges and to describe the phenotypes of reported food hypersensitivity in a cohort of Swedish schoolchildren.

Methods: In a population-based cohort of 11- to 12-year-old children, the parents of 2612 (96% of invited) returned a questionnaire. Specific IgE antibodies to foods were analyzed in a random sample ($n = 695$). Children reporting complete avoidance of cow's milk, hen's egg, cod, or wheat due to perceived hypersensitivity and without physician-diagnosed celiac disease were invited to undergo clinical examination that included specific IgE testing, a celiac screening test, and categorization into phe-

© 2015 The Authors

notypes of food hypersensitivity according to preset criteria. Children with food allergy were invited to further evaluation with double-blind challenges.
Results: In this cohort, the prevalence of reported food allergy to milk, egg, cod, or wheat was 4.8%. Food allergy was diagnosed in 1.4% of the children after clinical evaluation and in 0.6% following double-blind placebo-controlled food challenge. After clinical examination, children who completely avoided one or more essential foods due to perceived food hypersensitivity were categorized with the following phenotypes: allergy (29%), outgrown allergy (19%), lactose intolerance (40%), and unclear (12%).
Conclusions: Reported hypersensitivity to essential foods was common among the 11- to 12-year-olds, although there was a high discrepancy in the prevalence of allergy as assessed by reported data, clinical evaluation, and double-blind food challenges. Food hypersensitivity phenotyping according to preset criteria was helpful for identifying children with food allergy.

622
Is food allergy Increasing in Hong Kong Chinese children?

Leung, TF; Sy, HY; Tsen, CS; Tang, MF; Wong, GWK
Department of Paediatrics, Chinese University of Hong Kong, N.T., Hong Kong, China

Background: Food allergy is the leading cause of anaphylaxis in infants and toddlers, which appears as the second wave of allergy epidemic in developed countries. Our territory-wide study in 2006 found that food allergy as defined by parent-reported adverse food reactions (AFRs) were common in young Chinese children in Hong Kong, but there was no data on its trend over the past decade. This study investigated longitudinal change in food allergy prevalence among Chinese preschoolers over a 7-year period.
Methods: The present epidemiological study in 2013 adopted the same sampling strategy, questionnaire and analytical tool as our previous community survey conducted in 2006 (Leung TF, *et al*. Pediatr Allergy Immunol 2009; 20: 339–46). Briefly, Chinese children aged 2–6 years from 17 nurseries and kindergartens randomly recruited throughout Hong Kong were enrolled to ascertain the prevalence and clinical spectrum of parent-reported and parent-reported, doctor-diagnosed AFRs. All families were living in Hong Kong. Subjects' parents answered a Chinese self-administered questionnaire that was modified from ISAAC. Logistic regression was used to analyse AFR changes between the two studies.
Results: A total of 3687 (66.4%) of 5549 eligible children returned valid questionnaires, and data for AFR was analysable in 3525 (95.6%) children. The prevalence of parent-reported AFR was 9.7%, which was higher than 8.1% in 2006 ($P = 0.019$). Nonetheless, there was no change for parent-reported, doctor-diagnosed AFR (3.9% vs 4.6%; $P = 0.155$). Forty (1.1%) children had ≥3 AFR in the preceding 12 months. Wheeze ever and current wheeze also increased from 14.0% and 8.0% to 21.0% and 13.4% respectively ($P < 0.001$ for both). The leading foods causing parent-reported AFR were shellfish (2.1%), egg (1.9%), fish (1.1%), cow's milk (1.0%) and peanut (1.0%). Except for peanut, AFRs to these foods increased from 2006 to 2013. When adjusted for maternal education background, the prevalence for parent-reported AFR remained the same ($P = 0.521$) whereas that of parent-reported, doctor-diagnosed AFR decreased ($P = 0.008$) among our preschool children during this 7-year period.
Conclusions: Food allergy by AFR is common among Chinese preschool children in Hong Kong, and its epidemiology is comparable to that of White children. Nonetheless, this epidemiological study does not observe any increase in AFR prevalence over the past 7 years.
Funding: Direct Grants for Research (2012.2.020 and 2013.2.033), CUHK.

623
Self-reported adverse reactions and IgE sensitization to common foods in adults with asthma

Rentzos, G[1,2]; Johanson, L[3]; Sjölander, S[4]; Lundbäck, B[3]; Telemo, E[2]; Ekerljung, L[3]
[1]Section of Allergology Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [3]Department of Internal Medicine and Clinical Nutrition, Krefting Research Centre, University of Gothenburg, Gothenburg, Sweden; [4]R&D, ImmunoDiagnostics, Thermofischer Scientific, Uppsala, Sweden

Background: There is very few data available on the prevalence of food hypersensitivity and food allergy among adults with asthma. Epidemiological studies of food allergy have mostly been performed on children, and therefore it is still not clear to what extent the adult population is affected. The aim of this study was to explore the prevalence of self-reported adverse reactions to foods and to estimate the prevalence of IgE sensitization for the most common foods among adults with asthma compared to non-asthmatics. We also wanted to determine the spectrum and the prevalence of gastrointestinal symptoms caused by the most common foods in both asthmatics and non-asthmatics.
Methods: The present study was a cross sectional study based on interviews and questionnaire responses from 1527 subjects, 18–78 years of age, from Västra Götaland in Sweden, as part of the larger West Sweden Asthma Study (WSAS). IgE analyses were performed in sera from all subjects using three paneltests (Phadiatop, fx1 and fx5, Thermofischer Scientific, Uppsala, Sweden). The asthma patients were diagnosed with asthma according to the clinical protocol of the larger West Sweden Asthma Study (WSAS).
Results: Fifty three percent of adults with asthma reported adverse reactions to food intake compared to 30% in adults without asthma. The food item that most asthmatics reported symptoms from eating was hazelnut, followed by other nuts, birch-related foods, milk, peanut and shellfish. Furthermore, adults with asthma experienced significantly more gastrointestinal symptoms due to hazelnut, apple and milk compared to adults without asthma. When correlating self-reported symptoms and the IgE-sensitization, the asthmatics showed a correspondence between IgE sensitization and symptoms for hazelnut, milk and brazil nut. A significant correlation was found between IgE to birch and reported symptoms to hazelnut and to a lesser extent for almonds. In addition, a strong correlation between IgE for hazelnut and IgE for birch pollen was seen in asthmatics ($r = 0.904$, $P < 0.001$).
Conclusions: The prevalence of self-reported food hypersensitivity and food allergy is much higher among asthmatics compared to non-asthmatics. Hazelnut was the food that adults with asthma most frequently experienced adverse reactions from and the strong correlation between IgE to hazelnut and birch indicate that the reactions are caused partly due to pollen-food cross-reactions between allergens from the PR-10 family.

© 2015 The Authors

624
IgE to peanut in a low-risk population: the PIAMA cohort. Pollen cross-reactivity is poorly predicted by IgE to pollen or by the absence of IgE to Ara h 2

Aalberse, R[1]; van Leeuwen, A[2]; Wijga, AH[3]; Brunekreef, B[4]; PIAMA Birth Cohort Study Group[1]
[1]Sanquin Blood Supply Foundation, Amsterdam, The Netherlands; [2]Sanquin Blood Supply Foundation, Amssterdam, The Netherlands; [3]Centre for Prevention and Health Services Research, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; [4]Division of Environmental Epidemiology, Institute for Risk Assessment Sciences (IRAS), Utrecht, The Netherlands

Background: Asymptomatic sensitization to peanuts has been reported to be common, particularly in birch endemic areas. It is assumed to reflect cross-reactive sensitization to the major birch allergen Bet v 1, but grass cross-reactivity has also been implicated.
Methods: IgE to peanut was measured in 1299 sera from the PIAMA birth cohort at age 12. 'Peanut allergy' was based on reported allergy test positivity. IgE to Ara h 2 was measured in the peanut-positive sera. In the subgroup with IgE to peanut >3 kU_A/l, inhibition of peanut IgE by grass and/or birch pollen extract was investigated. 'Incomplete inhibition' was defined as <80% inhibition by an excess of the combined pollen extracts.
Results: IgE to peanut was >0.35 kU_A/l in 14.5% of the sera. IgE to Ara h 2 > 0.1 kU_A/l was found in 14 sera (1.1%), of which six were >0.35 kU_A/l. A positive peanut allergy test was reported in five cases, four of which were serologically Ara h 2 positive. Incomplete inhibition by pollen extract was found in 13 sera, 12 of which were strongly positive to grass- and/or birch pollen and eight of which were negative for Ara h 2. Cross-reactivity to profilins, to Bet v 1 homologues and to CCD's explained only part of the asymptomatic sensitization.
Conclusion: In Dutch adolescents the prevalence of non-symptomatic sensitization to peanut is extremely high (>10%), largely due to cross-reactive sensitization by grass pollen, with a smaller contribution by birch pollen. However, the finding of a negative test for IgE to Ara h 2 in combination with a strongly positive test for birch and/or grass pollen does not prove that a peanut-positive reaction is due to pollen-peanut cross-reactivity.

625
Asymmetrical distribution of specific IgG for peanut in the Italian population: an epidemiological perspective

Piuri, G[1]; Speciani, AF[1]; Soriano, J[2]; Ferrazzi, E[3]
[1]Food Intolerances Department, GEK Srl, Milano, Italy; [2]Department of Statistical Science, Duke University, Durham, United States; [3]Department of Woman, Mother and Neonate – Buzzi Children's Hospital, Biomedical Sciences School of Medicine, University of Milan, Milan, Italy

Background: Peanut allergy affects approximately 1% of the population worldwide. According to Finkelman's work on murine and human immune system, specific IgG for food are the expression of both reaction and tolerance. The former through the activation of the alternative pathway of anaphylaxis mediated by IgG, FcγRIII, macrophages, PAF and BAFF and the latter for the prevention of IgE-mediated anaphylaxis by intercepting antigens before their binding to mast-cell-associated specific IgE. IgG antibodies, therefore, block systemic anaphylaxis induced by small quantities of antigen but mediate systemic anaphylaxis induced by larger quantities. As discussed by Ligaarden, the concentration of specific IgG for food reflects the use of that food in the diet. The aim of this study is to investigate the distribution of IgG for peanuts in a large sample of non-peanut-allergic and healthy Italians in order to provide additional epidemiological information, useful to better understand the mechanisms of peanut hypersensitivity.
Method: This work is part of a larger study on non-IgE-mediated food reactions. The levels of specific IgG for peanuts were measured in 20 135 Italians patients (74.1% female, mean age 44.5 years ± 15.5 as standard deviation) without peanut allergy. The IgG antibodies were measured using the ELISA technique. From this data, a statistical analysis to study the frequency distribution has been performed.
Results: The analyzed data shows a highly asymmetrical distribution (skewness = 2.026) with median for IgG specific for peanut equal to 17.47 U/ml, first quartile 9.66 U/ml and the last quartile 30.94 U/ml as first and last part of distribution respectively. A 95% bootstrap confidence interval for the median ranges from 17.24 to 17.68 U/ml.
Conclusion: This data relates to a healthy population, not allergic to peanuts. The relatively quite low concentration of specific IgG for peanuts reflects the nutritional habits of Italians, the vast majority of which eats peanuts only occasionally. This knowledge can be useful to better understand the interaction between IgE and IgG in the pathogenesis of food allergy. If the level of IgG reflects the pattern of dietary intake, it is very likely that people with allergies to peanuts, systematically avoiding the intake of this food, have very low levels of specific IgG. Epidemiological knowledge is the first step in the understanding of these phenomena.

626
Web-based survey of food hypersensitivity in general adult population

Duman, H; Dursun, AB
Division of Immunology and Allergic Diseases, Department of Internal Medicine, School of Medicine, Recep Tayyip Erdoan University, Rize, Turkey

Background: There is limited and inconsistent data on the prevalence of food hypersensitivity (FH) in adult population. Sociocultural conditions and dietary habits may be responsible for the discordance. Furthermore there are serious knowledge gaps about the prevalence rates of FH in developing countries. The aim of the study was to determine the self-reported prevalence of FH in general adult population.
Method: A cross-sectional web-based structured survey was designed. The questionnaire was consisted of 12 items related to complaints about the food intake. Adults born, raised and still living in the Eastern Black Sea region of Turkey were chosen as a targeted population. Several invitations were made on the local virtual media during 3 months in the previous year.
Results: The study population consisted of 920 (F/M: 604/316) subjects who answered voluntarily. The mean age was 38.9 ± 15.2 (18–88) years. At least one atopic comorbidity was determined 36% of the subjects and 28.5% of their first-degree relatives. FH was reported by 157 (17.3%) subjects among them without statistical significant differences related to gender. The most culprit foods were spicery (15%), tomato (10.9%), cow milk (7.5%) and citrus fruit (4.1%). The most common reported symptom was urticaria (63.5%) followed by gastrointestinal symptoms (30.2%), worsening of rhinitis (15.1%) and oral allergy symptoms (11.3%). We found younger age ($P = 0.004$), personal and familial history of any atopic disease ($P = 0.001$) as a risk factor for having FH.
Conclusion: The prevalence of self-reported FH based on web based survey in Eastern Black Sea residents is relatively high and specific to the region.

© 2015 The Authors

Poster Discussion Session PDS 33

Drug allergy: Clinical aspects

627
Evaluation of drug provocation test related anxiety and possible related factors caused in patients with drug hypersensitivity

Soyyigit, S[1]; Aydın, Ö[1]; Yılmaz, İ[1]; Özdemir, SK[1]; Cankorur, VŞ[2]; Atbaşoğlu, C[2]; Çelik, G[1]
[1]Department of Immunology and Allergic Diseases, School of Medicine, Ankara University, Ankara, Turkey; [2]Department of Psychiatry, School of Medicine, Ankara University, Ankara, Turkey

Background: Drug provocation tests (DPT) carry certain risks for development of hypersensitivity reactions in patients with drug hypersensitivity (DH). As the patients are informed about the risks of the procedures prior to tests, this could lead them have some concerns. However, so far, no report has been available about how much anxiety of the patients have related to these tests. In this study, DPTs related anxiety of the patients admitted to our clinic to be tested for DH were primarily investigated.
Method: The study included all consecutive patients who are indicated to have DPT for evaluation of DH between 2009 and 2012. After recording of patients history and characteristics of DPTs, a variety of psychiatric measures [Hospital anxiety and depression (HAD) scale] were performed. DPTs related anxiety were also evaluated with VAS scale (between 0–10:10 is the most effected). Besides, these patients were re-questioned on whether they used the tested drug within 1 year.
Results: Total of 126 cases (F/M: 101/25, mean age: 38 ± 1 years) were included in the study. Analgesics were the most common reaction causing hypersensitivity reaction (55.2%). According to HAD scale, 34.9% and 23.9% of the patients had depression and anxiety symptoms, respectively. Prior to tests, in 58.3% of patients, DH related VAS anxiety scores were eight and above. The main reason for anxiety was the possibility of the recurrence of the reaction (n: 89, 70.6%). VAS anxiety scores before DPTs were higher than those of after negative test result (before: 5.2 vs after: 2 $P = 0.0001$). VAS scores in both conditions were correlated with anxiety scales. In the long term, 24.4% of the patients didn't use the drug because of ongoing anxiety related to drug reactions despite negative DPTs and presence of reason to use the drug.
Conclusion: This study showed that DPTs create a significant anxiety on patients who underwent these tests which was also influenced by the patients underlying general anxiety level. Importantly, this anxiety decreased after negative tests. However, considering the fact that one in four patients with negative test results didn't use the drug even if it was needed, supportive strategies to eliminate the anxiety are needed in these patients in order to facilitate to use the tested drug after negative test.

628
Drug allergy – results from drug provocation database in a Danish allergy clinic

Fransson, S; Mosbech, H; Kappel, M; Kvisselgaard, AD; Heinig, JH; Winther, L; Plaschke, P; Johnsen, C; Halkjær, LB; Torsnes, L; Elberling, J; Garvey, LH
Allergy Clinic, Copenhagen University Hospital Gentofte, Hellerup, Denmark

Background: Drug allergy is a widespread problem but large epidemiological studies are scarce. Most allergy centres use skin tests and only carry out drug provocation if skin tests are negative. However, drug provocation tests are the 'Gold Standard' and recent studies suggest that a negative drug provocation on first dose should be followed by a prolonged provocation over several days. This study presents results from drug allergy investigations based on drug provocation, including prolonged provocation, with suspected culprit drugs.
Method: Data from adult patients investigated for drug allergy (excluding perioperative reactions) in a Danish Allergy Clinic during 2010–2014 were consecutively entered in a database. Data were based on questionnaires completed by the attending doctors and included details on history, and results of specific IgE (only for penicillins) and provocation tests with suspected culprit drug, carried out if specific IgE tests were negative. If provocation was negative on first dose the drug was continued for 3–10 days depending on reported symptom onset. If culprit type of penicillin was unknown provocation with penicillin V was performed. Provocations were divided into positive on the first dose, or later.
Results: A total of 1913 provocations were carried out in 1659 patients, 1237 (74.6%) females and 422 (25.4%) men, mean age 47 (range 18–90). Drugs investigated were: Antibiotics 1776 (92.8%) of which 1590 (89.5%) were penicillins and 21 (1.2%) cephalosporins; analgesics 59 (3.1%) of which 45 (76.3%) were NSAIDS; local anaesthetics 33 (1.7%); other drugs 45 (2.4%) including vaccines, proton pump inhibitors, antifungals, biologicals and contrast media. In total 211 out of 1913 (11.0%) provocations were positive. Positive provocations were caused by: Antibiotics in 198 cases (93.8%) of which 167 (84.3%) were penicillins; 7 (3.3%) analgesics of which 6 (85.7%) were NSAIDS; local anaesthetics 0; 6 (2.8%) other drugs. Only 43 (20.4%) provocations were positive on first dose while 168 (79.6%) turned positive during prolonged provocation.
Conclusion: In this large study of 1913 recent drug provocations only 11.0% were positive. Interestingly, only 20.4% of provocations were positive on first dose, indicating that prolonged provocation is crucial when diagnosing drug allergy. The majority of provocations were with antibiotics, primarily penicillins, reflecting the pattern of antibiotic use in Denmark which differs from other countries.

629
Selective hypersensitivity to cefazolin – contribute of the basophil activation test

Almeida, JP[1]; Lopes, A[1]; Caiado, J[1]; Melo, A[2]; Santos, MC[2]; Barbosa, M[1]
[1]Department of Immunoallergology, Santa Maria Hospital, Lisbon, Portugal; [2]Laboratory of Clinical Immunology, Institute of Molecular Medicine, Faculty of Medicine of Lisbon, Lisbon, Portugal

Background: The prevalence of hypersensitivity to cephalosporins is increasing due to the rising number of prescriptions. Concerning cefazolin, reactions are often immediate and severe for its parenteral usage, and selective hypersensitivity seems to be the most common presentation. The immunoalergological evaluation includes skin testing and oral challenge tests. A basophil activation test (BAT) may add an additional contribute in those cases of immediate and severe allergy, in which confirmation of clinical suspicion (despite

© 2015 The Authors

negative skin test results) would avoid a risky drug challenge. The authors report two clinical cases of selective, immediate cefazolin hypersensitivity.

Case 1: A total of 49 year-old woman with a past history of a generalized urticaria 3 h following vertical-banded gastroplasty surgery in 2008. Revisional surgery was performed in 2009 with no adverse reaction. In 2012, anaphylaxis requiring mechanical ventilation support was documented 15 min after anesthesia induction for gastric sleeve surgery. Thedrugs used were rocuronium, propophol and cefazolin. Skin prick and intradermal tests were all negative except for the intradermal for cefazolin (immediate positive at 0.1 mg/ml). Oral challenge tests with penicillin, amoxicilin and other cephalosporins were negative. Specific IgEs were negative for all beta-lactams. The BAT was positive with cefazolin, with a percentage of activated basophils of 15.86% and a stimulation index of 10.

Case 2: A total of 36 year-old woman in labor was submitted to epidural block with ropivacain and sufentanil and experienced a facial erythema, lips angioedema and hypotension immediately after the administration of cefazolin, oxytocin and ephedrine. The skin prick and intradermal tests were negative for all drugs used, except for cefazolin which was immediate positive at 1 mg/ml. Challenge tests for other betalactams were also negative. Specific IgEs were negative for betalactams and the BAT with cefazolin was negative.

Discussion: In both cases, skin tests for culprit drugs were negative except for the intradermal test for cefalozin. The BAT performed for cefazolin was positive only in patient 1, corresponding to the most recent reaction (2 years before) as opposed to patient 2 who had reacted several years earlier. This selective hypersensitivity and reactivity pattern may be associated with the R1 side chain, which is known to be different in cefazolin when compared with other cephalosporins.

630
Trends in admissions for drugs anaphylaxis and for fatal drug anaphylaxis in the Spanish hospitals system (1998–2011)

Peña Acevedo, Y; Jimenez Ruiz, CE; Nieto Nieto, AM; Córdova Ramos, G; Rodriguez Alvarez, M; Tobón Franco, JD; Macias Iglesias, J; Tejedor Alonso, MA
Alergology, Hospital Universitario Fundación Alcorcon, Alcorcon, Spain

Background: In the present study, we examine trends both in admissions for drugs anaphylaxis and for fatal drugs anaphylaxis in the Spanish hospital system during the period 1998–2011.

Methods: The study was carried out using the Spanish Minimum Basic Data Set (MBDS), which is a database created by the Spanish Ministry of Health. The study was performed using data from the MBDS between 1998 and 2011.

Drug anaphylaxis: was designed using the next algorithm:

-Admission records with the code 995.0 (unspecified anaphylactic shock) and a code included in E930 to E949 (drugs and biologic substances which cause adverse effects when used as therapy).

-Admission records with the code 995.0 (unspecified anaphylactic shock) and the code 995.4 (shock caused by anesthesia).

-Unspecified adverse effect caused by appropriate administration of a drug or medicinal or biologic substance (995.2) and the combination of codes of two organs or systems usually involved in the anaphylaxis syndrome (skin and mucosa and respiratory system or gastrointestinal symptoms) or codes related to hypotension.

Results: During the study period, 4815 records from the MBDS met our inclusion criteria. We observed the highest incidences of admissions for drug anaphylaxis during 1998–2011 was patients aged >49 years. Increases in admissions for drug anaphylaxis were significant in the 10- to 14-year group (3.24-fold; 95% CI, 1.54–6.80), >75-year group (2.4-fold; 95% CI, 1.91–3.02), 15- to 49-year group (1.49-fold; 95% CI, 1.22–1.82), and 50- to 74-year group (1.49-fold; 95% CI, 1.28–1.72).

The highest increases in incidence were observed for other antibiotics (3.75-fold), general anesthetics (3.06-fold), anticancer drugs (2.33-fold), and diagnostic agents (2.01-fold).

Penicillin was the only drug family for which admissions decreased – We observed increase of drug anaphylaxis in the admissions for drugs anaphylaxis in Spain durind the period 1998–2011.

The highest increase in incidence were observed for other antibiotics, general anesthetics, anticancer drugs, and diagnostic agents. In the case of penicillin decreased its incidence.

Conclusions: We observed increase of drug anaphylaxis in the admissions for drugs anaphylaxis in Spain durind the period 1998–2011.

The highest increase in incidence were observed for other antibiotics, general anesthetics, anticancer drugs, and diagnostic agents. In the case of penicillin decreased its incidence.

631
Direct priming of mast cells with antihypertensive drugs – implications for the severity of anaphylaxis

Nassiri, M; Babina, M; Worm, M
Department of Dermatology and Allergy, Allergy-Center-Charité, Charité Universtiätsmedizin, Berlin, Germany

Background: Cardiovascular drugs like Beta-blockers and ACE-inhibitors are frequently used and have been suggested to influence the outcome of anaphylaxis. Whether and how they alter anaphylactic reactions is not well understood.

Method: We established a mouse model of passive systemic anaphylaxis (PSA) to mimic the long term treatment in cardiovascular therapy. Before the induction of PSA, Balb/c mice were pre-treated with the ACE-inhibitor ramipril and/or the beta-blocker metoprolol for 14 days. Core body temperature was determined for 70 min and the amount of mast cell mediators (histamine, serotonin, LTC_4, PGD_2) was analyzed via ELISA. In addition, murine bone marrow-derived cultured mast cells (BMcMCs) were incubated with various combinations of the drugs or bradykinin, which increases upon ACE-inhibitor treatment. The cells were sensitized with IgE over night and mast cell degranulation was induced via anti-IgE to determine histamine release.

Results: PSA (assessed by hypothermia) was aggravated by pre-treatment of mice with either ramipril or metoprolol alone. A combined treatment with the respective drugs, however, led to an even steeper increase in hypothermia. Accordingly, the preformed mediators histamine and serotonin as well as the de novo generated lipids LTC_4, PGD_2 were elevated in mouse sera upon combined drug treatment. Neither ramipril nor metoprolol (alone or in combination) affected the body core temperature of mice without induction of PSA.

In vitro, these findings were confirmed. Incubation of BMcMCs with either ramipril, metoprolol or bradykinin alone slightly elevated histamine release. However, the combination again turned out to be most potent. Metoprolol/ramipril elevated the release of histamine by up to 156% compared to an untreated control. A similar effect was obtained upon incubation of the cells with metoprolol/bradykinin (increase of histamine release by up to 92%). None of the employed substances altered histamine release, when cells were unstimulated.

Conclusion: Taken together our data indicate that ACE-inhibitors and beta-blockers can elevate the severity of anaphylaxis, which is in part mediated by a direct mast cell priming.

© 2015 The Authors

632
NSAIDs-exacerbated respiratory disease in adolescents

Ertoy Karagol, HI[1]; Yilmaz, O[1]; Topal, E[1]; Ceylan, A[2]; Bakirtas, A[1]
[1]Pediatric Allergy and Asthma, School of Medicine, Gazi University, Ankara, Turkey; [2]Department of Otorhinolaryngology Head & Neck Surgery, School of Medicine, Gazi University, Ankara, Turkey

Background: There has been no study investigating NSAIDs-exacerbated respiratory disease (NERD) exclusively in childhood. Therefore, in the current study, the authors aimed to evaluate the diagnostic features, clinical characteristics and follow-up of adolescents diagnosed as NERD.
Methods: The patients who were consecutively diagnosed with NERD between January, 2011 and November, 2013 included in the study. Oral provocation test (OPT) with at least two different NSAIDs was used to confirm NSAID hypersensitivity in patients with underlying asthma/chronic rhino sinusitis/nasal polyps. All patients were followed up regularly in 3 months intervals by the pediatric allergy and otorhinolaryngology department for asthma, allergic rhinitis, chronic rhinosinusitis with or without nasal polyps.
Results: A total of 10 adolescents with NERD were included in the study. The mean age of the patients at the time of diagnosis was 14.9 ± 1.5 years. Hives or angioedema accompanied respiratory complaints induced by NSAIDs. The mean duration of follow-up was 28.9 ± 12.4 months. All patients had asthma except one with asymptomatic bronchial hyperreactivity. Asthma of patients was well-controlled with moderate dose of inhaled corticosteroids. Chronic rhinosinusitis with or without nasal polyps developed in two patients. Aspirin desensitization was required in these two patients with recurrent nasal polyps.
Conclusions: NERD in childhood has much favorable clinical characteristics and course than in adulthood. Few adolescents with NERD refer with typical chronic upper respiratory tract complaints. Asthma component seems to be mild and is well-controlled in the short-term follow-up.

633
Sensitization to paracetamol in patients with intolerance to NSAIDS

Barasona Villarejo, MJ[1]; García Nuñez, I[2]; Moreno Aguilar, C[1]
[1]Allergology, Hospital Universitario Reina Sofía, Córdoba, Spain; [2]Hospital Quirón, Málaga, Spain

Background: After checking that there is an intolerance to NSAIDs a single blind controlled challenge test (CCT) is indicated, to confirm whether the patient tolerates other inhibitors of Cox-1 and Cox-2. We try to show the existence of cases in which, in addition to intolerance to NSAIDs, there is also sensitization to Paracetamol.
Method: We studied 445 patients considered intolerant to NSAIDs after being diagnosed by medical history and/or by positivity after CCT with ASA or another NSAID. All patients underwent three CCT with: Meloxicam, Paracetamol 2 g and Celecoxib (Piroxicam was also studied in 137 patients and Etoricoxib was studied in 48 patients), always with divided doses and separated by 1 week.
Results: Of the 445 patients, 391 (88%) tolerated all drugs studied. The remaining 54 had adverse reactions. There were 13 reactions with Celecoxib, 26 with Meloxicam, 9 with Piroxicam, 4 with Etoricoxib and 9 with Paracetamol. The nine patients who were allergic to Paracetamol (2%) had a mean age of 37 years (19–55) and 5 were male. All reactions occurred within the first 2 h after the CCT and all were mild skin reactions except 3 in which there were respiratory symptoms (rhinitis in two and dyspnoea in one). Five of the reactions occurred after the maximum dose of 2 g, 2 with 1 g and 2 with <750 mg. In seven patients tolerance dose of 1 g or less Paracetamol 500 mg was found. Two patients also presented reaction with Celecoxib and three with Meloxicam.
Conclusion: Although drugs administered are considered very safe, such as acetaminophen in patients with multiple sensitization to NSAIDs, caution should be exercised in the CCT and always before recommending their use, the patients' tolerance should be confirmed under a specialist's supervision.

634
Evaluation of diagnostics in patients with suspected allergic reactions during anaesthesia in the Netherlands

Purmer, MS[1]; Nederhoed, VC[1]; Oude Elberink, JNG[2]; van Maaren, MS[3]; Tupker, RA[4]; de Jong, NW[3]; Dhaliwal, R[2]; Rockmann, H[1]
[1]Dermatology/Allergology, UMC Utrecht, Utrecht, The Netherlands; [2]Allergology, UMCG, Groningen, The Netherlands; [3]Allergology, Erasmus MC, Rotterdam, The Netherlands; [4]Dermatology, St. Antonius Hospital, Nieuwegein, The Netherlands

Background: Allergic reactions during anaesthesia can contribute to a higher morbidity and delay of future anaesthetic procedures. It is important to perform diagnostic work-up to identify the causal drug. Despite thorough diagnostics, a causal drug cannot always be found. Furthermore, the most frequently identified causal drug varies per country. So far, no results have been published for the Netherlands.
Method: A retrospective multicentre study, in four Dutch medical centres, was performed. All patients referred between 2002 and 2014 for evaluation of a suspected allergic reaction related to general anaesthesia were included. Based on defined characteristics of the clinical presentation, reactions were differentiated in suggestive IgE-mediated (<30 min after drug exposure generalized cutaneous symptoms with or without hypotension) or non-suggestive IgE-mediated. Patient characteristics, symptoms of the reaction and results of the diagnostic work up (skintest, serology and provocation) were analysed.
Results: A total of 242 patients (66% female; mean age 47.7 years) were included. The 138 reactions (57%) were classified as suggestive IgE-mediated reaction (71.0% anaphylaxis). Of those, in 99 patients (71.7%) 104 possible causal drugs were identified. Most common causal drugs were antibiotics (23.8%), mainly cefazolin (68%). Neuromuscular blocking agents were identified as causal drug in 20.0% (rocuronium 53%; mivacurium 26%; atracurium 21%), patent blue dye in 14.3%, NSAIDs in 11.5% and chlorhexidine in 8.6%, respectively.

The reactions that were classified as not suggestive for an IgE mediated mechanism (n = 104; 43%) were likely due to non-specific histamine release (very mild cutaneous symptoms only; or infusion arm reaction; n = 62, 59.6%) or probably other side effects (n = 33, 31.7%).
Conclusion: Of all patients referred for evaluation of a suspected allergic reaction during general anaesthesia, 57% have been classified as having a suggestive IgE-mediated reaction. Of these, the causal drug could be identified in 71.7% of the patients, of which antibiotics, neuromuscular blocking agents and patent blue dye have been identified most frequently.

635
Diagnosis of allergy against beta-lactams in primary care: prevalence and diagnostic criteria

Salden, OAE[1]; Rockmann, H[2]; Verheij, TJM[1]; Broekhuizen, BDL[1]
[1]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands; [2]Department of Dermatology and Allergology, University Medical Center Utrecht, Utrecht, The Netherlands

Background: Beta-lactam antibiotics are among the most prescribed drugs in general practice worldwide. Unfortunately they account for a large fraction of the drug allergies noted in the medical records

© 2015 The Authors

of patients. Secondary care studies showed that a recorded allergy to beta-lactams could not be confirmed by valid allergy testing in >85% of cases. In daily practice beta-lactam allergy labels probably cause the prescription of secondary choice antibiotics. This overrating of beta-lactam allergy hampers appropriate use of narrow spectrum antibiotic and possibly generates unnecessary cost and bacterial resistance.

Objectives: To assess frequency of registration and criteria of suspected allergies against beta-lactam antibiotics in Dutch primary care.

Methods: A retrospective cohort study in 8288 primary care subjects was performed. Patients with recorded allergy were identified through International Classification for Primary Care coding. Signs and symptoms of the recorded allergic reaction and patient's characteristics were extracted from patient's files and from a standardized questionnaire sent to patients. The probability of allergy was based on a composite reference standard which was scored by two authors independently.

Results: A total of 163 (2.0%) subjects (66.9% female) had a recorded allergy. In 51.5% of cases, no characteristics of the recorded allergic reaction were reported in patients' medical files. Based on our composite reference standard, allergy was excluded in 19 subjects (11.7%). Risk factors for allergy registration were female gender, age <4 years, and the comorbidities: asthma, allergies and skin disorders.

Conclusion: The prevalence of recorded allergy against beta-lactam antibiotics in Dutch primary care is 2%. Due to lack of registration of accompanying signs and symptoms of the recorded allergy, this diagnosis is uncertain in the majority of patients. Better documentation and classification of possible allergic reactions to beta-lactams are needed in primary care setting to reduce unnecessary registration of allergy to beta-lactam antibiotics. This will help to target antibiotic treatment and reduce microbial resistance.

636
Nabumetone... a safe alternative NSAID for intolerant patients?

de la Osa Puebla, V; Sánchez Matas, I; Tapia de Pedro, G; Villalba Lorenzo, E; Senent Sánchez, C
Allergy, Hospital Virgen del Valle, Toledo, Spain

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) comprise a group of drugs widely prescribe in clinical practice due to their anti-inflammatory, antipyretic and analgesic properties.

Patients with intolerance to NSAIDs present respiratory (rhinitis/asthma) or skin symptoms (itching/angioedema) after taking these drugs. Therefore, diagnosis of hypersensitivity to NSAIDs is an important therapeutic limitation, so alternative drugs for these patients are required.

Nabumetone is a NSAID that is administered in prodrug form and then activated in the liver. It acts mainly upon activation in COX-2, so it may be a safe alternative drug for intolerant NSAIDs patients.

The aim of this study was to value the degree of tolerance to Nabumetone in patients, both children and adults, diagnosed with intolerance to NSAIDs.

Method: A descriptive, observational, retrospective study including 85 cases diagnosed with NSAID intolerance is presented in this work. From the 85 patients involved, 8 were children.

Patients were recruited for the study between January 2012 and M ay 2014. The diagnosis was establish by clear history of symptoms after taking two or more NSAIDs of different groups or history reactions with one NSAID and ASA positive provocation.

Subsequently, an oral provocation with Nabumetone to therapeutic doses was performed.

Results: It was found that 31.6% of adult diagnosed with intolerance to NSAID reported respiratory symptoms, 9.1% respiratory and skin symptoms, and 59.7% only skin symptoms.

Six of the eight children showed skin symptoms, one of the eight only respiratory and the other one, both respiratory and skin.

All our patients had negative oral challenge with Nabumetone until therapeutic dose, confirming their tolerance.

Conclusion: Nabumetone has been demonstrated to be a safe alternative NSAIDs, both for adults and children, in patients with previous diagnosis of intolerance to NSAIDs.

637
Infusion reactions to antineoplastic drugs (platines & taxanes) and re-administration with the standardized 12-step protocol: the Greek experience

Gkavogiannakis, N; Potika, M; Kontogiorgaki, I; Aggelides, X; Tyligada, A; Makris, M
Drug Allergy Outpatient Clinic, Allergy Unit 'D. Kalogeromitros', 2nd Dpt of Dermatology and Venereology, Medical School, University of Athens, Athens, Greece

Background: Platines and taxanes are frequently associated with drug hypersensitivity reactions nowadays. Drug desensitization is the only way to re-administer these drugs that in most cases are essential for the adequate therapy of the malignancy.

The aim of this study was to registry and diagnostic access of hypersensitivity infusion reactions of chemotherapeutics and the reintroduction of medications with desensitization.

Method: Patients receiving chemotherapeutics (platines&taxanes) between January 2011 and December 2014 in the Oncology Unit, were included in this cohort. All hypersensitivity reactions during the infusion or within 6 h after completion of infusion were evaluated and recorded. A detailed history, clinical assessment and subsequent grading of the reaction's severity were performed in all reactors; *in vivo* testing with non-irritant concentrations of the offending agent were carried out when available, 2 weeks later. In all subjects with severe reaction and/or positive skin testing, desensitization to the culprit agent was performed when alternative therapies were subordinate according to treating oncologist. A standardized 12-step, continuous infusion, desensitization protocol with use of three bags with 10-fold graded concentration of the drug, was used in desensitization procedures.

Results: Totally 87 patients (55♀, mean age ± SD: 63 ± 10) with hypersensitivity reaction to chemotherapeutics were evaluated; 53 (61%) experienced reactions to platines and 34 (39%) to taxanes respectively. Thirty-one out of 87 (35.6%) reported similar reactions, even milder in most cases, at previous chemotherapy cycles. Seventy seven (88.5%) pts developed anaphylaxis and 10 (11.5%) rather non immunological reactions. Cutaneous manifestations (75%) were the protrusive symptoms despite the culprit drug. The 44 out of 87 (51%) pts underwent skin tests: 27 (61%) turned positive while 17 (39%) were negative. Accordingly, 297 desensitizations were carried out in 67 (77%) pts (mean ± SD: 4.4 ± 3.5); 23/67 (34%) pts experienced hypersensitivity reaction during the desensitization, in the vast majority at the 12th step. All observed reactions were milder than the pre-desensitization reported.

Conclusion: Hypersensitivity reactions to chemotherapy agents require allergological evaluation. Rapid desensitization with the 12-step protocol, when performed properly in well-characterized patients, is the only safe and effective way to administer the full therapeutic dose of the first-line therapy.

© 2015 The Authors

638
Comparison of basophil activation test (BAT) and skin testing performances in the assessment of neuromuscular blocking drug-induced allergy

Dewachter, P[1,2]; Chollet-Martin, S[1,3]; Mouton-Faivre, C[4]; de Chaisemartin, L[1,3]; Nicaise-Roland, P[1]
[1]APHP, Paris, France; [2]INSERM UMR-S970, Paris, France; [3]INSERM UMRS996, Châtenay-Malabry, France; [4]CHU, Nancy, France

Background: Neuromuscular blocking drugs (NMBDs) are the main triggers involved during perioperative immediate hypersensitivity. The etiological diagnosis (IgE-mediated allergy vs non-allergy) is linked to the clinical presentation together with tryptase and skin tests results. The role of BAT needs to be better defined in this setting. The aims of this study were to compare the results of skin testing (identification of the culprit agent and cross-reactivity to other NMBDs) to those obtained using BAT in 31 patients experiencing perioperative immediate hypersensitivity potentially related to NMBD.
Methods: Plasma tryptase was quantified after the clinical reaction and compared to basal tryptase. Anesthetic drugs, including NMBDs (suxamethonium, rocuronium, vecuronium, atracurium, cis-atracurium), were skin-tested according to current guidelines. BAT was done using a technique adapted from a commercial test (FlowCAST®). CD63 and CD203c increased expression was measured following serial dilutions of NMBDs.
Results: Group *Allergy* involved 19/31 patients (61.3%) with positive skin tests (sensitivity: 100%) to the culprit NMBD. Skin cross-reactivity was identified in 36.8% of these patients. Group *Non-Allergy* included 12 patients (38.7%) with negative skin tests to the culprit NMBD and to the other NMBDs. Tryptase was increased in allergic (median: 42.4 µg/l) vs non-allergic patients (median: 5.6 µg/l; $P < 0.001$).

CD63 and CD203c increase in response to the culprit agent was respectively identified in 63.1% and 73.7% of the cases. When combining CD63 and CD203c, BAT positivity reached 78.9% (15/19 allergic patients). BAT was positive to other cross reactive NMBDs in 5/19 patients (26.3%). The concordance between skin tests and BAT (culprit and cross-reactive NMBDs) was 36.8%. In non-allergic patients, BAT was positive once for CD63. In this non-allergic group, the concordance between skin tests and BAT was 100% with the other skin-tested NMBDs. Negative skin-tested NMBDs were uneventfully injected in six of the allergic patients during further anesthetics.
Conclusion: In our technical conditions, BAT combining CD63 and CD203c markers is an interesting additional tool but it does not replace skin testing in NMBD-induced allergy.

639
Anaphylatic reactions ocurring during anaesthesia

Aguiar, R; Lopes, A; Fernandes, NP; Pereira-Barbosa, MA
Immunoallergology Department, Hospital de Santa Maria – Centro Hospitalar Lisboa Norte, Lisbon, Portugal

Background: Anaphylaxis incidence in the perioperative setting varies between 1:10 000 and 1:20 000. Although this value is yet to be determined in Portugal, an increase in the number of reactions has been reported. Clinical evaluation is important in order to identify risk factors and drugs that cause anaphylaxis, so that alternative options can be found. The aim of this study was to characterize the reactions of patients (pts) with perioperative anaphylaxis and for conducting medical techniques requiring sedation.
Methods: Retrospective analysis of medical records of 45 patients with perioperative anaphylaxis and anaphylaxis undergoing medical procedures with sedation, observed in the immunoallergologic outpatient clinic (2009–2014). The diagnostic investigation was carried out 6–8 weeks after the reaction, included detailed medical history, specific IgE assay to betalactams and latex, skin tests (ST) with the culprit drug and evidence of provocation when necessary, according to the recommendations of the SFAR / ENDA.
Results: We studied 45 patients (34 females) with mean age of 52 ± 15 years. Regarding the severity of anaphylaxis and according to Mertes classification, 17 cases (37.8%) had stage II reaction, five cases (11.1%) grade III and one case (2.2%) grade IV. An IgE-mediated mechanism has been established in 26 patients (57.8%). The major etiologic agents causing IgE-mediated reactions were muscle relaxants in 7 patients (26.9%), antibiotics in five cases (19.2%; three cefazolin, one aminopenicillin, one ciprofloxacin), metamizole in three patients (11.5%), two patients latex (7.6%), four patients reacted with less representative agents. On seven patients (15.5%) drugs responsible for the reactions were associated with non-IgE-mediated mechanisms, anti-inflammatory (NSAIDs) are the most frequent agents (five patients). In 12 patients (26.7%) it was not possible to determine the etiology of the reaction.
Conclusions: More than half of perioperative events (57.8%) have an IgE-mediated mechanism. Muscle relaxants, antibiotics and patent blue dye were the most frequently identified agents. In 15% of the reactions was involved a non-IgE-mediated mechanism, namely NSAIDs. It is important to determine the etiology of perioperative reactions for guidance in future surgery, either because the identified agents are often used outside the perioperative setting.

640
Angioedema triggered by medication blocking the renin/angiotensin system: retrospective study using the French national pharmacovigilance database

Faisant, C[1]; Bouillet, L[1]; Boccon Gibod, I[2]
[1]Grenoble Alpes University, La Tronche, France; [2]CHU Grenoble, La Tronche, France

Background: Bradykinin-mediated angioedema (AE) is a rare side effect of some medications, including angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB). In France, side-effects to treatments are reported to the national pharmacovigilance database. We used data from this database to determine the clinical characteristics and prognosis for this disease.
Method: The national MedDRA database was searched using the term 'angioedema". Patients were included if they met the clinical criteria corresponding to bradykinin-mediated AE (isolated AE without urticaria, lasting at least 12 h, resistant to treatment with anti-histamines), if their C1-inhibitor levels were normal, and if they were treated with an ACEi or an ARB.
Results: A ttoal of 7998 cases of AE were reported between 1994 and 2013. Among these, 436 patients met the criteria for bradykinin-mediated AE, 324 with no data on C-inhibitor and 112 (69 men, 43 women; mean age 65 years) with normal C1-inhibitor levels. On the 112 drug-AE, patients were treated with an ARB in 21% of cases (24 patients), or an ACEi in 77% of cases (88 patients), in combination with another treatment in 17 cases (mTORi for three patients, iDPP-4 for one patient, hormonal treatment for seven patients). ENT involvement was reported in 90% of cases (tongue: 48.2%, larynx: 23.2%). The median duration of treatment before the first attack was 720 days, and the mean duration of attacks was 36.6 h. 41% (19/46) of patients relapsed after discontinuing treatment.
Conclusion: It is important to sensitise the medical community to the need to report all incidences of AE to allow better characterisation of this side effect.

© 2015 The Authors

Poster Discussion Session PDS 34

Biomarkers in asthma

641
Prevalence and overlap of asthma phenotypes in a general asthma population

Tran, TN[1]; Ward, CK[2]
[1]AstraZeneca, Observational Research Center, Gaithersburg, United States; [2]MedImmune, Translational Medicine, Gaithersburg, United States

Background: Atopic, eosinophilic, and T helper cell type 2-high (Th2-high) asthma phenotypes may overlap. Several new biologic therapies targeting specific asthma phenotypes are either available, or in development; newer medications may also treat more than one phenotype. Understanding the overlap across asthma phenotypes may be useful in determining treatment guidance, optimization, and overall care. The objective of this study was to describe the prevalence and overlap of atopic, eosinophilic, and Th2-high phenotypes in a general asthma population.
Method: Data from the National Health and Nutrition Examination Survey (NHANES), an annual survey of a nationally representative sample of the non-institutionalized United States population, were analyzed. Asthma patients were identified based on the participants' self or parental report. Eosinophilic asthma was defined as a blood eosinophil count $\geq$300 cells/μl. Atopic asthma was identified as an allergen-specific immunoglobulin E (IgE) level of $\geq$0.35 IU/ml, for any of the nine tested perennial allergens. Th2-high asthma was defined as total serum IgE $\geq$100 IU/ml, and a blood eosinophil count of either $\geq$100 or $\geq$200 cells/μl (Corren et al. N Engl J Med 2011; 365: 1088–1098). The study included only survey years 2005 and 2006, for which IgE data were collected.
Results: A total of 265 children (aged 6–17 years), and 303 adults (aged 18–64 years) were included in the study. Approximately 57% of children and 41% of adults were classified as having eosinophilic asthma, 50% and 42% as Th2-high asthma, and 63% and 61% as atopic asthma, respectively. Among those with atopic asthma, 75% of children and 50% of adults were also eosinophilic, and 70% and 60%, respectively, were Th2-high. Among those with Th2-high asthma, 80% of children and 62% of adults were categorized as eosinophilic. Approximately 38% of children and 23% of adult asthma patients could be classified as eosinophilic, atopic, and Th2-high, simultaneously; 77% of children and 74% of adult asthma patients belonged to one of these three phenotypes.
Conclusion: This study showed a significant overlap among three asthma phenotypes (eosinophilic, atopic, and Th2-high), in a general asthma population, especially in children. Future studies will need to examine whether a similar overlap is present in moderate and severe asthma populations, who are the likely target of biologics and new asthma therapies.

642
New biomarkers for the diagnosis and prognosis of asthma

Baos, S[1]; Calzada, D[1]; Quiralte, J[2]; Florido, F[3]; Lahoz, C[1,4]; Cárdaba, B[1,4]
[1]Immunology, IIS-Fundación Jiménez Díaz-UAM, Madrid, Spain; [2]Allergy, Hospital Universitario Vírgen del Rocío, Sevilla, Spain; [3]Allergy, Hospital Universitario San Cecilio, Granada, Spain; [4]CIBERES, Ciber de Enfermedades Respiratorias, Madrid, Spain

Background: Asthma is a complex chronic respiratory disease that includes a wide clinical spectrum, probably responsible for the heterogeneity of treatment response. It can be considered a syndrome with a variety of 'endotypes". Characterize new diagnosis and/or therapeutic biomarkers is a challenge to further improve this disease. The aim of this study is to validate molecular biomarkers related to the asthmatic disease's types and severity.
Method: Ninety four genes selected from gene-studies previously published, were analyzed by qRT-PCR, in a population of 104 unrelated subjects, 30 healthy control, 30 non-allergic asthmatic (diagnosed as severe, moderated and mild), 30 asthmatic allergic (allergic to airborne allergens) and 14 non-asthmatic allergic subjects. RNA was extracted from the subject's peripheral blood mononuclear cells (PBMCs) by trizol method. Statistical analyses were performed by StatMinner program. After assessment of data quality and principal components analysis (PCA), differential gene-expression, by multiple testing was performed. Relevance was defined by RQ (relative quantification) and corrected *P* value (<0.05). For the significant genes, protein quantification was determined in sera by ELISA or in proteins extracted from PBMCs by Western Blot.
Results: Gene-expression profiling obtained by PCA clearly showed clusters that correlated with the control and non-allergic asthmatic groups. Furthermore, the analysis of differential gene expression between these two groups revealed a set of statistically relevant genes, highlighting IL10, MSR1, SERPINB2, PHLDA1, IL8, CHI3L1 and PI3. The qRT-PCR results of the asthmatic allergic and non-asthmatic allergic groups are being analyzed. Average levels of IL8 were higher in the serum of asthmatic patients (452.28 $\pm$ 357.72 pg/ml) than in control (286.09 $\pm$ 179.10 pg/ml) and asthmatic allergic subjects (244.82 $\pm$ 319.66), but there were no statistically significant differences. Furthermore, the analysis according the disease's severity associated significantly the increased expression of some of these genes, especially MSR1, with severe asthma phenotype.
Conclusion: Several differential molecular biomarkers of asthma have been defined, and some of them, could be useful for the diagnosis or prognosis of the disease's severity.

643
ACOS and asthma patients have similar levels of remodeling markers and basement membrane thickness

Regateiro, FS[1,2]; Robalo-Cordeiro, C[3,4,5]; Pereira, C[4,5,6]
[1]Service of Clinical Immunology and Allergy, Coimbra University Hospital Centre, Coimbra, Portugal; [2]Institute of Immunology, University of Coimbra, Coimbra, Portugal; [3]Service of Pneumology, Coimbra University Hospital Centre, Coimbra, Portugal; [4]Centro de Pneumologia, University of Coimbra, Coimbra, Portugal; [5]CIMAGO, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [6]Clinical Immunology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Background: Airway chronic inflammatory injury in asthmatic patients can lead to irreversible structural changes known as airway remodeling and to obstructive decline of lung function. Airway remodeling is also seen in chronic obstructive pulmonary disease (COPD). Recently, an asthma-COPD overlap syndrome (ACOS)

© 2015 The Authors

with features of both diseases was described and shown to aggravate symptoms and prognosis. We here analyze the expression of several markers associated with airway remodeling in the blood and broncho-alveolar lavage (BAL) of COPD, asthmatic and ACOS patients.

Methods: Serum levels of procollagen I, total and cellular fibronectin, and elastase were determined by ELISA methods in COPD patients ($n = 14$), ACOS patients ($n = 13$), 'young' asthmatic patients ($n = 15$), 'old' asthmatic patients ($n = 8$), and healthy controls ($n = 11$). The BAL levels were also measured in a subset of healthy control ($n = 5$), ACOS ($n = 7$) and asthmatic ($n = 5$) patients. Basement membrane thickening was determined in bronchial biopsies of ACOS and asthmatic patients.

Results: The levels of procollagen I, total and cellular fibronectin, and elastase in the blood were not significantly different between any of the groups in multiple comparison tests (ANOVA/Tuskey and Kruskal Wallis/Dunn, according to normality). BAL levels of procollagen I and cellular fibronectin were not different between controls, ACOS and asthmatic patients (Kruskal Wallis/Dunn) neither were BAL levels of cellular fibronectin and elastase levels between ACOS and asthmatic patients (Mann–Whitney test). Basement membrane thickness was increased in both asthma (12.10 mm ± 0.82) and ACOS (13.05 mm ± 4.36) groups but no significant differences were found between the disease groups (*t* test with Welsh correction).

Conclusion: Our results suggest that development of ACOS does not aggravate remodeling in asthmatic patients as measured by basement membrane thickening. Blood and/or BAL levels of procollagen I, total and cellular fibronectin, and elastase do not correlate with asthma, COPD or ACOS and do not emerge as ideal markers of airway remodeling.

645

The meaning of serum soluble CD93 level in asthmatics: a possibility to be a novel biomarker for allergic inflammation

Park, HJ[1,2]; Park, YH[2]; Han, H[2]; Kim, J[1,2]; Park, KH[1,2]; Park, J-W[1,2]; Lee, J-H[1,2]
[1]Division of Allergy and Immunology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea; [2]Institute of Allergy, Yonsei University College of Medicine, Seoul, Korea

Background: CD93 is receiving renewed attention as a biomarker of inflammation in various inflammatory and immune mediated diseases. Asthma is chronic inflammatory airway disease and has few serologic biomarkers for inflammation. However, the meanings and roles of soluble CD93 (sCD93) in asthmatics have never been studied. We intended to evaluate the possibility of sCD93's being a novel biomarker for inflammation in asthmatics.

Method: We measured the levels of sCD93 in serum and figured out comorbidity, medical condition, medication history, lung function, serologic and sputum markers in 116 asthmatics.

Results: Mean level of serum sCD93 was 115.4 ± 34.8 ng/ml (from 36.3 to 241.0 ng/ml). Serum neutrophil (standardized coefficient −0.232, *P*-value 0.027) and FEV_1/FVC (standardized coefficient −0.322, *P*-value 0.015) were negatively correlated with the levels of sCD93. However, levels of sCD93 have no significant correlation with serum eosinophil, sputum eosinophil, sputum neutrophil and FEV_1. Levels of sCD93 in high dose inhaled corticosteroid (ICS) users (84.1 ± 28.9 ng/ml) were significantly lower than those in ICS non-users (128.9 ± 35.8 ng/ml) and low-medium dose ICS users (114.5 ± 33.2 ng/ml). Smoking histories in sCD93 high group were significantly heavier than those in other groups.

Conclusion: The serum levels of sCD93 are associated with neutrophilic inflammation and dose of ICS in asthmatics. We suggested that sCD93 have a probability to be a novel biomarker reflecting level of inflammation and therapeutic results of ICS.

646

The combination of elevated FeNO and blood eosinophils relates to poorer asthma control in young patients with allergic asthma

Malinovschi, A[1]; Janson, C[1]; Borres, M[2]; Nordvall, L[2]; Alving, K[2]
[1]Medical Sciences, Uppsala University, Uppsala, Sweden; [2]Women's and Children's Health, Uppsala University, Uppsala, Sweden

Background: We have recently reported in a population-based study the independent and additive information of the fraction of exhaled NO (FeNO) and blood eosinophil (B-Eos) count in relation to asthma, wheeze and asthma exacerbations (Malinovschi et al. JACI 2012; 132: 821–7). In the present study, we investigated FeNO and B-Eos in relation to symptom control in an asthma cohort.

Method: FeNO at an exhalation flow-rate of 50 ml/s, B-Eos and asthma control, by means of Asthma Control Test (ACT), were assessed in 321 subjects (10–35 years) with allergic asthma and ongoing inhaled corticosteroid (ICS) treatment. Asthma control was defined as ACT ≥20. Subjects were characterised as having allergic asthma if they were positive to either a mix of aeroallergens or a mix of food allergens (≥0.35 kU_A/l). Subjects sensitized to a mix of aeroallergens were further characterised with regard to sensitisation to pet allergens (cat, dog, horse).

Results: Subjects with both elevated FeNO (≥25 ppb if ≥18 years and ≥20 ppb if <18 years) and B-Eos (≥0.3 × 10^9/l; $n = 73$) had controlled asthma to a lower extent than the group with normal FeNO och normal B-Eos ($n = 149$): 25% vs 40%, $P = 0.02$. Having both elevated FeNO and B-Eos related to absence of asthma control with an odds ratio (95% CI) of 2.1 (1.05, 4.2) after adjusting for gender, age, BMI, IgE sensitization to pet allergens, and current ICS dose ($P = 0.04$). Having only elevated FeNO ($n = 36$) or B-Eos count ($n = 63$) was not related to an increased likelihood of uncontrolled asthma ($P = 0.38$ and $P = 0.70$, respectively), when comparing to the group of subjects with normal FeNO and normal B-Eos.

Conclusion: Additive information of two easily accessible inflammatory biomarkers, exhaled nitric oxide and blood eosinophils, was found with regard to asthma control in young subjects with allergic asthma. Interestingly, single increase of either marker was not related to poorer control of asthma, suggesting a role for the combined monitoring of these markers in order to optimize treatment.

647

Periostin in exhaled breath condensate of patients with asthma and chronic obstructive pulmonary disease

Wardzyńska, A[1]; Makowska, JS[1]; Piechota-Polańczyk, A[1]; Jabłońska, A[1]; Jarzębska, M[1]; Kowalski, ML[1]
[1]Department of Clinical Immunology, Rheumatology and Allergy, Healthy Ageing Research Centre, Medical University of Łódź, Łódź, Poland

Background: Periostin is a matricellular protein involved in tissue repair processes. Serum periostin concentration has been proposed to be a biomarker of eosinophilic airway inflammation in asthmatics. The aim of the study was to evaluate periostin levels in exhaled breath condensates (EBC) and in serum of patients with asthma and chronic obstructive pulmonary disease (COPD).

Method: The study included 67 asthmatics with moderate to severe persistent asthma, nine patients with chronic obstructive pulmonary disease (COPD) and 20 healthy controls. All procedures (questionnaire, spirometry, FeNO measurement, skin prick tests, EBC collecting and venous blood sampling) were performed on the same

© 2015 The Authors

day. Periostin concentrations in EBC and serum were measured using commercial ELISA kits.

Results: Periostin was detectable in EBC from 62 of 67 asthmatics, all of COPD patients and 19 of 20 healthy subjects. The mean periostin level in EBC samples was lower than in serum (2.8 ± 1.6 ng/ml vs 25.6 ± 12.51 ng/ml; $P < 0.001$). Patients with COPD had higher periostin level in EBC than asthmatics (3.7 ± 1 ng/ml vs 2.6 ± 1.6 ng/ml, $P = 0.042$). Patients with non-atopic asthma had higher periostin level in EBC than atopic asthmatics (3 ± 1.6 ng/ml vs 2.1 ± 1.8 ng/ml; $P = 0.035$). EBC periostin level was significantly higher in asthmatics receiving a burst with oral GCS for asthma exacerbation during last year (3.2 ± 1.6 ng/ml vs 2.3 ± 1.6 ng/ml, $P = 0.045$). The concentration of periostin in EBC was higher in asthmatics with self-reported coronary heart disease (CHD) than without CHD (3.5 ± 1.7 ng/ml vs 2.3 ± 1.2 ng/ml; $P = 0.03$). Although there was a weak correlation between EBC and serum periostin concentration in all subjects ($r = 0.23$, $P = 0.03$), no difference in serum periostin concentration between patients with asthma, COPD and healthy controls was found.

Conclusion: We documented for the first time, that periostin is detectable in EBC of patients with inflammatory airway diseases, and asthma phenotype or comorbid conditions may influence periostin concentration.

648
Induced sputum contaminated by saliva can be used in assessing eosinophilic airway inflammation

Lee, B-J[1]; Yune, S[1]; Lee, J-Y[2]; Choi, D-C[1]
[1]Department of Medicine, Samsung Medical Center, Seoul, Korea; [2]Samsung Medical Center, Center for Health Promotion, Seoul, Korea

Background: Induced sputum analyses are used to assess airway inflammation in asthma and other airway diseases. Salivary contamination is thought to affect the differential cell count in induced sputum. This study aims to quantitatively evaluate the effect of salivary contamination of induced sputum in identifying eosinophilic airway inflammation.

Method: Data were analyzed from patients who underwent sputum induction test, with history indicating asthma or chronic cough. A receiver-operating characteristic (ROC) curve found the cutoff level of fractional exhaled nitric oxide (FeNO) that best identified sputum eosinophilia (induced sputum eosinophil of 3% or more). Subsequently, the patients were divided into six groups according to the squamous cell percentage in induced sputum. Clinical characteristics, as well as the sensitivity and specificity of sputum eosinophilia were compared among the groups.

Results: From May 2011 to May 2014, sputum induction was requested in 1021 patients. There were 12 (1.2%) patients who could not produce sputum following standard induction protocol. The 67 (6.6%) sputum samples containing more than 70% of squamous cells were not analyzed because of practical difficulty in differential cell counting. Among the remaining 942 patients, 366 (38.9%) produced sputum samples containing more than 30% of squamous cells. There were no significant differences among the six groups in the age, sex, level of FeNO, and the presence of atopy or airway hyperresponsiveness (AHR). The ROC curve found that the FeNO level at 48.5 ppb predicted sputum eosinophilia with sensitivity of 78.8% and specificity of 77.7%. The frequency of sputum eosinophilia and the percentage of eosinophils in induced sputum decreased with increasing squamous cell percentage. In predicting the presence of AHR and the FeNO level of 48.5 ppb, lower sensitivity and higher specificity were observed in the highly contaminated groups. See table. Data are presented as median with interquartile range.

Conclusion: Induced sputum containing more than 30% can be used in predicting airway inflammation and AHR with high specificity.

	Age (years) ($P = 0.096$)	Atopy ($P = 0.307$)	AHR ($P = 0.265$)	Sputum eosinophil (%) ($P < 0.001$)	FeNO (ppb) ($P = 0.091$)	Sputum eosinophilia predicting AHR, Sensivitivy ($P = 0.001$) (%)	Sputum eosinophilia predicting AHR, Specificity ($P = 0.069$) (%)	Sputum eosinophilia predicting FeNO ≥ 48.5 ppb, Sensitivity ($P = 0.056$) (%)	Sputum eosinophilia predicting FeNO ≥ 48.5 ppb, Specificity ($P = 0.004$) (%)
SqC < 10% ($n = 190$)	49 (35–60)	47.9% (91)	33.2% (63)	1.33 (0.33–8)	44.5 (28–78.3)	71.4	75.6	66.7	84.0
10% ≤ SqC < 20% ($n = 195$)	46 (31–57)	56.4% (110)	34.4% (67)	1.33 (0–8.33)	40 (25–90)	71.6	73.4	76.5	82.5
20% ≤ SqC < 30% ($n = 191$)	48 (36–60)	51.8% (99)	30.9% (59)	1.33 (0–7)	43 (26–91)	81.4	79.5	66.3	81.5
30% ≤ SqC < 40% ($n = 149$)	49 (36–57)	49.7% (74)	26.8% (40)	0.33 (0–3.33)	37 (25–70.7)	57.5	84.4	58.6	93.4
40% ≤ SqC < 50% ($n = 116$)	53 (36–60)	43.1% (50)	23.3% (27)	0.33 (0–2.46)	38 (23–86.8)	63.0	87.6	56.8	95.8
50% ≤ SqC < 70% ($n = 101$)	45 (30–57)	51.5% (52)	26.7% (27)	0 (0–1.83)	37 (20.8–64.6)	37.0	83.8	50.0	93.8

[Comparison of the six groups].

© 2015 The Authors

649
An inverse association between elafin protein and adult asthma

Tseng, YT[1]; Tsai, YS[1]; Lin, MC[2]; Wu, CC[2]; Wang, TN[1]
[1]Kaohsiung Medical University, Kaohsiung, Taiwan, China; [2]Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan, China

Background: With the arrival of microarray technologies, searches for smoke-related and asthma-related biomarkers can be implemented in a discovery-driven manner using datasets from the National Center for Biotechnology Information. This study analyzed whether candidate genes from freely available gene expression microarray datasets related to asthma and smoke play a role in asthma pathogenesis and evaluated their potential as biomarkers.
Methods: We used human genome microarray datasets from smoke- and asthma-related gene expression datasets and performed real time quantitative polymerase chain reaction to measure and validate gene expression. We recruited adult asthmatics and community controls and evaluated lung function and plasma elafin levels.
Results: A candidate gene, *PI3*, was analyzed and compared in the four asthma-related and five smoke-related datasets. Analysis of the A549 cells demonstrated that *PI3* mRNA was significantly regulated by nicotine exposure. Healthy control's elafin concentrations were significantly higher than those from asthmatic patients. The highest quartile of plasma elafin concentration (≥12.69 ng/ml) was inversely associated with asthma ($P < 0.001$), as compared to the lowest quartile (<5.82 ng/ml) after adjusting for age, gender, and smoking status.
Conclusions: Our study found that high elafin levels, identified from smoke- and asthma-related microarray databases, has a protective effect on asthma. Further studies on elafin as potential therapy for asthma are warranted.

650
The impact of angiomotin and angiostatin on bronchial asthma

Jang, A-S[1]; Lee, P-H[2]; Kim, B-G[2]; Park, C-S[2]
[1]Soonchunhyang University Bucheon Hospital, Internal Medicine, Bucheon, Korea; [2]Soonchunhyang University Bucheon Hospital, Bucheon, Korea

Background: Angiomotins (AMOT) are originally identified as angiostatin binding proteins and implicated in the regulation of endothelial cell migration. Angiostatin is an endogenous protein generated after serial enzymatic proteolysis of plasminogen. The relationship of AMOT and angiostatins to the pathogenesis and progression of asthma is still poorly understood.
Objective: The aim of study was to identify the role of AMOT and angiostatin in asthmatic patients.
Methods: Fifty patients with asthma (stable and exacerbated state, respectively) and 25 healthy subjects were recruited. AMOT and angiostatins plasma level were checked using ELISA.
Results: AMOT and angiostatin in stable patients with asthma were not increased compared to that of control subjects. AMOT in patients of asthma was increased in exacerbation state than in stable state and in control subjects. Angiostatin in patients of asthma was decreased in exacerbation state than in stable state and in control subjects. AMT correlated with WBC count ($r = 0.321$, $P < 0.01$) and eosinophil proportion ($r = -0.282$, $P < 0.01$). AMT correlated with FEV1% predicted ($r = -0.252$, $P < 0.01$) and FVC % predicted ($r = -0.332$, $P < 0.01$).
Conclusion: These results indicate that angiomotin and angiostatin may be a maker for exacerbation of bronchial asthma.

651
Staphyloccocus aureus enterotoxin-specific IgE and asthma severity

Vilà-Nadal, G[1]; Pola-Bibián, B[1]; Romero, D[2]; Domínguez, J[1]; Barranco, P[1]; Quirce, S[1]
[1]Hospital La Paz Institute for Health Research (IdiPAZ), Allergology, Madrid, Spain; [2]Hospital Universitario La Paz, Neumology, Madrid, Spain

Background: Specific IgE to *Staphylococcus aureus* enterotoxins (SE-IgE) has been associated with rhinitis, rhinosinusitis, atopic dermatitis and asthma. However, this association has never been studied in the Spanish population. We aimed to determine the prevalence of SE-IgE in patients with severe uncontrolled asthma from our outpatient clinic.
Methods: We performed an epidemiological, open, observational and retrospective study with the data previously collected in our Department. We included all patients diagnosed with severe uncontrolled asthma from whom results of blood measurement of total IgE and SE IgE where available. The variables studied were: age, sex, severity and level of asthma control, presence of allergic rhinitis, chronic rhino-sinusitis with or without polyps, atopic status, SE-IgE (kU/L), total IgE (kU/L) and eosinophilia in peripheral blood. SE-IgE and total IgE concentrations were measured in serum with immunoassays. Asthma severity was classified following GEMA 2009 (*Spanish Guidelines for Asthma Management)*: intermittent, mild persistent, moderate persistent, severe persistent. Statistical analyses were performed using SPSS 13.2.
Results: A total of 266 patients were included (200 women, 66 men) of which 44 (16.5%) presented SE-IgE ≥0.35 kU/l. Mean value was 2.14 kU/l (SD: 5.6). We did not find any correlation between asthma severity and the presence of elevated levels of SE-IgE. The classification of the patients according to level of asthma control did not improve the significance of the possible relationship.
Conclusion: Unlike previous studies in which SE-IgE had been recognized as a risk factor for asthma severity, there is no evidence of this association in our study group. Further studies should be done in order to clarify the role of SE-IgE in the pathogenesis of asthma.

652
Serum and sputum level of sCD14 depend on a polymorphism of the CD14 promoter (CD14-C159T) in Crimean adults with early and late-onset asthma

Bisyuk, YA[1]; Kurchenko, AI[1]; Beloglazov, VA[2]; Dubovyi, AI[2]; Znamenska, LK[2]; DuBuske, LM[3,4]
[1]Bogomolets National Medical University, Kiev, Ukraine; [2]Crimean State Medical University, Simferopol, Ukraine; [3]Immunology Research Institute of New England, Gardner, United States; [4]George Washington University, MFA, Washington, DC, United States

Background: Patients with the TT genotype of the CD14 receptor have increased sCD14 concentrations and reduced total IgE levels compared to other genotypes. The association of the CD14 C-159T SNP and levels of sCD14 in early and late-onset asthma patients is unknwon.
Methods: A total of 262 early-onset and 69 late-onset persistent asthma patients were studied. Early-onset asthma as first symptoms before 40 years old and late onset after this age by GINA 2012 guidelines. The control group included 285 non-atopic volunteers. Single nucleotide polymorphisms of CD14 C-159T were detected by PCR. Serum and induced sputum levels of sCD14 were detected by ELISA. Patients and volunteers were recruited at the Crimean State Medical University, Simferopol, Crimea, providing written informed consent for the study.
Results: Serum levels of sCD14 in early-onset (Me = 5.53, Q1 = 3.94, Q3 = 7.51 μg/ml) and late-onset (Me = 5.90, Q1 = 4.13, Q3 = 8.05 μg/ml) asthma did not differ ($P = 0.064$) from control (Me = 4.99, Q1 = 3.53, Q3 = 6.90 μg/ml). Induced sputum levels of CD14 were significantly greater

© 2015 The Authors

in late-onset (Me = 11.5, Q1 = 6.2, Q3 = 21.4 ng/ml) vs early-onset asthma (Me = 8.8, Q1 = 5.8, Q3 = 11.6 ng/ml) and controls (Me = 6.7, Q1 = 4.3, Q3 = 9.3 ng/ml). In early-onset asthma patients CC genotype was detected in 74 patients (serum sCD14 – Me = 4.99, Q1 = 3.49, Q3 = 7.04 μg/ml, sputum sCD14 – Me = 8.8, Q1 = 5.4, Q3 = 10.6 ng/ml), CT in 142 (serum sCD14 – Me = 5.20, Q1 = 3.66, Q3 = 6.57 μg/ml, sputum sCD14 – Me = 8.1, Q1 = 5.6, Q3 = 10.6 ng/ml) and TT (serum sCD14 – Me = 10.72, Q1 = 6.53, Q3 = 13.18 μg/ml, sputum sCD14 – Me = 15.0, Q1 = 9.5, Q3 = 19.5 ng/ml) in 46; in late-onset asthma: CC (serum sCD14 – Me = 6.81, Q1 = 4.48, Q3 = 8.31 μg/ml, sputum sCD14 – Me = 14.7, Q1 = 8.1, Q3 = 22.3 ng/ml) in 31, CT (serum sCD14 – Me = 4.60, Q1 = 3.92, Q3 = 6.28 μg/ml, sputum sCD14 – Me = 7.4, Q1 = 3.8, Q3 = 10.7 ng/ml) in 27, and TT (serum sCD14 – Me = 11.47, Q1 = 6.11, Q3 = 12.93 μg/ml, sputum sCD14 – Me = 23.4, Q1 = 19.6, Q3 = 29.6 ng/ml) in 11; while in the control group CC in 97, CT in 146, and TT in 42. In early and late-onset asthma the serum and sputum levels of sCD14 in patients with TT genotype were significantly greater ($P < 0.01$) vs CC and CT genotypes. Patients with late onset asthma and TT genotype had the greatest levels of sputum sCD14.

Conclusion: Greatest levels of CD14 are associated with the TT genotype of the CD14-C159T polymorphism in Crimean adult late-onset asthma patients.

653
The changing curve of serum inhibitory activity for facilitated allergen-IgE binding during house dust mite immunotherapy

Mulin, F; Zhaowei, Y; Jing, L
The First Affiliated Hospital, Guangzhou Medical University, Guangzhou, China

Background: Specific allergen immunotherapy (SIT) is an effective treatment for IgE-mediated allergic disease and involve with specific IgG4 (sIgG4) level increase. Elevation of sIgG4 is accompanied by increase in IgG-dependent serum inhibitory activity for IgE-facilitated allergen binding (FAB) assay. As this 'functional' assay of inhibitory antibodies may be more predictive of clinical efficacy, we investigated the changing curve of serum inhibitory activity for FAB during different period of subcutaneous Der p immunotherapy in rhinitis and/or asthma patient.

Method: This study involved 20 patients with allergic rhinitis and/or asthma receiving a 156-week course of subcutaneous Der p immunotherapy, and 20 patients with allergic rhinitis and/or asthma receiving drug therapy only as control. Symptom and medication scores, forced expiratory volume in 1 s (FEV1), Der p-sIgG4 levels and the serum inhibitory activity at weeks 0, 4, 12, 16, 52, 104 and 156 were analyzed.

Results: Rhinitis and/or asthma symptom and medication scores, as well as FEV1% predicted showed improvement at week 52, 104 and 156 than 0 week with significant difference in SIT patients ($P < 0.05$). Levels of Der p-sIgG4 showed a significant increase after 16 weeks of subcutaneous SIT ($P < 0.01$) and continued to increase during the 156-week SIT period. Serum obtained during SIT significantly inhibited Der p-IgE binding to B-cells (Der p-FAB) after 16 weeks of SIT (% relative Der p-IgE complex binding to B cells: 0 week = 104 ± 24%; 4 weeks = 104 ± 20%, 12 weeks = 105 ± 17%, 16 weeks = 53 ± 22%; 52 weeks = 35 ± 22%; 104 weeks = 29 ± 18%; 156 weeks = 27 ± 15%; $P < 0.001$) when compared with serum obtained from controls. However, Der p-FAB, but not Der p-sIgG4, is positively associated with clinical improvements.

Conclusion: Serum sIgG4 levels and inhibitory activity for FAB increased significantly during SIT. Inhibitory activity for FAB may be more relevant for clinical efficacy of subcutaneous Der p immunotherapy.

654
Aspirin desensitization in aspirin-exacerbated respiratory disease: some new insights into the molecular mechanisms

Bobolea, ID[1]; del Pozo, V[2]; Sanz, V[2]; Cabañas, R[3]; Fiandor, A[3]; Quirce, S[3]
[1]Allergy, Hospital 12 de Octubre, Madrid, Spain; [2]Fundacion Jimenez Diaz, Madrid, Spain; [3]Hospital Universitario La Paz, Madrid, Spain

Background: Numerous trials have demonstrated the beneficial clinical effects of aspirin desensitization (AD) in patients with aspirin-exacerbated respiratory disease (AERD), but the mechanisms of this phenomenon remain unknown. Thymic stromal lymphopoietin (TSLP), a cytokine produced by epithelial cells, is a potent inducer of Th2-type responses and seems to play a critical role in the pathogenesis of allergic asthma. Clinical trials with TSLP downstream blockade are currently underway. The aim of this study was to characterize the airway inflammatory response to AD followed by treatment with high-dose aspirin for 6 months. TSPL dynamics were analysed in order to assess its role in the pathogenesis of AD.

Method: Adult patients with aspirin challenged-confirmed AERD who had AD indications and no contraindications, underwent an oral AD over a 3-day period, followed by daily ingestion of aspirin starting with 650 mg bid during 1 month, then tapering the dose in each case. Nasal symptoms, Asthma control (ACQ7, ACT), quality of life (RSDI, AQLQ), FeNO, spirometric parameters, controller asthma treatment, were assessed before and on a monthly basis after AD. Induced sputum differential cell count and levels of sputum supernatant leukotriene C_4, prostaglandin (PG) D y E_2, and TSLP were measured by ELISA, before AD and after 1 and 6 months of treatment.

Results: About 13/14 patients underwent successful AD, but for different reasons only 10 of them [6 females, 4 males, mean age 44 (21–65) years] reached 6 months of aspirin treatment. All clinical outcomes significantly improved at 1 and 6 months. The spirometry slightly improved and FeNO levels and sputum eosinophilia decreased in spite of a marked reduction of inhaled corticosteroids. Sputum PGD2 decreased compared to baseline and PGE2 increased 1 and 6 months ($P < 0.05$) after AD, but LTC4 also increased slightly (ns), maintaining a constant LTC4/PGE2 before and after AD. Unexpectedly, TSLP level increased 1 and 6 months after AD in all patients (mean baseline 0.1 ± 0.05; 1 month 3.68 ± 7; 6 months 212.3 ± 47 pg/ml, $P < 0.01$ compared to baseline and to 1 month).

Conclusion: In our study, TSLP paradoxically increased 6 months after AD, in spite of the clinical and lung function improvement. Although our findings need further validation in larger studies, we hypothesize that TSLP can act not only as an effector molecule but also as regulator, confirming thus the pleiotopric effects of TSLP in different asthma endotypes.

655
Th2-induced transcriptome is antagonized by IFN-gamma in airway epithelial cells

Zissler, U[1]; Guerth, F[1]; Ulrich, M[1,2]; Suttner, K[1]; Chaker, A[1,2]; Schmidt-Weber, C[1]
[1]Center for Allergy and Environment (ZAUM) – Member of DZL, Technical University Munich and Helmholtz Center Munich, Munich, Germany; [2]Klinikum rechts der Isar, ENT-Department, Technical University Munich, Munich, Germany

Background: Allergic inflammation is dominated by allergen-specific IgE and IL-4 producing lymphocytes, which is commonly defined as Th2 immunity. In contrast, anti-viral or anti-microbial immunity is dependent on Th1 lymphocytes produc-

© 2015 The Authors

ing interferons. Th1 and Th2 immunity is competing and antagonizing on the level of lymphocytes regulation. However, this balance and its impact on function and pathology of airway epithelial cells has not been systematically investigated.

Objective: To identify Th2-related gene expression changes in primary human bronchial epithelial cells and secreted proteins.

Methods: The responsiveness of primary human epithelial cells to IL-4 was investigated. NHBEs and DHBEs were exposed to IL-4 for 5, 20, 60 min and 6 h and an immunocytochemical staining for IL-4Ra, STAT-6, T-bet and GATA-3 was performed. For NHBEs stimulated with IL-4 or IFN-g and the combination of both for 6 h, a transcriptome analysis was performed using RNA-arrays. Significantly regulated genes were verified by qRT-PCR *in vitro* and *in vivo* using nasal scrapings and nasal secretions on protein levels.

Results: IL-4Ra, STAT-6, T-bet and GATA-3 are expressed on/in NHBEs and DHBEs. IL-4 is a strong regulator of epithelial gene expression and induces similar numbers of entities as IFN-g. Furthermore, IL-4 and IFN-g orchestrate gene expression in an antagonistic manner. Additionally, IL-4 induces a distinct transcription factor profile that is antagonistically regulated by IFN-g, both *in vitro* and *in vivo*. Additionally, some novel potential Th2 biomarkers were identified and validated on protein level *in vivo*.

Conclusion: This study demonstrates for the first time that epithelial immune competence is subject to the Th1 – Th2 balance. IL-4Ra is expressed on the surface of airway epithelial cells, signaling is transduced via STAT-6. IL-4-induced genes, e.g. CCL-26, ANO-1, RUNX-2, which are IFN-g-antagonized contribute to allergic disease in airway epithelial cells. This result not only allows to track Th2 immunity in airway epithelial cells, but also offers novel potential intervention strategies.

© 2015 The Authors