Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI

Poster Session 28

Innate and adaptive immune responses

872
Impact of neonatal mono-colonization of mice with *Lactobacillus casei* on oral sensitisation to cow's milk proteins

Morin, S[1]; Przybylski-Nicaise, L[1]; Bernard, H[1]; Rabot, S[2]; Wal, J-M[1]; Hazebrouck, S[1]
[1]INRA, UR496, Unité d'Immuno-Allergie Alimentaire, Gif-sur-Yvette, France; [2]INRA, UMR 1319, MICALIS, Jouy-en-Josas, France

Background: Colonization of the gut by commensal bacteria is critical for the proper maturation of the host immune system. Recent studies suggest that early establishment of lactobacilli in the gut could prevent the development of allergic disorders. Here, we investigated the effect of neonatal mono-colonization of BALB/c mice by *Lactobacillus casei* BL23 on the development of an allergic sensitisation toward cow's milk proteins.
Method: Mono-associated (MX) mice were obtained by inoculating *L. casei* to germ-free (GF) mothers. Nine week-old mice were then orally sensitised to cow's milk in the presence of cholera toxin. Antibody responses specific of whey proteins, i.e. β-lactoglobulin (BLG) and α-lactalbumin (αLac) and of whole casein (CAS) were measured in sera and in fecal samples. Cytokine productions were assayed in splenocytes and mesenteric lymph nodes (MLN) cell cultures after *in vitro* reactivation by BLG or CAS.
Results: Compared to GF mice, MX mice developed higher CAS-specific IgG1, IgG2a and IgA responses, particularly against αS1-casein. In contrast, no significant difference between GF and MX mice was observed for the production of serum antibodies against BLG or αLac and for the presence of specific IgA antibodies in feces. After reactivation by BLG and CAS, secretions of IL-17 by spleen and MLN cells were higher in MX mice than in GF mice.
Conclusion: The increase of CAS-specific antibody responses in sensitised MX mice could be induced by the bacterial proteolytic degradation of CAS into more immunogenic peptides. Furthermore, enhanced IL-17 secretion by reactivated spleen and MLN cells from MX mice supports the concept that early gut colonization by lactobacilli could significantly modulate the host immune response toward food antigens.

874
Manumycin A inhibition of IL-1 beta and IL-6 release from human monocytes and macrophages

Striz, I[1]; Brabcova, E[1]; Petrickova, K[2]; Kolesar, L[1]; Sekerkova, A[1]; Jaresova, M[1]; Petricek, M[2]
[1]Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Background: Polyketide antibiotics including macrolides are known to affect signalling pathways of transcription factors and regulate a number of genes involved in their potential antiinflammatory effects. Manumycin A is a streptomycete derived farnesyltransferase inhibitor with a capacity to inhibit Ras and down-regulate the expression of adhesion molecules required for cell-cell interactions of immune cells. The aim of our study was to assess the effect of manumycin A on a release and gene expression of proinflammatory cytokines from human peripheral blood monocytes and THP-1 monocytes/macrophages.
Method: Separated human peripheral blood monocytes and THP-1 monocyte/macrophage line were cultured in RPMI1640 with 5% fetal calf serum and then stimulated with TNF alpha (20 ng/ml) under serum free conditions in the presence or absence of different concentrations of manumycin (0.25; 0.50; 1.0; 2.0; 5.0 μM). The concentrations of IL-1 beta, IL-6, and IL-18 in culture supernatants were measured by ELISA or Luminex. The total mRNA was extracted with RNAeasy Plus Mini Kit (Qiagen) and quantitative RT-PCR (SABiosciences) was used for the evaluation of 84 different gene expressions in TNF alpha and manumycin stimulated cultures.
Results: Manumycin A significantly inhibited IL-1 beta and IL-6 secretion from human peripheral blood monocytes in a dose dependent manner without affecting cell viability. After a 24 culture of cells with TNF alpha and manumycin A, the concentrations of IL-1 beta and IL-6 dropt below the values detected in unstimulated cells. In THP-1 cells, the same level of inhibition was observed with additional inhibitory effect on the release of IL-18 which was not detectable in reasonable concentrations in cultures of monocytes. When looking at the transcription level, downregulation of mRNA expression of IL-1 beta and IL-6 genes by manumycin A was accompanied by inhibition of additional pro-inflammatory genes including IL-8/CXCL8, ICAM-1 (CD54) and TLR8. Among the genes upregulated in response to manumycin, HMOX1, gene for heme oxigenase 1, showed the highest mRNA induction.
Conclusion: We assume from our study that manumycin A exerts *in vitro* potent inhibition of pro-inflammatory cytokines release from human monocytes and macrophages and might be a perspective substance for potential antiinflammatory effects. Some of the proinflammatory genes are downregulated by manumycin A on the level of transcription.

Supported by IGA MZCR grant NT/13012-4.

875
Indices of innate immunity in children with recurrent wheezing

Gromadina, O[1]; Lokshina, E[1]; Zaytseva, O[1]; Dmitrieva, E[2]; Malinovskaya, V[2]; Guseva, T[2]; Parshina, O[2]; Shklyaeva, I[1]
[1]Department of Pediatrics, Moscow State University of Medicine and Dentistry named after A.I. Evdokimov, Moscow, Russia; [2]Gamaleya Research Institute of Epidemiology and Microbiology, Moscow, Russia

Background: Wheezing is a very common symptom in young children with acute respiratory infections (AIR). But a lot of questions of immune response are unclear for subsequent prediction in recurrently wheezy children.

The aim was to study clinical importance of indicators of innate immunity in children with recurrent wheezing and the effectiveness of recombinant interferon alpha 2b in complex treatment of these children.
Method: One hundred and eighty-seven children with recurrent wheezing and 16 healthy children aged from 1 to 10 years were included in this study. We diagnosed bronchial asthma at 31 kids (16.6%). Examination included detecting of eosinophilia, total IgE, level of subpopulations of blood lymphocytes (CD3+, CD3+ CD4+, CD3+ CD8+, CD3− CD19+, CD3− CD16+56+, CD3− CD8+) and proinflammatory monocytes (CD14+ CD16+),

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

immunoregulation index, the expression level of TLR-2 and TLR-4, and also we evaluated the effectiveness of complex therapy with using recombinant interferon alpha 2b.

Results: Eosinofilia was identified by 18.7% ($n = 35$), and the level of total IgE exceeded the age norm in 6.70 ± 1.1 times at 30% ($n = 56$) of the children. We estimated immunological status at 37 children with recurrent wheezing and 16 healthy kids. We revealed increase of natural killers, proinflammatory monocytes, eosinophils, with increased expression of TLR-2, which is in inverse proportion to the frequency of the ARI in children with recurrent wheezing. The expression level of TLR-2 was higher in wheezy children – 8.28 ± 0.44 ($P = 0.028$) compared with 6.59 ± 0.98 in the group of healthy kids. In the group of children with asthma has been registered decrease in the level of TLR-4 ($P < 0.05$), which correlated with the level of IgE ($r = -0.81$, $P < 0.05$). One year after the treatment with use of recombinant interferon alpha 2b we found reduction ARIs, decrease of episodes and duration of wheezing in children.

Conclusion: Revealed changes of innate immunity and the reduction of activity some indicators of adaptive management showed signs of deficiency of the immune system in the group of children with recurrent wheezing. Use of interferon alpha-2b interferon in complex therapy of children with recurrent wheezing has a positive therapeutic and protective effect, leads to reduction ARIs and frequency of wheezing.

876
Vitamin D3 treatment decreases frequencies of CD14++CD16+ and CD14+CD16++ monocytes

Moniuszko, M[1]; Grubczak, K[2]; Lipinska, D[3]; Jablonska, E[2]; Bodzenta-Lukaszyk, A[1]
[1]Departments of Allergology, Internal Medicine, Regenerative Medicine and Immune Regulation, Medical University of Bialystok, Bialystok, Poland; [2]Department of Immunology, Medical University of Bialystok, Bialystok, Poland; [3]Department of Endocrionology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland

Background: Monocyte subsets expressing CD16 exhibit potent pro-inflammatory properties and are highly enriched in numerous inflammatory disorders including asthma. Previously we demonstrated that *in vivo* glucocorticoid (GC) treatment of asthmatic patients resuIted in significant decrease in CD16-positive monocyte numbers. Here we tested hypothesis whether vitamin D3 (vit.D3), known for its immunomodulatory potential, could affect GC-mediated actions exerted on monocyte subsets.

Methods: We recruited 17 healthy volunteers in order to isolate peripheral blood mononuclear cells (PBMCs) that were cultured for next 24 h in the presence or absence of methylprednisolone (MP, 10^{-6} M) and vit. D3 (100 nM). Monocyte subsets were evaluated by flow cytometry based on forward and sideward light scatter properties and varying levels of expression of CD14 and CD16. Accordingly, CD16-positive monocytes were divided into CD14++CD16+ (named intermediate) and CD14+CD16++ (non-classical) monocytes.

Results: Twenty-four-hour stimulation with methylprednisolone resulted in a significant decrease of intermediate CD14++16+ monocyte subset ($P = 0.006$). Similarly, culture with vit. D3 led to significant decrease of CD14++16+ positive monocytes ($P = 0.004$). However, co-culture with MP and vit. D3 resulted in robust decrease of CD14++CD16+ monocytes ($P < 0.0001$). Next we analyzed detailed actions of MP and vit. D3 on non-classical CD14+CD16++ monocytes. Twenty-four-hour culture with MP alone tended to decrease CD14+CD16++ monocyte frequencies. In contrast, stimulation with vit. D3 led to significant decrease of same subset. Consequently, co-culture with both MP and vit. D3 most signifcantly decreased CD14+CD16++ monocytes ($P < 0.0001$).

Conclusions: In our study we demonstrated for the first time that some of immunomodulatory actions of vit. D3 could be attributed to its depleting effects on pro-inflammatory monocyte subsets. Moreover, we showed that vit. D3 can strongly enhance GC-induced decrease of CD16-positive monocytes. Based on these findings, we could hypothesise that addition of vit. D3 to GC treatment could potentially become an attractive tool to reduce GC dose.

877
Predictive clinical significance of tumor infiltrating neutrophils in patients with colorectal cancer

Galdiero, MR[1]; Grizzi, F[2]; Di Caro, G[2]; Laghi, L[2]; Garlanda, C[1]; Mantovani, A[1,3]; Jaillon, S[1]
[1]Department of Inflammation and Immunology, Humanitas Clinical and Research Center, Rozzano, Italy; [2]Laboratory of Molecular Gastroenterology, Humanitas Clinical and Research Center, Rozzano, Italy; [3]Department of Medical Biotechnology and Translational Medicine, University of Milan, Rozzano, Italy

Background: Myeloid cells, mainly macrophages, infiltrating the stroma have been involved in tumor onset and progression. Neutrophils have emerged as candidate immune cells that may modulate the tumorigenic process as well as the antitumor immunity. Epidemiological studies and animal models suggest that tumor-infiltrating neutrophils may be associated with poor clinical outcome. The present study was aimed at investigating the clinical significance of tumor-infiltrating neutrophils in 77 characterised patients with primary stage II-III colorectal cancer (CRC).

Method: Preliminary data showed that CD66b is a more specific neutrophil marker compared to MPO, which is also expressed in monocytes/macrophages. CRC histological sections were immunohistochemically treated with monoclonal antibody raised against CD66b. For each section, intratumoral and peritumoral neutrophils densities were assessed and expressed as immunoreactive area ($CD66b^+$ IRA) in three randomly selected and non-contiguous fields using a computer-aided image analysis system.

Results: We did not find any correlation between intratumoral $CD66b^+$ IRA and clinico-pathological features, including age, sex, microsatellite status, anatomical site, histological grade, tumor cell type, vascular invasion. In contrast, $CD66b^+$ IRA at the tumor invasive front was positively correlated with clinical stage (nodal status) in CRC ($P = 0.03$). Assessment of survival demonstrated that high percentage of $CD66b^+$ IRA (based on the median value), assessed both at the tumor invasive front as well as in the intratumoral compartment, was associated with better Disease Specific Survival (DSS) in CRC patients ($P = 0.02$ and $P = 0.07$, respectively). Moreover, multivariate analysis demonstrated that the increased $CD66b^+$ IRA at the invasive tumor front is an independent risk factor for better patient DSS (HR 0.22; 95% CI: 0.07–0.69; $P = 0.009$). Among the other variables, nodal status was found to be an independent prognostic predictor for DSS, although in the opposite direction (HR = 2.73; 95% CI 1.52–4.89; $P < 0.001$).

Conclusions: Collectively, these findings suggest that neutrophils located at the tumor invasive front are associated with a positive clinical outcome in stages II-III CRC patients and might be considered as a novel independent prognostic factor in stage II and III CRC patients.

880
Epidemiological association and some immuunological peculiarities of atopic disesases and TB infection in view of allergy hygiene hypothesis

Machavariani, K[1]; Mitskevich, N[2]; Omiadze, K[3]; Telia, A[1]; Khechinashvili, G[3]
[1]Allergology and Clinical Immunology, Tbilisi State Medical University, Tbilisi, Georgia; [2]Immunology and Microbiology, Iv. Javakhishvili Tbilisi State University, Tbilisi, Georgia; [3]Georgian Foundation of Tuberculoses and Lung Diseases, Tbilisi, Georgia

Background: The epidemiological association between allergy and TB has become especially topical over the last 10 years, since the time when the new wave of TB epidemic arose worldwide. The goal of the research is to define the epidemiological association of mycobacterial infection with bronchial asthma and allergies under high TB prevalence condition and, also, to determine some immunological peculiarities of this relation.
Method: The study was conducted in three key directions, determining the specificity of the study material and methodology: I. Establishing association of BCG immunisation and TB infection with symptoms of bronchial asthma and allergies in internally dislocated children. II. Determining atopy frequency rate at a tender age following TB latent infection emerged through confirmed close contacts. III. Evaluating peculiarities of IgE regulation and delayed hypersensitivity to PPD in cases of pulmonary TB, bronchial asthma and other clinical forms of atopy.
Results: The epidemiological association of BCG immunisation with the symptoms of bronchial asthma and allergies in 7–10 year-old target population has not been statistically confirmed, which leaves doubtful feasible application of TB vaccination in allergy hygiene hypothesis to a certain extent. Bronchial asthma and allergies symptom rate in 7–10 year-old TB infected target population has been proved to be twice lower, compared with the non-infected group. Prospective study of tender age has demonstrated statistically valid epidemiological inverse association between latent TB, developed through close family contacts and later emerged atopic diseases. Herewith, atopic diseases in TB infected children population is by far later manifested in comparison with non-infected group.
Conclusion: It is suggested, that epidemiological and immunological evaluation of pro and con arguments of allergy hygiene hypotheses should be extended in Georgia, not only in view of TB and atopy association study, but atopy and other infectious diseases. TB infection and allergy epidemiological association should be investigated considering regional peculiarities of the country. Herewith, extended allergological examination and the study of Th1/Th2 immunological links should be conducted after determination of bronchial asthma and symptoms of other allergies in prospective and retrospective research.

881
Role of IL-10 in the pathogenesis of dengue infections

Malavige, GN[1,2]; Jeewandara, C[1]; Alles, L[1]; Gomes, L[1]; Salimi, M[2]; Jayaratne, SD[1]; Ogg, GS[2]
[1]Centre for Dengue Research, University of Sri Jayawardenapura, Nugegoda, Sri Lanka; [2]Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom

Background: IL-10 has been shown to be associated with severe dengue infection. We proceeded to investigate the role of IL-10 in the pathogenesis of acute dengue infection.
Materials and methods: *Ex vivo* IFNγ ELISpot assays for dengue virus (DV) NS3 protein and non dengue viral proteins were carried out in 32 patients with acute dengue infection (12 with DHF) and 12 healthy dengue seropositive individuals. Serum IL-10 and IL-21 levels were determined by using quantitative ELISAs. IL-10 levels were also determined in ELISPot culture supernatants. The effect of IL-10 blockage on antiviral responses was determined using anti IL-10 and IL-10 receptor blocking antibodies.
Results: DV-NS3 specific *ex vivo* IFNγ ELISpot responses were significantly higher ($P = 0.04$) in patients with dengue fever (DF) (mean 693.3, SD ± 1109 spot forming units/1 million PBMCs) when compared to those with DHF (mean 45.45, SD ± 34.09 SFU/1 million PBMCs). Serum IL-10 levels correlated significantly ($P = 0.03$) and inversely (Spearmans $R = -0.45$) with *ex vivo* DV-NS3 specific responses but not with non DV specific responses (Spearmans $R = -014$, $P = 0.5$). The IL-10 levels in the ELISpot supernatants were higher in the unstimulated wells when compared to wells with PBMCs stimulated with NS3. The difference in the IL-10 levels in the NS3 stimulated and unstimulated wells positively (Spearmans $R = 0.81$) and significantly ($P < 0.0001$) correlated with *ex vivo* IFNγ DV-NS3 specific ELISpot responses. However, the difference in IL-10 levels in stimulated and unstimulated ELISpot supernatants inversely (Spearmans $R = -0.51$) and significantly ($P = 0.01$) correlated with serum IL-10 levels. Blockage of IL-10 significantly increased the *ex vivo* IFNγ ELISpot DV-NS3 specific responses but had no effect on responses to non DV proteins. IL-10 blockage also increased other antiviral responses such as CD107a expression by DV-NS3 specific CD8+ T cells.
Conclusion: IL-10 appears to contribute to the pathogenesis of acute dengue infections by inhibiting DV-specific T cell responses, which can be restored by blocking IL-10.

882
Role of hypothalamic proline rich polypeptide galarmin in regulation of blood cellular counts following methicillin-resistant *Staphylococcus aureus* (MRSA) infection

Durgaryan, A; Matevosyan, M; Galoyan, A
H. Buniatian Institut of Biochemistry, Neurohormones Biochemistry, Yerevan, Armenia

Background: Since few years the importance of interactions between the central nervous system (CNS) and the immune system are being recognised and the question arose whether the CNS can positively influence the immune reaction during severe infection. Data received by A. Galoyan that the neurosecretory cells of the hypothalamus produce cytokines and immunomodulators of theoretical and clinical significance and namely proline-rich polypeptides PRP-1 (galarmin) and its analogues being shown efficient for the treatment of a number of infectious, immune, and neurodegenerative diseases supports the theory of neuroendocrine immune system of brain. The impact of galarmin on complete blood count (CBC) in mice with significant inflammatory background with methicillin-resistant Staphylococcus aureus (MRSA) infection was investigated.
Method: To address immuno-stimulatory effect of galarmin and especially its influence on blood parameters and cellular count of MRSA infected mice, the putative analysis of white blood cells (WBC), red blood cells (RBC) and platelets with their indices were performed at 96 h post-infection (p.i.) period. CBC was performed with automatic hematology analyzer Horiba ABX Micros 60 (*HORIBA* ABX, France) designed for veterinary use.
Results: The administration of galarmin had a pronounced protective effect during the period of development of the infection and increased significantly the overall WBC and especially platelets count (PC) that were consistently higher than those in controls.

For the given absolute number of WBC, the highest rate was observed at the concentrations of galarmin 1 μg per mice ($10.4 \times 103/mm^3$), with an increase of more than 80% ($P = 0.0064$) as compared with untreated group ($5.6 \times 103/mm^3$). We

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

observed a significant increase in the number of PC under the effect of galarmin, most of which was observed at concentrations of 1 μg ($888 \times 10^3/mm^3$), more than two-fold increase ($P = 0.0056$) compared with MRSA infection without treatment. These results suggest a previously undescribed role of galarmin to stimulate recovery of monocytopenia and thrombocytopenia following MRSA infection and the importance of interactions between the CNS and the immune system.

Conclusion: Received results support our previous data that via signal molecules the brain controls the immune system that includes the control of synthesis, development and function of WBC and other key cells made by the immune system.

884
Expression of the genes encoding the transcription factors controlling differentiation of adaptive CD4+-T-lymphocyte subpopulations in allergic patients

Sidorovich, O; Nikonova, M; Donetskova, A; Luss, L; Yarilin, A
RNC Institute of Immunology, Moscow, Russia

Background: In this work we studied the expression of the genes of transcription factors controlling differentiation of adaptive CD4+-T-lymphocyte subpopulations in patients with allergic rhinoconjunctivitis (ARC).

Method: Peripheral blood mononuclear cells of 20 healthy persons and patients with ARC in acute process (five patients) and during remission (17 patients) were isolated by A. Boyum method. The expression of GATA-3, T-bet, RORc и FOXP3 was measured by real-time PCR-method.

Results: The exacerbations of the allergic disease enhanced the expression of the genes encoding transcription factors (GATA-3, T-bet and FOXP3) responsible for the differentiation of CD4+-T-cells (Th2, Th1 and Treg respectively) in blood limphocytes. The expression of the RORc gene encoding Th17 remained unaltered. The genes expression decreased during remission of ARC, with the exception of the T-bet gene which expression was abnormally high. The expression decrease of the 'proallergic' GATA-3 gene during remission was pronounced stronger than that of the other genes, which is shown through the values of the corresponding indexes.

Conclusion: These findings indicate that the expression of the genes of transcription factors controlling differentiation of adaptive CD4+-T-lymphocyte subpopulations changes during the allergic process. It is also shown that the deviation of the degree of expression from the respective normal values reflects the acuteness of the allergic disease.

885
Inhibiting the binding of allergens having glycosylated structures by dextrans as a potential immunomodulatory strategy

Pustylnikov, S[1]; DuBuske, LM[2]
[1]Novosibirsk TB Research Institute, Novosibirsk, Russia; [2]Immunology Research Institute of New England, Gardner, United States

Background: Immune responses to pathogens and allergens are similar for Th2-activating allergens and Th2-activating intracellular pathogens including bacteria, fungi, viruses or protozoa. Mannose receptor (MR) and DC-SIGN receptor of dendritic cells have important roles in the uptake and formation of immune responses to diverse antigens and impact Th polarisation. Mite, dog, cockroach and peanut allergens are recognised by mannose receptors due to glycosylation. Silencing of mannose receptors on dendritic cells reverses Th2 response to such allergens. Pollen and mite allergens are recognised by DC-SIGN which influences immune response of dendritic cells.

Method: The data on MR and DC-SIGN family receptor (DFRs) properties were reviewed in infectious diseases. It was noted that nteractions of these receptors with pathogens can drive T cell response towards Th2. Two main types of cellular reaction to MR/DRFs ligands were noted to occur: Th1 response limiting (some structures of pathogens, allergens such as Der p1) or seemingly neutral reactions (dextrans). The review of the influences of dextrans derivatives (MR/DFRs ligands) in treatment of infectious disease showed a gap in knowledge regarding their precise mechanisms of action. Oxidized dextrans reduced the size and quantity of granulomas in BCG granulomatosis in mice and significantly decreased death as a preventive therapy against influenza H5N1.

Results: We hypothesise that dextrans acted as an immuno-modulatory molecule reducing suppression of Th1 response induced by pathogens. If we assume that complex allergens (as many pathogens) activate both antagonistic Th1 and Th2 responses, and interaction with immune cells leads preferably to Th2 over-reaction, the unbalanced response may be caused by limitation of Th1 via MR/DFRs. Ligands like dextrans that induce neutral responses may block MR/DFRs, reversing the limitation of Th1 responses.

Conclusion: Dextrans, as ligands of MR/DFRs that have shown to be of therapeutic and prophylactic use in infectious disease models, might also be of use as an immunomodulator in allergic disorders.

886
Types of NK cells responses to interferon-alpha in patient with recurrent herpes simplex

Karsonova, A[1,2]; Shulzhenko, A[3]; Karaulov, A[1]
[1]Department of Clinical Immunology and Allergology, I.M. Sechenov First Moscow State Medical University, Moscow, Russia; [2]Laboratory of Clinical Immunology, National Research Center 'Institute of Immunology of the Federal Medical-Biological Agency', Moscow, Russia; [3]Department of Allergy and Immunotherapy, National Research Center 'Institute of Immunology of the Federal Medical-Biological Agency', Moscow, Russia

Background: NK cells, which are principal antiviral effector cells, accomplish their effector function by degranulation, i.e. by releasing cytotoxic proteins from lytic granules onto the surface of target cells. This study aimed to investigate k562-induced NK cells degranulation and cytotoxicity under the IFN-α influence in patient with recurrent herpes simplex (RHS).

Method: MNCs were obtained from 48 patients with RHS and from 31 age-matched healthy donors. MNCs were incubated in presence of IFN-α2b (10, 100 and 1000 IU/ml) for 24 h. Control aliquot was incubated without IFN-α2b. After 24 h NK cells response to IFN-a was assessed by NK cell degranulation and NK cytotoxicity assay. Degranulation was induced by adding K562 target cells and assessed by flow cytometry with monoclonal antibodies against CD107. In order to assess NK cytotoxicity effector MNCs were incubated 4 h with k562, labeled with carboxyfluorescein diacetate succinimidyl ester. The dead K-562 cells were stained with propidium iodide and analysed by flow cytometry.

Results: We identified a group of patients who had no significant degranulation increase in response to increasing concentration of IFN-a from 10 to 1000 IU/ml (marked as type B). Rest of patients had degranulation increase like donors (marked as type A). Identified groups (A and B) had principal differences in NK-cytotoxicity. During remission group A was characterised by normal non-stimulated NK-cytotoxicity and low IFN-stimulated NK-cytotoxicity. During recurrence significant difference between group A and healthy donors wasn't observed. On the contrary, during remission group B had normal non-stimulated and normal IFN-stimulated NK-cytotoxicity. During recurrence in group B non-stimulated and IFN-stimulated

NK-cytotoxicity was in three times lower than in healthy donors.
Conclusion: Thus, patients with RHS are heterogeneous group and have different defects in system 'IFN type I/NK cell cytotoxicity'. We identified two types of NK cells responses to IFN-a. In group A NK cells had low 'susceptibility' to IFN-a during remission and didn't have any defects during exacerbation, whereas group B had normal 'susceptibility' to IFN-a during remission and strong immunodeficiency during exacerbation. Immunotherapeutic approaches should be based on the type of response.

887

Characterisation of equine macrophage-derived chemokine and its expression in the skin of horses with recurrent urticaria

Janda, J[1,2]; Roosje, P[3]; Marti, E[1]
[1]Department of Clinical Research-VPH, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [2]The Academy of Sciences of the Czech Republic, Institute of Animal Physiology and Genetics, Libechov, Czech Republic; [3]Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Background: Macrophage-derived chemokine (MDC/CCL22) plays an important role in the pathogenesis of allergic diseases, since it attracts T helper 2 cells bearing the corresponding receptor CCR4 to the site of allergic inflammation. However, the role of MDC in equine allergic diseases is not known. Therefore, our aim was to characterise equine *MDC* and to assess the expression of TSLP in the skin of horses with recurrent urticaria and in stimulated blood leukocytes under various conditions.
Method: Basic bioinformatics was used to characterise equine *MDC* gene and real-time reverse-transcription PCR was used to assess the expression of equine MDC.
Results: Equine *MDC* gene is localised on chromosome 3 and is organised in three exons. It has a high degree of homology with other species (86%, 83%, 80%, 73% nucleotide identity with bovine, canine, human, and murine *MDC*, respectively). The expression of MDC was higher in skin lesions of horses with recurrent urticaria ($n = 8$) than in non lesional samples ($P \leq 0.05$) or samples from healthy horses ($n = 8$, $P \leq 0.05$). Stimulation of horse leukocytes with concanavalin A resulted in an increased expression of MDC compared to incubation with medium alone. MDC expression could furthermore be enhanced by the addition of IL-4 and inhibited using IL-4- or TSLP-specific neutralising antibodies, or by the addition of regulatory cytokines IL-10 and TGFbeta1.
Conclusion: These results complement our previous finding of high expression of IL-4 and TSLP in lesional skin of horses with recurrent urticaria and identify MDC as an important player in the pathogenesis of equine recurrent urticaria.

Poster Session 29

Immunogenetics and immunogenomics

888
Genetic susceptibility to bronchiolitis in infants

Moore, DB[1]; Boechat, JL[1]; Pestana, S[2]; Azevedo, ZM[3]
[1]Medicina Clínica, Universidade Federal Fluminense, Niterói, Brazil; [2]Pediatria, Universidade Federal Fluminense, Niterói, Brazil; [3]Pediatria, Instituto Fernandes Figueira, Rio de Janeiro, Brazil

Background: The recurrent wheezing is a common problem among infants. Epidemiological studies suggest that the risk of asthma after bronchiolitis is related to the severity of the episode. It was proposed a hypothesis in which the response to viral infection is given by the conjunction of several factors: the genetic makeup of the child, concomitant exposure to environmental antigens and the degree of maturity of the infant immune system and airway at the time of infection. Therefore, knowing polymorphisms of genes related to inflammation may help to better understand the genetic makeup of these infants and thereby assist in identifying phenotypes of allergic and respiratory symptoms that can be used to assess the heterogeneity of the disease in children and improve understanding of disease progression and management.
Method: We carried out a comparison of the genotypic frequency of SNP LTA+252, TNF-863, IL6-174 and MIF-173 between a group of 85 infants with bronchiolitis that was admitted to an intensive care unit and a group of 143 healthy children using retrospective data from a study that intend to identify biomarkers of severity in the pediatric intensive care unit. Deviations from Hardy-Weinberg equilibrium (HWE) predictions were assessed by comparing the detected genotype distribution with the expected distribution estimated on the basis of the SNP allelic frequencies, using chi-square tests. Cases and controls were compared regarding the frequencies of genotypes. Odds ratios (ORs) were obtained through unconditional logistic regression models. All analyses were performed using the R software version 2.9.1, with the package 'genetics'.
Results: No association of susceptibility to severe bronchiolitis in respect to SNP TNF-863, IL6-174 and MIF-173. However, we found a risk association (OR = 2.55; CI = 1.01–6.42; $P = 0.04$) between the SNP LTA+252 GG and the susceptibility to severe bronchiolitis.
Conclusion: A preliminary data suggest a role of the SNPLTA+252GG and susceptibility of severe forms of bronchiolitis. Further studies should address the impact of this SNP in a larger sample, stratifying the population between atopic and non atopic infants.

889
Interactions between *IL13, IL4* and *IL4R* gene polymorphisms and association with atopy in a Portuguese population

Mareco, RM[1,2]; Trindade, H[3]; Fonseca, AM[1]; Lourenço, O[1]; Taborda-Barata, L[1,4]
[1]CICS – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal; [2]University Atlântica, Oeiras, Portugal; [3]Centro de Histocompatibilidade do Sul, Lisboa, Portugal; [4]Department of Allergy & Clinical Immunology, Centro Hospitalar Cova da Beira, Covilhã, Portugal

Background: The effects of IL-4 and IL-13 on IgE production are mediated via a common receptor, IL4R. Polymorphisms in the genes for IL-4, IL-13 and IL-4R may be associated with the presence or absence of increased IgE prodution, eventually giving rise to greater or lesser susceptibility to atopy. The aim of the present study was to analyse the association between genetic factors and atopy.
Method: We studied 67 atopic (with asthma and/or rhinitis and with total serum IgE levels >250 IU/ml associated with positive Phadiatop and positive skin prick tests to aeroallergens) and 50 non atopic subjects, and used SSP-PCR to characterise the frequency of the following polymorphisms: IL 4 (pos-1098/pos-590/pos-33); IL-4Rα (cod 50, cod 478, cod 551); IL-13 (pos −1055).
Results: A significant association between the IL-4 receptor (IL-4Ra) codon 50 and the A/G genotype ($P = 0.001$), in atopic patients and an interesting association between two combined genotypes was found with A/G of the IL-4Rα cod 50 with the IL-4 TCC/TCC genotype ($P = 0.004$) and also with the IL-13 C/C polymorphism ($P = 0.033$). We also found IL-4Rα cod 50 G/G to be significantly associated with lower total serum IgE levels ($P = 0.016$).
Conclusion: These results suggest that some genotypes or the combination of them, may be associated with a higher predisposition to atopy and some others may also constitute a protection. Furthermore, a distinct role for IL4Ra is also suggested, especially in the regulation of the production of IgE.

891
Study of *IL18* and *IL21* gene polymorphisms in atopy

Mareco, RM[1,2]; Trindade, H[3]; Fonseca, AM[1]; Lourenço, O[1]; Taborda-Barata, L[1,4]
[1]CICS – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal; [2]University Atlântica, Oeiras, Portugal; [3]Centro de Histocompatibilidade do Sul, Lisboa, Portugal; [4]Department of Allergy & Clinical Immunology, Centro Hospitalar Cova da Beira, Covilhã, Portugal

Background: Cytokines such as IL-18 and IL-21 may be associated with the induction of Th1-type pro-inflammatory responses in detriment of Th2-type inflammatory allergic responses. Polymorphisms in *IL18* and *IL21* genes may underlie a genetic predisposition towards a higher or lower production of the related cytokines. Thus, the study of these polymorphisms may contribute towards a better understanding of the phenotypic diversity of atopy.
Method: We studied 67 atopic (with asthma and/or rhinitis and with total serum IgE levels >250 IU/ml associated with positive Phadiatop and positive skin prick tests to aeroallergens) and 50 non atopic subjects, and used SSP-PCR to characterise the frequency of the following polymorphisms: *IL18* (pos +127/pos −137); *IL21* (pos-22/pos+234).
Results: Our results showed that the *IL18* G/T genotype (pos +127) and the *IL18* C/G genotype (pos −137) were associated with atopy ($P = 0.015$ and $P = 0.05$, respectively), in the Portuguese population studied. These results suggest that *IL-18* may have a role in atopy. Meanwhile, none of the studied polymorphisms were predictive of total serum IgE levels.
Conclusion: Thus, further research, is needed to better clarify the role of these cytokines in atopy. Acknowledgements: Project Pest-C/SAU/U0709/2011 by the Portuguese Foundation for Science and Technology through the COMPETE program.

892
Angiotensin converting enzyme polymorphism in asthmatic patients

Castro, MMTCC[1]; Matos, A[2]; Joana, F[2]; Lopes, L[2]; Manuel, B[2]
[1]CHLN-HSM, Immunoallergy, Lisbon, Portugal; [2]Genetic Department, Lisbon Medical School, Lisbon, Portugal

Background: The aim of this study is to analyse if there is an association between angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism (287 base pairs, on chromosome 17q23, intron 16(rs4340)) with asthma severity.
Method: Asthmatics: $n = 99$; were compared with a control group of $n = 201$ healthy blood donors. The (I/D) polymorphism was determined by PCR- Polymerase chain reaction. Control of asthma assessed by validated instrument (ACQ7 and PAQLQ). Statistical analysis was performed with PASW 18, establishing a significance level of $P < 0.05$.
Results: Mean age of 99 asthmatics: 38.54 ± 17.45 years (7–74 years); 64 females and 35 males; 84 atopic and 15 non-atopic. Mean age of control-group ($n = 201$) was 41.14 ± 11.87 years (19–69 years); 69 females and 132 males. In asthmatics the frequencies of D- Allele (ACE-D) 0.621 and of I-Allele (ACE-I) 0.379; in controls: 0.669 and 0.331 respectively. There is no statistical difference between these groups ($P > 0.05$). Genotypes in the asthmatics- DD: 50.5%; ID: 23.2%; II: 26.3%; in control group- DD: 47.3%; ID: 39.3%; II: 13.4%. There is statistical difference ($P = 0.003$). When we associate ACE genotypes (II vs DD+ID; $P = 0.01$), II genotype was more frequent in the asthma-group. The risk (OR) associated is 2.295 (CI 95% [1.255–4.199], ($P = 0.01$). In asthmatics, there is no statistical differences in genotype frequencies ($P > 0.05$) between: atopics and non atopics; controlled and uncontrolled asthma; males and females; and in the different age-groups.
Conclusion: The role of ACE (I/D) polymorphism, in asthmatic patients is a controversy risk factor to the severity of asthma, but we concluded that II genotype is more prevalent in asthmatics, in this hospital – based population.

893
The role of type 1 angiotensin 2 receptor polymorphism in asthmatic patients

Cortez e Castro, MMTC[1]; Matos, A[2]; Ferreira, J[2]; Lopes, L[2]; Bicho, M[2]
[1]Immunoallergy, CHLN-HSM, Lisbon, Portugal; [2]Genetic Department, Lisbon Medical School, Lisbon, Portugal

Background: Type 1 angiotensin II (Ang II) receptor (AGTR1) could be related with the pathogenesis of bronchial asthma. The purpose of this study is to analyze the association between AGTR1 1166A/C (rs5186) gene polymorphism with asthma severity.
Method: Asthmatic patients: $n = 98$ were compared with a control group of $n = 50$ healthy blood donors. The AGTR1 1166A/C polymorphism was determined by PCR-RFLP (Polymerase chain reaction- restriction fragment length polymorphism). Control of asthma assessed by validated instrument (ACQ7 and PAQLQ). Statistical analysis was performed with PASW version 18 establishing a significance level of $P < 0.05$.
Results: Ninety-eight asthmatics, mean age 38.9 ± 17.5 (7–74 years), 64 females and 34 males; 84 atopics and 14 non-atopics; 68 with controlled and 30 with uncontrolled asthma. The control group, $n = 50$, mean age 40.9 ± 11.8 (19–69 years), 37 females and 13 males. The control group for this polymorphism is in Hardy-Weinberg equilibrium ($P > 0.05$). In asthmatics the frequencies of the allele A is 57% and the allele C is 43%; in controls: 55% and 45%, respectively. There was no statistical differences between these groups ($P > 0.05$). Genotypes in the asthmatics-AA: 28.6%; AC: 57.1%; CC: 14.3%; in control group-AA: 26%; AC: 58%; CC: 16%. There is no statistical difference between these groups ($P > 0.05$). In asthmatics, there is no statistical difference ($P > 0.05$) in genotypes: between atopics and non atopics; controlled and uncontrolled asthma; males and females and in the different age-groups.
Conclusion: In this study group there is not a significant evidence, that AGTR1 gene A1166C polymorphism could be a genetic marker for the pathophysiology of asthmatic disease.

894
TNF-alpha single nucleotide polymorphisms in atopic dermatitis

Behniafard, N[1]; Rezaei, N[2]; Gharagozlou, M[3]
[1]Allergy & Clinical Immunology, Tehran University, Tehran, Iran; [2]Research Center for Immunodeficiencies, Tehran University of Medical Sciences, Tehran, Iran; [3]Allergy and Clinical Immunology, Tehran University of Medical Sciences, Tehran, Iran

Background: Tumor necrosis factor-alpha (TNF-α) could be considered as potential biomarkers in atopic dermatitis (AD), while their levels could be influenced by cytokine single gene polymorphisms (SNP).
Method: This study was performed in 89 pediatric patients with AD and 137 controls to assess polymorphisms of TNF-α gene at positions −308 and −238, using polymerase chain reaction with sequence-specific primers method.
Results: The highest positive allelic association that makes the patients susceptible for AD was seen for TNF-α −238/G ($P < 0.001$) and TNF-α −308/G ($P = 0.003$). The GG genotypes at TNF-α −238 and TNF-α −308 were both significantly higher in the patients with AD, compared to the controls ($P < 0.01$). The GG haplotype at TNF-α (−308, −238) was seen in 92.7% of the patients, which was significantly higher than the controls ($P < 0.001$), while the haplotypic negative association for AD was seen in TNF-α (−308, −238) AG and GA ($P < 0.01$).
Conclusion: This study showed that AG genotype of TNF-α −308, associated with high production of cytokines, was significantly decreased in the patients with AD, while the low-producing GG genotype was over-expressed in the atopic patients, which could lead to low production of TNF-α in the atopic patients.

895
Design of gene delivery system based on lipopeptides

Koloskova, O[1]; Shilovskiy, I[2]; Andreev, S[2]; Khaitov, M[2]
[1]Institut of Immunology, Moscow State University of Fine Chemical Technology named after M.V. Lomonosov, Mosocw, Russia; [2]Institut of Immunology, Mosocw, Russia

Background: Currently, cationic liposomes are widely used as a non-viral gene delivery systems. Nucleic acids in this case are protected from the adverse effects of the environment. However, the relatively low efficiency and high toxicity of the commercial transfection agents stimulate an active search for new amphiphiles.
Method: Lipopeptides were synthesised by solid-phase method on rink resin. To form a cationic polar head group we used L amino acids, such as L-lysine, L-tryptophan, L-cystine. To form a hydrophobic domain we used a hydrocarbon chains with length in 16 carbon atoms. The structure and purity of all substances were confirmed by HPLC, NMR-spectroscopy and mass-spectrometry. Liposomes were prepared by the method of hydration the lipid layer. To decrease the size of the particles we used an ultrasonic treatment. To standartisation a liposomal dispersion in diametre we used extrusion. Effectiveness of gene delivery was evaluated by analyzing the degree of transfection on HEK 293 cells with plasmid of green fluorescent protein (GFP), complexed with lipopeptides. Effectiveness of targeted delivery of luciferase reporter gene (pCMV-Luc) to the various organs of laboratory animals was measured in female

mice of BALB, 6–8 weeks old, weighing 18–20. The level of expression of luciferase reporter gene in the organs of mice in 24 h after intravenous injection of plasmid/lipopeptide complexes was determined by the method of quantitative PCR analysis.

Results: We synthesised a new type of lipopeptides based on amino acids. Liposomal dispersion based on new compounds have an attractive properties. The size of the particle measured by photon-correlation spectroscopy on LSTM 13320 (Beckman Coulter) are less 100 nm. The stability over time at room temperature and transfection efficiency exceed 4 months. Transfection efficiency is comparable with 'golden standard' lipofectamine. And our delivery system is non toxic for cells and animals. There was no death among laboratory animals and significant changes in the weight were not found. Our lipopeptides facilitate gene penetration in to spleen and bone marrow in mice. And the efficiency of our gene delivery system was in two time better then in case of commercial transfection agent for animals 'TurboFect'.

Conclusion: We synthesised a non-toxic lipopeptides capable to effective gene delivery in various organs of mammals. Our liposomes promotes the penetration of the transgene mainly in to the spleen.

896
Influence of olive pollen stimuli on the gene- expression profile in allergic patients

Calzada, D[1]; Aguerri, M[1]; Baos, S[1]; Montaner, D[2]; Mata, M[3,4]; Dopazo, J[2]; Lahoz, C[1,4]; Cárdaba, B[1,4]
[1]Immunology, IIS- Fundación Jiménez Díaz, Madrid, Spain; [2]Functional Genomics Unit Bioinformatics Department, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain; [3]Genomic Department, Fundación de Investigación, Hospital General Universitario, Valencia, Spain; [4]CIBER de Enfermedades Respiratorias, Palma de Mallorca, Spain

Background: Analysis of gene-expression profiles with microarrays can be very useful to dissect specific responses and to characterise with a global view, new elements for improving the diagnosis, treatment and understanding of allergic diseases. We have used this approach for studying the olive pollen response, taking advantage our previous results of T-cell epitope mapping on Ole e 1 molecule (the major allergen from olive pollen) in order to analyze the stimuli influence on the gene-expression of olive pollen allergic patients.

Methods: Peripheral blood mononuclear cells (PBMCs) from six allergic subjects were stimulated 24–36 h with olive pollen stimuli: Ole e 1 molecule and two Ole e 1 peptides previously defined as P2 + 3 (aa10–31), mainly recognised by non-allergic subjects (possible immunoregulatory epitope) and P10 + 12 + 13 (aa90–130), immunodominant T-cell epitope.

RNA extracted from basal and stimulated PBMCs was analyzed by HuGeU133 plus 2.0 GeneChip, Affymetrix (38 500 genes). After assessment of data quality by standard quality checks and principal components analysis (PCA), differential gene-expression by experimental conditions was performed by multiple testing, using microarrays specific software. Differences in functional analysis were performed by KEGG, for pathways and Gene-Ontology for biological process. Relevance was defined by fold change (>2 or <−2) and corrected P values (<0.05). The most differential genes were reanalyzed by qRT-PCR (micro-fluidic gene cards) in an independent set of individuals.

Results: The results of gene-expression by PCA showed differential clusters that correlated with the experimental conditions. Analyses of gene-expression and functionality revealed differential genes and pathways among the four experimental conditions.

The 193 most significant genes were validated by qRT-PCR in a new cohort of 10 subjects. A set of 50 genes specifically defined the P2 + 3 responses. Some of these genes are essentials for the maintenance of the peripheral T cell tolerance and homeostasis.

Conclusions: Besides to have demonstrated that the stimuli can differentially affect the gene-expression, we have found specific genes and pathways related with Ole e 1 response. Interestingly, our data explain why Ole e 1 P2 + 3 could be implicated in the regulation of the olive pollen response. These results open new research ways for the regulation of this disease.

897
Serum 25-hydroxyvitamin D levels and atopic diseases

Ginaldi, L[1]; Ciccarelli, F[2]; Nocelli, C[1]; de Martinis, M[1]
[1]l'Aquila University, l'Aquila, Italy; [2]l'Aquila University, Porto San Giorgio, Italy

Background: Vitamin D has immunomodulatory properties, in particular by influencing T regulatory cells and the TH1/TH2 balance, modulating the development of allergies. Evidence for an association between vitamin D levels and allergic conditions is well known; however existing data are controversial. Our aim was to investigate the relationship between serum 25-hydroxyvitamin D (25(OH)D) levels and both clinical manifestations of atopic diseases and serum IgE concentrations.

Methods: 25(OH)D was measured in blood samples of 70 allergic patients and 61 non atopic healthy controls, ranging in age from 5 to 75 years. The patient population was affected by respiratory (38 patients) or cutaneous (32 patients) manifestations of atopy. Moreover, patients and controls were divided into three groups: under 20 (20 patients and 15 controls), between 20 and 50 (25 patients and 20 controls) and over 50 years of age (25 patients and 26 controls). In all of them serum IgE levels were also measured and compared with serum 25(OH)D concentrations. Mean and standard deviations of 25(OH)D and IgE serum levels in both atopic and non atopic subjects were compared by T -student test and a P value < 0.05 was assumed as significant.

Results: In atopic patients 25(OH)D concentrations were significantly lower (17.84 ± 8 ng/ml) compared to non allergic subjects (21 ± 11 ng/ml). 25(OH)D levels were not significantly different between atopic patients with respiratory symptoms and patients with cutaneous manifestations (17.54 ± 9.03 and 18.33 ± 8.65 respectively). In the first group the 25(OH)D levels were significantly lower among atopic patients compared to healthy individuals, wereas this difference was statistically less significant in older subjects. The values of IgE were higher (365 ± 68) in subjects with marked hypovitaminosis D (<10 ng/ml) compared to the other groups, respectively 147 ± 70 in subjects with levels of 25(OH)D ranging from 11 to 30 ng/ml and 163 ± 35 in subjects with higher 25(OH)D levels.

Conclusion: These preliminary data evidentiate lower 25(OH)D levels in patients with atopic diseases, associated with elevated serum IgE concentrations, suggesting that a condition of hypovitaminosis D may represent a risk factor for the development of allergies.

898
Importance of CoQ10 and omega-3 fatty acids as novel therapeutics for allergy

Ahmed, N[1]; Barrow, C[1]; Adcock, J[1]; Sinclair, A[2]; Suphioglu, C[1]
[1]School of Life and Environmental Sciences, Deakin University, Geelong, Australia; [2]School of Medicine, Deakin University, Geelong, Australia

Background: In recent times, allergy has become a financial, physical and psychological burden to the society as a whole. Allergic reactions can result in life-threatening situations causing morbidity and high economic cost. Therefore, more effective reagents are needed for allergy treatment. The role of CoQ10 and omega-3 fatty acids has gained much attention in allergic studies mainly due to their

antiinflammatory properties. Literature suggests atopic individuals suffering from allergies such as asthma may have inadequate dietary intake of CoQ10 and/or DHA. In an allergic cascade, cytokines IL-4 and IL-13 bind to IL-4 receptor (IL-4R) which activates the STAT6 phosphorylation pathway leading to gene activation of allergen-specific IgE production by B cells. Consequently, IgE production leads to clinical symptoms of allergy. Therefore, characterisation of novel allergy therapeutics such as CoQ10 and DHA, that inhibit IgE production, forms the overall aim of this study.

Method: CoQ10 and DHA were tested *in vitro* with a HEK-Blue IL-4/IL-13 reporter cell line model, transfected with a reporter gene that produces an enzyme, secreted embryonic alkaline phosphatase (SEAP). SEAP acts as a substitute to IgE when cells are stimulated with bioactive cytokines IL-4 and IL-13. QUANTI-Blue was added as a substrate that breaks down in the presence of SEAP, producing blue coloration. The blue color was used to confirm the activation of the STAT6 pathway that leads to SEAP secretion using a spectrophotometer.

Results: We have successfully used CoQ10 and DHA in our studies that demonstrated the specific inhibition of the allergic cascade leading to a decrease in SEAP production in HEK-Blue cells. A colorimetric analysis showed a >50% inhibition with CoQ10 as well as DHA, resulting in less SEAP and hence, less color production. A statistical Student's *t*-test revealed the significance of the results, confirming our initial hypothesis.

Conclusion: We have successfully identified and characterised CoQ10 and DHA as potent inhibitors of IL-4R signalling, which effectively down-regulates the STAT6 induced pathway in allergic cascades *in vitro*. Since IL-4 and IL-13 interaction with IL-4R is a common pathway for many allergies, a prophylactic treatment can be devised by inhibiting this interaction for future treatment of allergies.

901
Griscelli syndrome: case report

Castañeda Macazaga, T[1]; Rosas Herrera, B[2]; Treviño, G[3]; Rangel, M[3]
[1]Pediatrics, Hospital San Jose Tec de Monterrey, Monterrey, Mexico; [2]Hospital San Jose Tec de Monterrey, Monterrey, Mexico; [3]Hospital Regional Materno Infantil de Alta Especialidad, Monterrey, Mexico

Introduction: Griscelli syndrome, is very rare autosomal dominant disease, with <10 cases reported in the United States, and 60 cases reported worldwide. It is characterised by partial albinism and variable immunodeficiency

Case: Three years old, masculine, with familiar history of consanguinity, maternal uncles perished 6 and 7 years old, and a sister died at four because of anemia and gait disturbances.

Personal history of psychomotor regression and an episode of gait disturbance 6 months before his admisison. Three months before his admission he developed intermittent fever of 39°C and in the last 3 weeks progressive ataxia. Physical exam: hair with silvery metallic sheen, generalised lymphadenopathy, hepatosplenomegaly and ataxia. Hematic biometry with pancytopenia, blood chemistry with elevated liver enzymes and triglycerides. Magnetic resonance imaging with multiple areas of demyelination manifested as diffuse hiperintensities on T2 with cerebellar affection, as well as right cortical atrophy. Hair microscopy with mature melanosomes in the cytoplasm of melanocytes, with no pigmented keratinocytes. Positive RAB27A mutation.

Conclusion: While waiting for bone marrow transplantation (definite treatment), the patient presented intracranial bleeding, which conducted to its death.

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

Poster Session 30

Clinical immunology

902
Cytokines and micronutrient deficiencies in pregnant adolescents

Leal, JY; Romero, T; Ortega, P; Chavez, C; Escalona, C
Faculty of Medicine, Biological Research Institute, Malnutrition Research Laboratory, Zulia Unversity, Maracaibo, Venezuela

Background: A bias of T cell immunity towards type 2 (Th2) is thought to be critical for normal pregnancy. Pathological pregnancies, such as pre-eclampsia, are characterised by cell-mediated (Th1) immune dominance. The Th1/Th2 paradigm, however, is too simplistic. Normal pregnancy is associated with a systemic inflammatory response which increases throughout gestation. Studies done with human and animals have shown that iron deficiency (FeD) and vitamin A deficiency (VAD) affects the immunomodulated response mediated by cytokines. FeD and Anemia with FeD were associated with high serum levels of gamma-IFN; Also, VAD was correlated with high levels of gamma-IFN. However, these studies are controvesial and there are not studies about the complex interactions.
Method: The purpose of the present study was to analyze Interleukin-10 and gamma-Interferon serum concentrations in 154 female pregnant adolescents of a low socioeconomic condition. Anemia in pregnant adolescents was determined during the first and third trimesters when Hb < 110 g/l, second trimester Hb ≤ 105 g/l. FeD: ferritin < 15 μg/l; insufficient reserves of iron IRI = 15–20 μg/l and normal reserves of iron (RIN) > 20 μg/l, as recommended by WHO and the International Anemia Consultative Group. Serum retinol was determined by HPLC using the Bieri method. International reference standards were considered to define VAD (serum retinol < 20 μg/dl), risk of VAD (20–30 μg/dl) and vitamin A sufficiency (>30 μg/dl). Serum concentrations of Interleukine-10 (IL-10) and gamma-Interferon (gamma-IFN) were detected by an ELISA method (pg/ml). The data were analyzed using the SAS/STAT statistical program; the results were presented as mean ± Standard deviation and the differences between mean values were analyzed by the ANOVA test.
Results: The prevalence of anemia was 55.20%. FeD = 22.07%; Anemia + IRI = 37.66%, VAD = 15.58%; risk of VAD = 29.87% and Anemia + FeD + VAD = 3.89%. IL-10 and gamma-IFN serum concentration didn't show differences between the groups of pregnant adolescents with micronutrient deficiencies; however, IL-10 and gamma-IFN showed not significantly increased in pregnant adolescents with anemia + VAD.
Conclusion: Nutritional disorders may affect cytokines necessary for the optimal development of pregnancy. Strategies for recovery nutrition and education in primary health care and maternal health programs are needed to maintain immunoregulation that occurs in pregnancy.

906
Splenomegaly as a manifestation of a rare disease

Almeida, JP[1]; Neves, M[2]; Raposo, J[2]; Caiado, J[1]; Leitão, MJ[3]
[1]Imunnoallergology Department, Santa Maria Hospital, Lisbon, Portugal; [2]Department of Hematology, Santa Maria Hospital, Lisbon, Portugal; [3]Department of Medicine I, Santa Maria Hospital, Lisbon, Portugal

Case report: A 75-year-old caucasian man presented with complaints of increasingly left abdominal pain over a 12-month period accompanied by intense asthenia, anorexia and marked weight loss (12 kg). The patient had been in overall good health despite the diagnoses of mild chronic obstructive pulmonary disease, hypertension and type 2 diabetes mellitus diagnosed 15 years ago, and controlled psoriatic arthritis for the past 10 years under systemic corticosteroids (deflazacort 3 mg/day) and metrotexate.

Current pertinent findings at physical examination included cutaneous paleness, a slightly protuberant, mildly tender abdomen with a firm liver edge at approximately 3 cm below the costal margin, and a palpable massive splenomegaly reaching the iliac crest.

His complete blood count and the blood smear revealed a normochromic anemia (hemoglobin 10.2 g/dl, haematocrit 30%) with anysocytosis. Liver enzymes, kidney function and LDH were normal, but the erythrocyte sedimentation rate was approximately two times normal. Viral serology was negative for hepatitis, herpes, HIV and cytomegalovirus. Radiological evaluation revealed a normal chest x-ray, but the abdominal x-ray showed marked enlargement of the spleen with collapsed gastric bubble. The CT scan confirmed a homogenous splenomegaly reaching the iliac crest and enlarged lomboaortic lymph nodes.

Besides these findings, further evaluation included

1 a bone marrow aspirate smear which showed that more than 20% of cells were mast cells;
2 an osteomedullary biopsy documenting the presence of dense and multi-focal mast cells infiltration; and
3 a spleen biopsy showing the presence of multifocal, dense infiltrate of mast cells (> 15 mast cells in aggregates).

This strongly suggests the diagnosis of systemic mastocytosis. The serum tryptase levels were compatible with the diagnosis: 271.5 μg/l (N < 11.4). The patient was then referred to the Hematology Department and remained clinically stable for a 12-month period, after which started on a cladribin regimen due to increased painful splenomegaly, with good response to the treatment.
Conclusion: This is a case of a rare disease with late onset and non-cutaneous involvement (such as flushing, pruritus). According to the current classification, our patient had indolent mastocytosis, with some poor prognosis factors including age, anemia and weight loss.

909
NK cells and thyroid disorders in women with reproductive failures

Triggianese, P[1]; Bifolchi, A[1]; Perricone, R[1]; De Carolis, C[2]
[1]Rheumatology, Allergology and Clinical Immunology, Department of Internal Medicine, University of Rome Tor Vergata, Rome, Italy; [2]Gynaecology and Obstetrics II, Azienda Ospedaliera San Giovanni-Addolorata, Rome, Italy

Background: Abnormalities in both numbers and activation of peripheral blood natural killer (NK) cells have been related to unexplained reproductive failures (infertility and recurrent miscarriages). Those conditions have been also associated with thyroid dysfunctions.

In this study, we test out the recent evidences in the literature that relate high peripheral NK cell mass to thyroid autoimmunity (TA) in women with reproductive failures. The association between abnormalities in NK cells and non-autoimmune thyroid diseases (NAT) such as hypothyroidism and non-toxic goiter is also, for the first time, examined.

Methods: This study involves 277 women with reproductive failures. In addition to routine hormonal checks, all patients were screened for the presence of anti-thyroglobulin and anti-thyroid peroxidase antibodies. A dynamic evaluation of thyrotrope function (short-TRH test) was performed in order to identify a possible transient hypothyroid status. A quantitative analysis of peripheral NK cells was carried out through peripheral blood immunophenotype assay by using monoclonal antibody against CD56. Physical and ultrasonographic examinations of thyroid were performed.

Results: NK cells (as a percentage of lymphocytes) were found to be elevated in all the considered women (15.2 ± 6%). Women with TA ($n = 67$) exhibited significantly ($P = 0.03$) lower NK levels (13.8 ± 5.9%) than the ones corresponding to women with NAT ($n = 173$, 15.63 ± 6%). Women with TA showed a proportion (41.8%) of patients with NK cell levels >15% being significantly smaller than the one corresponding to both the women without thyroid diseases ($n = 37$, 67.8%) and the women with NAT (56%) where $P < 0.001$ and $P < 0.05$ respectively. All the investigated women with hypothyroidism ($n = 124$) showed no significant difference ($P = 0.74$) in NK levels (15.5 ± 6%) when compared to euthyroid patients ($n = 86$, 15.8 ± 5.6%).

Conclusions: While immunological implications in reproductive failures involve NK cells-related immunity abnormalities, thyroid diseases (AT and NAT) appear to constitute an independent risk factor. In this context, the dysregulation of NK cells cannot be inferred by TSH levels.

910
Evaluation of levels of IL-2 in *Mycoplasma pneumoniae* infection in children with respiratory tract diseases

Sciuca, S[1]; Neamtu, L[1]; Magalu, V[2]
[1]Pediatrics, State Medical and Pharmaceutical University, Chisinau, Moldova; [2]Laboratory of Immunology, Scientific Research Institute for Maternal and Child Health Care, Chisinau, Moldova

Background: The role of cytokines in mycoplasma infections has gained much attention. IL-2 is normally produced by T cells during an immune response. Aim of our study was to evaluate level of IL-2 in peripheral blood in children during an episode of acute lower respiratory tract infection caused by *Mycoplasma pneumoniae*.

Method: Thirty-three patients (17 boys and 16 girls) aged 6 months–7 years old suffering from acute pneumonia concomitant with obstructive bronchitis were included in our study. Determination of specific antibodies of *M. pneumoniae* (IgM, IgG), the peripheral blood concentrations of IL-2 was performed by using *ELISA* in all 33 children.

Results: There were two study visits: first at admission and second – after 1 month. Thirty-three children completed the study: 22 patients with *M. pneumoniae* infection (I group) and 13 children without *M. pneumoniae* infection (II group). In I group levels of specific IgM was 0.73 ± 0.13 (cut-off 0.67 ± 0.02) and IgG was 1.19 ± 0.13 (cut-off 0.53 ± 0.02) and in II group IgM – 0.29 ± 0.04 (cut-off 0.66 ± 0.05), IgG – 0.26 ± 0.04 (cut-off 0.5 ± 0.03), respectively. IL-2 levels in the *Mycoplasma*-positive group was 31.80 ± 2.26 pg/ml and *Mycoplasma*-negative group 39.27 ± 3.17 pg/ml. Levels of IL-2 in our study group had decreased in comparison with control group ($P < 0.05$). Pneumonia with atypical agents develops with poor inflammatory reaction of IL-2, it can suggest an insufficiency of mechanisms of T-cell mediated immunity. The second visit occurred after 1 month from the beginning of the assessment and only eight mycoplasma-positive patients were evaluated for the levels of specific antibodies and serum levels of IL-2. After 1 month in *mycoplasma-p*ositive group levels of IL-2 had increased (39.68 ± 6.84 pg/ml).

Conclusion: There was an imbalance in IL-2 secretion in children with Mycoplasmal pneumonia at the acute phase in our study, suggesting necessity of administration of adequate therapeutic program for control of infectious inflammatory process in Mycoplasmal pneumonia.

912
The level of IL-4 after treatment with prophylthiouracil at hyperthyroid autoimmune disease (Graves disease)

Masri, R
Internal Medicine, Faculty of Medicine Andalas University Padang, Padang, Indonesia

Background: Graves disease is the autoimmune hyperthyroid disease. Hyper production of thyroid hormone cause of the thyroid-stimulating antibody (TSAb) can make hypertrophy and hyperplasia of thyroid gland. Autoimmune disorder of Graves disease suggest cause of limited clonal T suppressor cell that responsibility to TSAb production. Il-4 can increase the expression of MHC class II at B cell, dendrite cell, macrophage cell and put in order of CD 23 expression at B cell that produce of TSAb. Prophylthiouracil (PTU) can inhibit of intrathyroid hormogenesis and conversion of T4 to T3. PTU also action to immunosuppressant effect with supressed of TSAb production. Aim of the study: To look the decreased of IL-4 level after treatment with PTU at Graves disease patients after 3 and 6 month of the treatment.

Method: Prospective study with follow of 25 Graves disease patients at M Jamil Hospital Padang West Sumatera Indonesia at 2010 until 2011. We measured of T3, T4, TSH and Il-4 level at before, 3 and 6 month after treatment. Statistical analysis with chi-square test.

Results: After 3 month treatment we found normal of T3 and T4 level for all of the patients but normal TSH level only at 10 (40%) patients. We found decreased of Il-4 level for all of the patients but still above of normal limit (13.29 ± 4.73 pg/ml). After 6 month treatment we found normal of T3 and T4 level for all of the patients and normal TSH level at 17 (68%) patients. The level of Il-4 for all of the patients after 6 month are decreased but still above of normal limit (3.84 ± 0.42 pg/ml).

Conclusion: The normal level of thyroid hormone after 3 and 6 month PTU treatment at Graves disease not follow by normally of Il-4 level.

915
Melkersson-Rosenthal Syndrome associated with autoimmune hypothyroidism

Aksu, K[1]; Kurt, E[2]
[1]Division of Allergy and Clinical Immunology, Department of Chest Diseases, Ankara Numune Training and Research Hospital, Ankara, Turkey; [2]Division of Allergy and Clinical Immunology, Department of Chest Diseases, Eskişehir Osmangazi University, Eskişehir, Turkey

Melkersson-Rosenthal Syndrome (MRS) is a rarely encountered clinical entitiy characterised with the triad of facial nerve palsy, recurrent episodes of orofacial oedema and fissured tongue. The etiology of MRS is not yet clearly understood though autosomal dominant inheritance is reported in some cases. Some factors possibly involved in the pathogenesis are infections, atopy, contact allergy and hypersensitivity to food additives. Autoimmunity may be also important in the pathogenesis since some cases are associated with Crohn's disease and sarcoidosis.

Case report: A 57 years old female patient was admitted to allergy clinic complaining of persistant swelling of upper lip for the last 2 years. She did not describe accompanying rash or pruritus or swelling in any other part of the body. She had a history of facial nerve palsy recurred for several times since her mid-twenties. She did not report any adverse drug or food reactions and she was not on angiotensin-converting enzyme inhibitor treatment. Previously she was treated with intralesional corticosteroid for swollen lip which revealed partial response. In her first degree relatives fissured tongue was present but the family history was negative for any other related symptoms. Physical examination revealed prominently swollen upper lip and fissured tongue with no other findings. The laboratory examinations including complete blood count, C-reactive protein, renal and liver function tests, blood glucose and serum electrolytes, urinalysis, C3 and C4 levels were all within normal limits. Skin prick tests with aeroallergens and food allergen extracts as well as patch tests with European baseline series came out to be negative. Radiologic evaluation of the lungs, pulmonary function test and carbon monoxide diffusion capacity showed no abnormalities. No pathologic findings were detected in opthalmologic and gastroentorologic evaluations. The patient also received diagnosis of autoimmune hypotyroidism based on elevated tyroid stimulating hormone level and anti-TPO antibody positivity. In conclusion a case of MRS with classical triad who also received diagnosis of autoimmune hypothyroism is reported. The case is presented to point out that MRS should be suspected in cases presented with angioedema and that investigating autoimmune diseases including autoimmune hypothyroidism in patients with MRS is important.

916
Adaptive T cell response in patients with agammaglobulinaemia

Siziakina, L; Andreeva, I
Rostov State Medical University, Rostov-on-Don, Russia

Background: The aim of the research was to carry out the complex analysis of adaptive T cell response parameters in patients with infectious form of CVID (agammaglobulinaemia).
Method: Seven patients in clinical remission aged from 22 to 54 years old who do not take immunonoglobulin replacement therapy and 10 healthy individuals have been followed-up. The disease was clinically manifestated with bacterial infections of the respiratory tract in all patients.
Results: The study of humoral immunity parameters revealed that the total level of immunoglobulins in serum was considerably decreased, not exceeding 2 g/l, while mature B cell count appeared to be of normal age values (8.2 ± 0.15%). The T-cell count was higher than in the control group (83.0 ± 3.4% and 65.7 ± 2.2% respectivety). CD4/CD8 ratio was skewed towards CD8. The decrease in the number of CD4-cells was accompanied by the disorder of their functional potential, i.s. reduction in the number of CD4+CD25+Foxp3+ cells (1.1 ± 0.3%, 3.2 ± 1.2% in the control group) and changes in Th2/Th1 ratio due to Th2 count decrease (1.0 ± 0.2%, 1.5 ± 0.1% in the control group) and Th1 count increase (5.2 ± 0.2%, 3.5 ± 0.2% in the control group). Th1 predominance over Th2 was associated with the elevation of INFγ level (119.5 ± 29 and 6.2 ± 3.3 pkg/ml in the control group). At the same time in case of additional T cell stimulation *in vitro* Th1 count (9.0 ± 0.9%) didn't differ from that in the control group (9.5 ± 0.2%). CD4 lymphocyte count decrease was compensated by CD8 count increase. A sharp increase in the number of granzyme-containing T cells (43.2 ± 2.1%), four times as marked as in the control group (9.4 ± 2.2%), was registered. The late activation marker (HLA DR) expression on CD8 T cells was found to be elevated (7.6 ± 3.4%, 0.94 ± 0.07% in the control group).
Conclusion: Therefore, in patients with CVID (agammaglobulinaemia) compensatory activation of cell immunity was revealed. Exhaustion of CTL adaptive resources obviously determines clinical manifestation of immune deficiency.

918
Chronic granulomatous disease: a case report

Seoane, ME[1,2]; Saavedra, J[3]; Navarro, ML[3]; Pais, P[3]; Campos, M[4]; Carbo, E[5]; Villa, AJ[5]; Noguerado, B[2]; Gil, J[6]; Hernadez-Sampelayo, T[3]; Zubeldia, J[2]; Gurbindo, D[1]
[1]Paediatric Clinical Immunology Unit, Gregorio Marañón General University Hospital, Madrid, Spain; [2]Allergy and Clinical Immunology Department, Gregorio Marañón General University Hospital, Madrid, Spain; [3]Paediatric Infectious Diseases Unit, Gregorio Marañón General University Hospital, Madrid, Spain; [4]Paediatric Dermatology Unit, Gregorio Marañón General University Hospital, Madrid, Spain; [5]Paediatric Traumatology Unit, Gregorio Marañón General University Hospital, Madrid, Spain; [6]Cellular Immunology Laboratory, Gregorio Marañón General University Hospital, Madrid, Spain

Background: Chronic granulomatous disease (CGD) is a primary immunodeficiency disorder characterised by defects in superoxide-generating systems of phagocytes leading to recurrent bacterial and fungal infections. Has an incidence of one in 200 000–250 000 live births and is more common in male. Granuloma formation is characteristic, they often require surgical drainage.
Case report: A previously well 10 months old girl, was admitted from the emergency department for persistent fever 38.3–37.9°C, begins about 1 month ago. Physical examination was normal except: Temperature 38.2°C and two purplish nodular lesions one of 3–4 cm and 1 cm diameter in right leg and pain when moving it. She had no family history related to primary immunodeficiency history of local trauma or puncture wound or travel abroad, coexistence, or contact with animals, no contact with chronic sick. No consumption of unpasteurised milk.
Results: Her total leukocytes count was 28 100/μl, with 59.8% neutrophils and 36% lymphocytes. Platelet 964 000/μl The ESR was 104 mm/h, and CRP 5.5 g/dl. Blood chemistry, including kidney and hepatic function was normal Negative study RSV and influenza. Other studies shown: Negative Mantoux skin test, normal chest X-ray. Soft parts Ultrasonography shown Subcutaneous nodules with central necrosis in the right leg. Finally the granulomas were drained: Gram stain revealed abundant polymorphonuclear leukocytes and Gram-negative bacteria growing Serratia marcescens in culture Bone scintigraphy shown: tracer increased concentration in right tibia support diagnosis of osteomyelitis. Investigations for her immunologic work-up showed Ig G, Ig A, Ig M, Ig E, C3, C4, and CH50 levels to be within her age specific reference range. Lymphocyte subset analysis revealed normal B, T cells and NK cells for her age. Flow cytometry analysis revealed an inability of neutrophils from the patient to undergo a respiratory burst after phagocytosis or phorbol myristate acetate (PMA) and pokeweed (PWM) stimulation, that is, to generate superoxide ions findings consistent with CGD. Neutrophil counts are normal and the chemotactic, adherence, and phagocytic functions of these cells were normal
Conclusion: We try to make clinical physician keep in mind the diagnosis of CGD. Diagnosis is fast by flow cytometry analysis. In case of a history of persistent granulomas or infections this immune deficiency should be investigated.

919
Structural and functional features of the immune system of patients with ulcerative colitis and atopy

Chigaeva, E[1]; Siziakina, L[1,2]; Mkrtychan, L[2]
[1]Rostov State Medical University, Rostov-on-Don, Russia; [2]Immunological Department, Rostov State Medical University, Rostov-on-Don, Russia

Background: Coexistence of several syndromes or diseases, which are patho-genetically related to each other in one patient, is the most common problems of clinical practice. Autoimmune processes, running at the back of atopy, have their own characteristics. The aim is to determine characteristics of immune system of patients with allergy-autoimmune pathology.
Method: Twenty-six patients at the age of 18–58 years old, who have been suffering from ulcerative colitis for more than 5 years and receiving therapy of glucocorticosteroids and biological agents for more than 6 months (infliximab), are being observed. Patients are divided into two groups: I group ($n = 21$, control) without allergic pathology, II group ($n = 5$) with heavy allergoanamnez, where four patients have combined pathology (heavy long-lasting bronchial asthma combined with grass pollen allergy and medicamentous allergy) and one patient has only medicamentous allergy.
Results: Immune monitoring of II group determined high level of IgE/IgG4 against medicine, domestic and pollen allergy without eosinophilia, increase of TNF-α level in blood serum to 10.01 ± 0.46 pg/ml (against 6.0 ± 0.5), interleukine 4 and interleukine 6 till 4.7 pg/ml (against 1.9 ± 0.2) and 3.9 pg/ml (against 2 ± 1.3) respectively, concentration of IFN-γ had trace quantity. A law was revealed in subpopulation content of lymphoid cell of II group patients – the lower level of $CD3^+CD4^+$ lymphoid cells – $29 \pm 2\%$ (against $39 \pm 5\%$), the higher level of $CD3^-CD16^+$ – $16 \pm 3\%$ (against $12 \pm 2\%$) and $CD3^-CD16^+GranzymesB^+$ – $15 \pm 3\%$ (against $9 \pm 2\%$). At referential values of $CD3^+CD4^+$ the level of $CD3^-CD16^+$% is accurately reduced – 3 ± 2 (against 12 ± 2), also there is a lack of their lytic potency: $CD3^-CD16^+GranzymesB^+$ $1 \pm 1\%$ (against 9 ± 2). The level of activated lymphocyte $CD3^+CD8^+GranzymesB^+$ was accurately higher at $19.20 \pm 1.72\%$ (against 9.38 ± 2.21). Clinically, patients with atopy have a lack of stabile remission of ulcerative colitis in comparison with the patients of I group, moreover, elaboration of intrusive fungal infection of intestinal tract was registered.
Conclusion: Progress of bronchial asthma is out of control, there is a tendency for recrudescent progress of herpetic infection and ARVI (till 4–5 times per year) revealing the presence of immunodeficiency that requires correction.

920
A case of SJS-TEN with diverse etiologies: successful treatment with intravenous immunoglobulin and effect on sTRAIL, SCD200, CXCL8 levels

Yalcin, AD[1]; Akman Karakas, A[2]; Soykam, G[3]; Gorczynski, R[4]; Sezer, C[5]; Bisgin, A[6]; Strauss, LG[7]
[1]Internal Medicine, Allergy and Clinical Immunology, Antalya Education and Research Hospital, Antalya, Turkey; [2]Department of Dermatology and Venerology, Akdeniz University School of Medicine, Antalya, Turkey; [3]Department of Internal Medicine, Allergy and Clinical Immunology-Intensive Care Unit, Antalya Training and Research Hospital, Antalya, Turkey; [4]Division of Cellular & Molecular Biology, Toronto Hospital, University Health Network, Toronto, ON, Canada; [5]Department of Pathology, Antalya Training and Research Hospital, Antalya, Turkey; [6]Department of Medical Genetics, Cukurova University Hospitals and Clinics, Adana, Turkey; [7]Clinical Cooperation Unit Nuclear Medicine, German Cancer Research Center, Heidelberg, Germany

Background: SCORTEN is a scoring system used to predict mortality in TEN patients. If SCORTEN index is five or more, mortality rate is more than 90% SJS-TENs pathogenesis is not completely explained and its immunological symptoms are similar to graft versus host disease so; it is possible to say that SJS-TEN is a disorder of the cell-mediated immunity Here, we report a first case of patient with intracranial tumors (ICT) who developed a cutaneous adverse drug reaction during lansoprazole and prophylactic anticonvulsants treatment.
Case: Our patient is a 64 year-old female, who had glioma and had been on post-op prophylactic anticonvulsants therapy. On the 3rd day after she had an operation, lansoprazole was added to the therapy. After the first lansoprazole dose, erythematous dusky red macules were occurred in extremities and trunk and on the following day confluent purpuric lesions tended to run together in 95% of the whole body including scalp and, oral and genital mucosa. Nikolsky's Sign was positive on the skin. Physical examination; body temparature was 38.4 °C with heart rate of 146 beats/min and 80/50 mmHg arterial blood pressure, Glascow Coma Scale was E1 M1e, pupillary light reflex was 2/2+/+ and she had a confusion. Severity scores for TEN (SCORTEN) was calculated as 5 and her biopsy resulted as toxic epidermal necrolysis. Moreover, sTRAIL, CXCL8 and sCD200 levels of serum and blister fluid were investigated as an apoptotic marker and a negative marker for inflammation.
Results and conclusions: sTRAIL and sCD200 were evaluated both in the sera and blister fluid. sTRAIL level was lower than the healthy individuals with high levels in blister fluid; and sCD200 level was depressed in to 10% of the normal values of healthy individuals but with high levels in the blister fluid during the active phase of the disease. After our successful treatment with human albumin, prednisolone pulse therapy and IVIG in dose of 400 mg/kg, she was discharged from the hospital on the 23rd day and followed up after 2 months. The increase in sTRAIL (up to two folds) and sCD200 (up to six folds) levels may provide useful information in understanding disease pathogenesis and monitoring treatment efficacy.

921
Association between allergy and cancer in Korean adults

Lee, S-H[1,2,3]; Kang, H-R[1,2]; Kim, S-S[1,2,3]; Min, K-U[1,2]; Yong, S-J[4]; Cho, S-H[1,2,3]
[1]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Institute of Allergy and Clinical Immunology, Seoul National University Medical Research Center, Seoul, Korea; [3]Healthcare System Gangnam Center, Seoul National University Hospital, Seoul, Korea; [4]Department of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea

Background: Although numerous epidemiological studies have evaluated the association between allergy and cancer, it is still unclear whether allergic diathesis plays as a risk factor or protective factor for the development of cancer.
Method: A retrospective review was done for the subjects who had visited the Seoul-National UniversityHospital Gangnam-Healthcare Center for private health check ups from October 2003 to May 2011. Atopy was defined if a subject had positive response to at least one allergen in skin prick test or *in vitro* allergen-specific IgE tests. Airway hyperresponsiveness (AHR) was defined when PC_{20} was <25 mg/dl in methacholine bronchial provocation test (MBPT). Information on allergic diseases was obtained through questionnaires. Information on previous or current cancer morbidity was obtained from medical history, endoscopic or ultrasonography-guided biopsy, and imaging studies such as computed tomography or magnetic resonance imaging.
Results: Among 12 462 subjects, atopy was detected in 5224 subjects (41.9%), allergic diseases in 918 (7.4%), AHR in 546 (4.4%) and cancers in 552 (4.4%). Overall rate of cancer did not differ according to the presence of atopy, AHR,

or allergic diseases. The incidence of kidney cancer was significantly lower in men with atopy (0.21% vs 0.6%, $P = 0.02$) but not in female. The incidence of colorectal cancer was significantly higher in subjects with allergic rhinitis compared with those who were free of allergic rhinitis (1.0% vs 0.4%, $P = 0.009$) and Breast cancer was more prevalent in women with allergic diseases (4.1% vs 1.4%, $P = 0.009$). Lung cancer was significantly more prevalent in men with AHR (1.93% vs 0.84%, $P = 0.036$) and this difference was still significant after adjustment of smoking history and age ($P = 0.046$, RR = 2.306, CI: 1.015–5.237).

Conclusion: We observed that the incidence of some cancers was different according to the state of atopy, AHR or allergic diseases. Further research is needed to elucidate their relation to cancer pathogenesis.

Poster Session 31

Allergic inflammation

922

Effect of highly selective inhibitor for hematopoietic prostaglandin D synthase on experimental allergic rhinitis model. Pharmacological character of a novel inhibitor

Aoyagi, H; Kajiwara, D; Shigeno, K; Tanaka, K; Miyoshi, K; Sasaki, E
Taiho Pharmaceutical Co., Ltd., Drug Discovery & Development II, Tsukuba Research Center, Tsukuba-shi, Japan

Background: Hematopoietic prostaglandin D synthase (HPGDS) is an enzyme of prostaglandin D_2 (PGD_2) synthase expressed on mast cells, Th2 cells, and basophils, which are known to be major players in allergic responses. Although some reports have suggested therapeutic effect of HPGDS inhibitor on allergic and inflammatory diseases, it has never been clear on allergic rhinitis. To investigate beneficial effect of HPGDS inhibition in allergic rhinitis, we found a highly selective inhibitor, a 5-benzoylbenzimidazole derivative (TAS-204). In this study, we evaluated the specificity and potency of TAS-204.

Method:

1 Inhibitory effect on HPGDS activity was assessed by measuring the amount of PGD_2 in the enzyme reaction mixture and in supernatants of a human basophilic leukemia (KU812) stimulated with calcium ionophore. Specificity on enzyme activity was also assessed on activities of other arachidonic acid-related enzymes including lopocalin-type PGDS, cyclooxygenase, 5-lipoxygenase, leukotriene C4 synthase and thromboxane synthase. Specificity to allergy-related receptors was assessed on the binding of each ligand to the receptors including histamine H1 receptor, CysLT1 receptor, TP receptor and DP receptor.
2 On allergic rhinitis model in guinea pigs, the inhibitory activity on PGD_2 production was evaluated on the levels of PGD_2 concentration of nasal lavage fluids at 30 min after the challenge with ovalbumin.

Results:

1 TAS-204 inhibited HPGDS with the IC_{50} of 18 nM and reduced PGD_2 production in KU812 with the IC_{50} of 4.6 nM. TAS-204 at 10 μM did not show significant effect in various other activities of arachidonic acid-related enzyme or in the binding of each ligand to allergy-related receptors.
2 The challenge with ovalbumin caused increase in PGD_2 concentration of nasal lavage fluids. TAS-204 dose-dependently inhibited PGD_2 production in nasal cavity.

Conclusion: These results suggest that TAS-204 is a highly selective inhibitor for HPGDS and that it inhibit PGD_2 production induced by antigen-challenge. Therefore, TAS-204 is expected to improve allergic rhinitis symptoms such as nasal obstruction.

923

Effect of highly selective inhibitor for hematopoietic prostaglandin D synthase on experimental allergic rhinitis model in guinea pigs. Therapeutic effects of a novel inhibitor

Kajiwara, D; Aoyagi, H; Shigeno, K; Tanaka, K; Miyoshi, K; Sasaki, E
Taiho Pharmaceutical Co., Ltd., Drug Discovery & Development II, Tsukuba Research Center, Tsukuba-shi, Japan

Background: We found that a 5-benzoylbenzimidazole derivative (TAS-204) is a highly selective inhibitor for hematopoietic prostaglandin D synthase (HPGDS) and that it inhibits prostaglandin D_2 (PGD_2) production induced by antigen-challenge. To investigate beneficial effect of HPGDS inhibition in allergic rhinitis, we evaluated the effects of TAS-204 against symptoms in experimental allergic rhinitis model of guinea pigs.

Method: Guinea pigs sensitised to ovalbumin were challenged with intranasal exposure to ovalbumin once a week. TAS-204 was administrated orally 1 h before the 3rd challenge, or once a day for 15 days from the 1st challenge to the 3rd challenge. Eosinophilic accumulation in nasal mucosa was evaluated on the number of eosinophil in nasal lavage fluid at 6 h after the 3rd challenge. Nasal responsiveness to histamine was assessed by counting the number of sneezing for 20 min following the provocation with histamine 1 day after the 3rd challenge. To evaluate the effects on biphasic increase of nasal resistance, specific airway resistance was measured before and after the 3rd antigen challenge.

Results: The increase in number of eosinophil in nasal lavage fluid was significantly suppressed by repeated administration of TAS-204 (30 mg/kg/day). Number of sneezing after the provocation of histamine was increased as compared with non-challenged animal. The increase was also significantly suppressed by repeated administration of TAS-204 (30 mg/kg/day). Single dosing of TAS-204 at 30 mg/kg resulted in suppression of the increase in nasal airway resistance, especially on late phase response. When TAS-204 was administered for 15 days, the increase in nasal airway resistance was suppressed not only on late phase but also on early phase.

Conclusion: We found that HPGDS plays a critical role in the development of biphasic nasal obstruction and eosinophilic inflammation. These results suggest that suppression of PGD_2 production by repetitive HPGDS-inhibition interrupts development of disease. Therefore, early treatment of HPGDS inhibitor may be very useful therapeutic strategy for allergic rhinitis.

924

The release of IL-10, IL-17, IL-31, IL-33, soluble ST2, and other cytokines in allergic rhinitis after nasal allergen challenge and during natural allergen exposure

Wagenmann, M; Baumann, R; Werminghaus, P; Stenin, I; Rabaszowski, M; Tilgner, L; Scheckenbach, K
Department of Otorhinolaryngology, Head & Neck Surgery, Heinrich-Heine University Duesseldorf, Duesseldorf, Germany

Background: For some of the newest cytokines only limited data is available concerning their possible role in the pathophysiology of allergic rhinitis. To study the release and the kinetics of these cytokines in allergic rhinitis we performed nasal allergen challenges and collected nasal secretions of allergics during pollination season.

Method: Two studies were carried out. In the first study, unilateral nasal allergen and control challenge with the disc method were performed out of the allergy season in seven allergic volunteers with allergic rhinitis and sensitisation to seasonal

allergens. Nasal symptom scores were obtained and bilateral nasal secretions were quantified before and until 24 h after allergen provocation. In the second study, we collected nasal lavages and obtained symptom scores from 15 allergic and 14 non-allergic volunteers during the pollination season. The levels of IL-4, IL-5, IL-10, IL-13, IL-17, IL-31, IL-33, soluble ST2, and Eotaxin-3 in nasal secretions and lavages were measured using electrochemiluminescent assays or with ELISA.

Results: Nasal allergen challenge induced the typical clinical symptoms and physiological changes. The levels of IL-4, IL-5, IL-10, IL-13, IL-17, IL-31, soluble ST2, and Eotaxin-3 in nasal secretions significantly increased after allergen provocation. Correlations were found between IL-13 and IL-31, and IL-17 and Eotaxin-3. Soluble ST2 was inversely correlated with IL-5. Symptom scores correlated with IL-31 in nasal secretions. During natural allergen exposure, IL-5, soluble ST2, and Eotaxin-3 levels in nasal lavages were significantly elevated compared to non-allergic controls. IL-10 and IL-4 were significantly lower in allergics. Nasal soluble ST2 concentrations were inversely correlated with nasal symptoms. IL-33 could neither be detected after nasal allergen challenge nor during natural allergen exposure.

Conclusion: We were able to detect several new cytokines that seem relevant in the regulation of the allergic inflammation in allergic rhinitis and describe their time-course of release into nasal secretions. Correlations between cytokine levels and symptoms point to their relevance. Additional studies to examine interactions between pro- and anti-inflammatory cytokines might elucidate pathophysiological networks.

925

Meta-analysis of the efficacy of ectoine nasal spray and eye drops in the treatment of allergic rhinoconjunctivitis

Eichel, A[1]; Werkhaeuser, N[2]; Bilstein, A[2]; Moesges, R[1]
[1]Institute of Medical Statistics, Informatics and Epidemiology, University of Cologne, Cologne, Germany; [2]Medical Device Development, Bitop AG, Witten, Germany

Background: Nasal spray and eye drops containing the compatible solute ectoine have been applied in several trials in the treatment of allergic rhinoconjunctivitis. This meta-analysis aimed to investigate the overall efficacy of the ectoine products in reducing nasal and ocular symptoms.

Method: Based on results of four studies, effects of ectoine products on nasal and ocular symptoms were analysed using the ANOVA model. Total improvement of symptoms on day 7 of treatment was assessed. Additionally, individual symptom scores on day 7 of treatment were evaluated in comparison to baseline values at day 1. Reduction of symptom scores was compared to that of comparator or placebo products. SPSS version 19 and Review Manager 5 were used for statistical analyses and quantitative data synthesis.

Results: Both nasal and ocular symptoms decreased significantly upon treatment with ectoine products. A strong reduction of symptom severity was shown for the parameters rhinorrhoea (31.76% reduction) and nasal obstruction (29.94% reduction). The strength of effects of ectoine products was assessed by comparison of symptom scores on day 7 and baseline values on day 1. A strong effect (effect size in accordance with Ferguson et al) of 0.53 ± 0.26 was shown for the improvement of nasal obstruction, and moderate effect sizes were demonstrated for the parameters rhinorrhea (0.47 ± 0.24), nasal itching (0.47 ± 0.24) and sneezing (0.37 ± 0.26). Results of studies using both nasal spray and eye drops demonstrated moderate to strong effect sizes in the improvement of the symptoms itching of eyes and redness of eyes. The comparison of ectoine products with reference products (antihistamine, glucocorticoid, or cromoglycic acid) or placebo treatment showed comparable (nasal obstruction and rhinorrhea) or better (nasal itching and sneezing) efficacy of the ectoine products in comparison to control substances. Whereas the symptom of teary eyes was significantly better improved by ectoine treatment in comparison to control, the parameters itching of eyes and redness of eyes were improved comparably well.

Conclusion: Treatment of allergic rhinoconjunctivitis with ectoine products showed comparable efficacy as treatment with standard pharmaceutical products. Those results together with a very positive safety profile of ectoine products might open promising new treatment strategies.

927

Increase in fractional exhaled nitric oxide induced by bronchial allergen challenge in allergic individuals lasts at least 2 weeks

Siergiejko, P[1]; Siergiejko, D[1]; Siergiejko, G[2]; Świebocka, EM[2]; Sierakowski, S[1]; Siergiejko, Z[3,4]
[1]Internal Medicine and Rheumatology Department, Medical University of Bialystok, Bialystok, Poland; [2]Pediatrics, Gastroenterology and Children Allergology Department, University Children's Hospital, Bialystok, Poland; [3]Respiratory System Diagnostics and Bronchoscopy Department, Medical University of Bialystok, Bialystok, Poland; [4]Prywatny Gabinet Internistyczno-Alergologiczny, Bialystok, Poland

Background: A bronchial allergen challenge (BAC) causes a bronchial obstruction in some subjects sensitive to grass pollen and an increase in fractional exhaled nitric oxide (FeNO) lasting at least 72 h in all of them (1). The purpose of the study was to evaluate how long after BAC the level of FeNO remains elevated.

Method: Studies were performed on a group of 25 subjects suffering from seasonal or perennial allergic rhinitis. BAC was performed according to Siergiejko et al. method (2) using commercial allergens of grass pollen, birch and Dermatophagoides pteronyssinus (Dpt) (Allergopharma, Germany). Sensitisation to the allergens were confirmed by serum sIgE level evaluation (Polycheck, Germany). KoKo Digidoser (n-Spire, USA) was used both as a spirometer and a dosimeter. Early and late asthmatic reactions were evaluated during the challenge. FeNO were evaluated before, 8 h after BAC, and daily morning for a period of 14 days using Niox Mino® (Sweden).

Results: In serum of three individuals among challenged no specific IgE against the allergens used for BAC were found. No increase in FeNO after BAC was observed in them. Their results were excluded from analysis. All analyzed subjects independent of bronchoconstriction demonstrated a significant increase in FeNO lasting until the last day of the observation. The elevation of FeNO started 24 h after BAC and gradually increased until to 4th day in the group provoked by Dpt ($n = 12$) and to 6th day by pollen allergens ($n = 11$) and than slowly recovered. Fourteen days after challenge FeNO was still significantly elevated. BAC with pollen allergens stronger than Dpt caused the increase in FeNO (higher value of FeNO and longer lasting increasing).

Conclusion: Bronchial allergen challenge in allergic subjects causes an elevation in FeNO lasting at least 2 weeks.

928

Rupatadine inhibits platelet activating factor-induced mast cell degranulation in both cell line (LAD-2) and primary human lung mast cells

Muñoz-Cano, R[1]; Torres-Atencio, I[1]; Ainsua, E[1]; Martin, M[1]; Sánchez-López, J[1]; Bartra, J[1]; Picado, C[1]; Mullol, J[1]; Valero, A[2]
[1]Hospital Clinic, IDIBAPS, Barcelona, Spain; [2]Pneumology and Respiratory Allergy, Hospital Clinic, ICT, Barcelona, Spain

Background: Platelet activating factor (PAF) is a lipid mediator involved in the amplification of mast cell (MC) activation in anaphylaxis and other allergic reactions. The role of rupatadine as PAF antagonist, although described in several models, has not been investigated in human MC. The objective of this study was to investigate

the expression of PAF receptors in MC and the effect of rupatadine on PAF-induced MC degranulation compared with other second generation antihistamines (desloratadine, levocetirine) and aspecific pure PAF antagonist (CV6209).

Methods: Investigations were done in both a human MC line (LAD-2) and primary MC from human lung. MC degranulation was evaluated by the β-hexosaminidase and histamine release and PAF receptor expression was evaluated by western blot. After stimulation with PAF in a dose-response and time course manner, the optimal PAF conditions to induce optimal LAD-2 (10 μM and 30 min) and primary lung (10 μM and 15 days) degranulation were identified. The effects of rupatadine, desloratadine, and levocetirizine from 1 to 100 μM on PAF-induced MC activation were investigated. The inhibitory effect of CV6209 (specific anti-PAF) at 2 μM was used as positive control in all experiments.

Results: PAF receptor protein was found expressed in both LAD-2 and human lung mast cells. In LAD-2 cell line, rupatadine inhibited PAF-induced β-hexosaminidase release from 5 to 10 μM ($P < 0.005$). In a lower extent, both levocetirizine (5 μM, $P < 0.01$) but not desloratadine also showed some inhibitory effect. Rupatadine also inhibited PAF-induced histamine release from 1 to 10 μM ($P < 0.01$) as well as levocetirizine (1–25 μM, $P < 0.05$) and desloratadine (10 μM, $P < 0.05$). In addition, rupatadine but not levocetirizine or desloratadine also inhibited PAF-induced histamine release in human lung mast cells at 10 μM ($P < 0.05$).

Conclusions: This study shows that the anti-H1 compounds rupatadine, and to a lower extent levocetirizine, have an anti-PAF effect in MC from both a cell line and MC from human lung origin, suggesting that rupatadine could be more effective than other antihistamine drugs in those allergic disorders where PAF may act as an important inflammatory mediator.

929

Gene expression profiles of peripheral blood mononuclear leukocytes from patients with psoriasis

Titov, LP[1]; Liauchenia, M[1]; DuBuske, LM[2]
[1]Republican Scientific and Practical Center for Epidemiology and Microbiology, Minsk, Belarus; [2]Immunology Research Institute of New England, Gardner, United States

Background: Psoriasis is characterised by hyper proliferation and aberrant differentiation of keratinocytes, vascular hyperplasia and infiltration by mononuclear cells. This study assesses how genes or group of genes differentially change expression in peripheral blood mononuclear cells (PBMCs) in patients with psoriasis compared with a control group of healthy donors.

Method: Twelve patients (nine men, three women), age 47.6 ± 13.8 years (mean ± SD) and disease duration 12.3 ± 10.4 years were studied. The control group consisted of age and sex matched healthy individuals ($n = 5$). PBMCs were collected from blood of each participant. Total RNA was isolated from PBMCs using *Tri-Reagent* (Sigma, USA). cDNA was synthesised and labeled using 1 mg of total RNA and the Superscript *cDNA Synthesis* Kit (*Invitrogen,* CA). Analysis of gene expression was carried out with custom e-chip (ArrayIT, USA) containing a total of 652 known genes. Five μg of labeled cDNA was hybridized to an e-chip and scanned on Innoscan 700 (Carbone, France).

Results: Expression of 652 genes on the e-chip showed the number of genes which significantly ($P < 0.05$) changed expression by 1.5-fold or more compared with the control was 70 (51 up and 19 down regulated). The greatest changes were found in the genes responsible for cell cycle, proliferation and apoptosis ($n = 23$), the signaling pathways and cytokines ($n = 25$), genes involved in B-cell and T-cell immunity ($n = 12$) and cell adhesion ($n = 7$). Significant differences were found for TNFa, IL-8, annexin A, HSP60, IL-2, IL-4, IL-10, CD28, IL-1 and IL-6.

Conclusion: Gene expression profiling of mononuclear cells from the peripheral blood patients with psoriasis identified a number of novel genes and genes having down and up regulation which might serve as future targets for therapeutic intervention or markers of disease activity.

930

In vitro and *in vivo* functional assays for human mesenchymal stem cell adherence, invasivity, and migration towards inflammatory lesions after transfection with CD29 specific siRNA

Panaitescu, C; Marusciac, L; Bojin, FM; Paunescu, V
University of Medicine and Pharmacy Victor Babes Timisoara, Functional Sciences, Timisoara, Romania

Background: CD29 (integrin β1) is one of the mediators involved in human mesenchymal stem cell (hMSCs) adhesion and migration. The aim of this study was to investigate the significance of CD29 for hMSCs migration, by evaluating *in vitro* the adherence of hMSCs on inflammation mediators, and by determining *in vivo* the hMSCs migratory potential towards inflammatory skin lesions, after specific inhibition of CD29.

Method: hMSCs were isolated from donor iliac crest and characterised. After hMSCs expansion, the expression of CD29 was inhibited specifically by transfection with siRNA duplexes. Transfection efficiency at the gene and protein levels was evaluated at different time-points by RT-PCR, flow cytometry, flow chamber assay, and immunohistochemistry. The transfected hMSCs were cultured in the presence of several inflammation mediators (vascular cell adhesion molecule 1, VCAM-1; intercellular adhesion molecule, ICAM; tumor necrosis factor alpha, TNF-α; transforming growth factor beta1, TGF-β1), using a real-time cell analyzer to measure cell adhesion. Transfected hMSCs were injected subcutaneously in CD1 Nu/Nu mice, after producing an inflammatory skin lesion, and hMSCs migration towards the wound site was evaluated macroscopically by an *in vivo* imaging system, and microscopically by immunofluorescence for CD29, Ki67, cytokeratin, and vimentin.

Results: hMSCs were successfully expanded and kept in culture for up to eight passages. The isolated cells were positive for CD44, CD73, CD90, and CD105. Flow cytometry showed an abundance of CD29 on the hMSCs surface (78.24 ± 4.7%). Inhibition of CD29 was successful, transfected cells showing approximately 80% depletion of CD29 at 72 h (18.02% of transfected hMSCs expressed CD29). CD29 inhibition also decreased CD90 (98.42–74%) and CD105 (95.67–68.73%) expression. Adherence of transfected hMSCs to VCAM-1 was significantly reduced, while their adhesion to ICAM and TGF-β1 was not affected. When compared with control hMSCs, transfected hMSCs migrated more slowly to the skin wound site, and their proliferation rate was higher, but there were no significant differences between overall healing times.

Conclusion: CD29 inhibition modulates hMSC proliferation and has downstream effects by modulating the expression of other hMSCs markers. The specific interactions between CD29 and VCAM-1 are involved in hMSCs migration towards inflammation sites. CD29 is a possible therapeutic target in inflammatory disorders.

931
Identification of specific markers for type 2 (pro-allergic) human dendritic cells: interest as follow-up markers for immunotherapy

Guéguen, C; Moussu, H; Baron-Bodo, V; Mascarell, L; Moingeon, P
Stallergenes France, Research and Pharmaceutical Development, Antony, France

Background: Dendritic cells (DCs) are involved in the initiation of regulatory and effector adaptive immune responses. Thus, markers of polarised DCs represent promising molecules to monitor immune responses following immunotherapy. Herein, we aimed to identify specific markers for type 2 DCs (DC2), ie. monocyte-derived dendritic cells (MoDCs) which promote Th2 allergic responses.
Method: Immature MoDCs were treated with either lipopolysaccharide (LPS) from *E. coli* to produce effector type 1 DCs (DC1), a cocktail of molecules capable of differentiating DC2, or with dexamethasone (DEX) to induce regulatory DCs (DCreg). Patterns of cytokine secretion were monitored in DCs using multiplex cytokine quantification assays. The expression of effector or regulatory genes was followed by quantitative PCR in treated-DCs. Cytokine production by co-cultures of MoDCs with allogeneic $CD4^+$ T cells was assessed as a read-out of DCs polarisation. Whole genome mRNA expression was conducted in DC1, DC2 and DCreg using microarrays covering 50 684 sequences.
Results: After screening more than one hundred biological and pharmaceutical agents, a cocktail of molecules capable of differentiating DC2 was selected. Such DC2 were confirmed to support the differentiation of IL-5 and IL-13 secreting $CD4^+$ T cells. While DC1 produced IL-1β, IL-6, IL-8, IL-10, IL-12p70 as well as TNF-α, DC2 secreted a distinct panel of effector cytokines ($IL\text{-}1\beta^-$, $IL\text{-}12p70^-$, $TNF\text{-}\alpha^-$, $IL\text{-}10^{low}$, $IL\text{-}6^+$ and $IL\text{-}8^+$). Both DC1 and DC2 overexpressed effector genes (e.g. MX1, NMES1, FSCN1 and IRF4) while downregulating the expression of regulatory genes (e.g. C1QA, CATC, GILZ, STAB 1 and RALDH1) specific for DCreg. Whole genome transcriptome comparison of DC1, DC2 and DCreg revealed extensive differences between these three DC subsets. When compared to non-treated MoDCs, DC1, DC2 and DCreg up-regulated 1617, 1493 and 186 genes and down-regulated 1917, 1882 and 197 genes, respectively. Interestingly, in DC2, 104 and 36 genes were specifically over-expressed and under-expressed, respectively, when compared with DC1 and DCreg.
Conclusion: Specific DC2 markers have been identified, allowing to distinguish such cells from effector DC1 or regulatory DC subsets. Those markers are being tested as follow-up read outs of efficacy for allergen immunotherapy.

932
IL17 in allergic diseases

Porr, C[1]; Porr, PJ[2]
[1]Allergology, County Clinical Hospital, Sibiu, Romania; [2]Internal Medicine, University Lucian Blaga, Sibiu, Romania

Background: Interleukin 17 is an interleukin with potent pro-inflammatory effect by inducing proinflammatory chemokines release and autoimmune effect. Given the inflammatory component of asthma pathogenesis, we considered useful to study the behaviour of this interleukin in allergic diseases.
Method: We studied the behaviour of interleukin-17 at 31 patients with allergic rhinitis, 15 patients with intrinsic asthma, 22 patients with asthma and allergic rhinitis and 20 controls. At all patients were made skin prick tests to aeroallergens. Determination of serum interleukin-17 was made by ELISA kit also with a sandwich method, using monoclonal antibodies.
Results: Correlated with coexisting obesity, we observed higher values of interleukin-17 in patients with asthma and obesity, but without statistical significance. No significant correlation with the area of origin and smoking could be done. Regarding the gender of patients, increased values were observed in women from the group with asthma and allergic rhinitis. Correlated with family history, we found elevated levels of interleukin-17 in all patients with positive family history from all groups of patients, but at patients group with allergic rhinitis this increase was statistically significant.
Conclusion: These results suggest an important role of interleukin-17 in allergic diseases with increasing disease severity.

933
Leptin in allergic diseases

Porr, C[1]; Porr, PJ[2]
[1]Allergology, County Clinical Hospital, Sibiu, Romania; [2]Internal Medicine, University Lucian Blaga, Sibiu, Romania

Background: Leptin is one of the most important adipokines with proinflammatory role. There is also a study which shows that high levels of leptin are associated with increased asthma severity. We analyzed the level of leptin of patients with atopic dermatitis, allergic rhinitis and asthma.
Method: We determined serum level of leptin by ELISA kit with a sandwich method at 31 patients with allergic rhinitis, 15 patients with intrinsic asthma, 22 patients with asthma and allergic rhinitis and 20 controls. At all patients were made skin prick tests to aeroallergens.
Results: There is a lower average of leptin for those with moderately/severe intermittent allergic rhinitis, than those with mild persistent allergic rhinitis. In patients with asthma and associated allergic rhinitis with intermittent asthma, leptin was lower than in those with moderate persistent asthma. It was observed an increase in leptin values with increasing asthma severity. In allergic rhinitis, leptin levels increased with increasing disease severity.
Conclusion: These results suggest a possible role of leptin in allergic diseases with increasing disease severity.

934
Pollen-associated phytoprostane E1 directly enhances IgE production in B cells

Oeder, S[1]; Braun, A[2]; Kamml, M[1]; Alessandrini, F[1]; Kanter, U[3]; Hauser, M[4]; Ernst, D[3]; Ferreira, F[4]; Ring, J[5]; Buters, J[1]; Mempel, M[2]; Traidl-Hoffmann, C[1]; Schmidt-Weber, C[1]; Behrendt, H[1]; Gutermuth, J[1,6]
[1]TU München/Helmholtz Zentrum München, Center for Allergy and Environment (ZAUM), Munich, Germany; [2]Department of Dermatology, Venereology and Allergology, University of Göttingen, Göttingen, Germany; [3]Helmholtz Zentrum München, Institute of Biochemical Plant Pathology, Neuherberg, Germany; [4]Department of Molecular Biology, University of Salzburg, Salzburg, Austria; [5]Department of Dermatology and Allergy Biederstein, Technische Universität München, Munich, Germany; [6]Department of Dermatology, Vrije Universiteit Brussel, Brussels, Belgium

Background: As providers of IgE, B cells play a central role in allergies. In nasal and bronchial epithelium B cells can have direct contact to aeroallergens and switch their immunoglobulin isotypes locally towards IgE. In contrast to other cell types, B cells are not well characterised after direct exposure to pollen constituents.
Method: To assess, whether direct exposure of B cells towards pollen can increase allergic reactions, we incubated murine B cells to aqueous pollen extracts (APE) from ragweed plants. Naïve B cells were isolated from C57BL/6-splenocytes by untouched MACS sorting. B cells were incubated under IgE-inducing conditions

with anti-CD40 and IL-4 (or under IgG-inducing conditions with anti-CD40 and IFN-γ) +/−different ragweed (Amb) APEs or a protein-free fraction (<3 kD) of the extract for 8 days. The production of total IgM/IgG/or IgE was quantified by ELISA. Number of viable cells was assessed by measuring intracellular ATP (CellTiter-Glo). Transcripts necessary for immunoglobulin isotype switch towards IgE (AID, ε germline transcript) were quantified by real-time PCR. Additionally single substances that are contained in pollen grain were tested: the major allergen of ragweed Amb a 1 and the low molecular weight substance phytoprostane E1 (PPE1).

Results: Amb-APE alone had no effect on IgE production, but in anti-CD40 and IL-4 stimulated B cells, the extract caused a dose dependent increase of IgE production, while IgG and IgM were not affected. Under Th1 conditions, Amb-APE did not influence the production of any Ig. The IgE-enhancing effect under Th2 conditions was correlated with the number of viable B cells, but not with an increase in IgE class switch (AID and ε germline transcript were not induced). While Amb a 1 alone had no effect on IgE production, the low molecular weight fraction caused an increase in IgE production similar to total Amb-APE. A specific substance that can be contained in this fraction, PPE1 was found to increase IgE production in the same way.

Conclusion: Pollen extracts specifically enhance IgE-production by Th2-stimulated B cells. PPE1 is one causing substance and probably a major player that mediates the allergy-aggravating effect of pollen.

936
Myrtol enhances ciliation and mucin secretion in human nasal epithelial cells from healthy subjects and patients with nasal polyposis *in vitro*

Li, YY[1]; Jin, P[1,2]; Li, CW[1]; Chao, SS[1]; Liu, J[1]; Shi, L[2]; Wang, DY[1]
[1]Otolaryngology, National University of Singapore, Singapore, Singapore; [2]Department of Otolaryngology, Shandong University, Qilu Hospital, Jinan, China

Background: Myrtol (Gelomyrtol forte) is effective in controlling the nasal symptoms of acute and chronic sinusitis by promoting cilia beat frequency and mucociliary clearance. We sought to study the cellular and molecular changes of airway epithelial cells from healthy subjects and patients with nasal polyposis (NP) in response to Myrtol treatment *in vitro*.

Method: Primary epithelial cells were isolated from biopsies of healthy inferior turbinates ($n = 3$) and nasal polyps ($n = 3$). Cells were cultured in the differentiation medium in air liquid interface. 0.1% Myrtol was applied in the cell cultures at four different time points (day 0, 10, 21, and 28) and then it was maintained in the cell cultures throughout the differentiation. The cellular patterns as well as the molecular changes were assessed by immunofluorescence and quantitative PCR.

Results: When achieving fully ciliated cultures (day 35), epithelial cells (from both healthy and NP subjects) treated with Myrtol on day 0 and day 10 showed a significant increase of beta-tubulin IV staining (ciliated cell marker) and MUC5AC staining (goblet cell markers) as compared to those without treatment. In addition, epithelial cells (from both healthy and NP subjects) stimulated on day 21 and day 28 exhibited greater ciliogenesis and mucin secretion than those treated on day 0 and day 10. mRNA levels of mucin genes (MUC5AC, MUC1, MUC4, and MUC16) were also changed in different stimulation time points.

Conclusion: Myrtol has positive effects on ciliation and mucin secretion in epithelial cells derived from both healthy and diseased nasal mucosa, which is concordant to its clinical effect on mucociliary clearance *in vivo*.

937
Parietaria-induced allergic rhinitis: how immune inflammation process in nasal mucosa changes over time

Gelardi, M[1]; Squeo, V[1]; Quaranta, N[1]; La Mantia, I[2]; Pintaldi, L[2]; Scolari, M[3]; Dell'Albani, I[3]; Incorvaia, C[4]; Frati, F[3]
[1]Section of Otolaryngology, Department of Neuroscience and Sensory Organs, University of Bari, Bari, Italy; [2]ASP 3 Catania, Otolaringology – P.O Santa Marta – Acireale, Catania, Italy; [3]Medical and Scientific Department, Stallergenes Italy srl, Milan, Italy; [4]Allergy/Pulmonary Rehabilitation Unit, ICP Hospital, Milan, Italy

Background: Parietaria-induced pollinosis is an important clinical feature in Italy especially in the South area. The extraordinary long persistence of Parietaria pollen in the atmosphere in this area is often responsible for almost perennial symptoms. Concerning allergic rhinitis (AR), different clinical stages are associated with different cytological patterns in nasal mucosa, which is the site of the disease. In fact, during acute phases a greater inflammatory infiltrate composed of eosinophils, mast cells and lymphocytes is present. The goal of our pilot study was to follow the modifications of immune inflammation process in the nasal mucosa over 1 year.

Method: Seventy-four patients (35 males and 39 females, mean age 44.4 years, range 12–75) with persistent AR caused by monosensitisation to Parietaria were included. In order to perform nasal cytology, patients had to be untreated for 7 days. In all subjects, nasal cytology was monthly performed by Rhino-probe sampling, staining and reading by optical microscope.

Results: The results showed that during 1 year of follow-up eosinophils are the cells most commonly detected. The most important finding is the presence of two specific periods (spring and late summer) where an immune inflammation process was actively ongoing. In fact, in May and August/September there was a peak of eosinophils, lymphocytes/plasma cells and mast cells in the nasal mucosa according to the pollen period.

Conclusion: In patients with Parietaria-induced AR, we observed a greater inflammatory infiltrate of eosinophils, mast cells and lymphocytes/plasma cells in two different periods (spring and late summer) and this is probably involved in the worsening of the clinical stage. These findings could pave the way to a seasonal treatment of Parietaria-induced AR in accordance with the status of immune inflammation process in nasal mucosa.

939
Serine proteases inhibitor reduce allergic inflammation by regulatory T cells in a house dust mite allergic rhinitis mouse model

Kim, SW; Kim, B-Y; Shin, J-H; Park, HR; Kim, SW
Otolaryngology, The Catholic University of Korea, Seoul, Korea

Background: Serine proteases inhibitors are involved in immune development, anti-inflammation and tissue repair. In the present study, 4-(2-Aminoethyl) benzene sulfonyl fluoride hydrochloride (AEBSF) a serine protease inhibitor was evaluated for prophylactic and therapeutic treatment in mouse model of allergic rhinitis (AR).

Method: BALB/c mice were divided into control, Derf, Pre-S, Pre-C and steroid groups. The allergen was Dermatophagoides farinae (Derf). AEBSF was administered before sensitisation (Pre-S) or before challenge (Pre-C). Allergic symptom scores, eosinophil counts in nasal mucosa, proteolytic activity, Interferone-γ and interleukin (IL)-10 levels in nasal lavage fluid and serum Derf-specific IgE levels were measured. T-bet, GATA-3, and Foxp3 mRNA expression in spleen and IL-13, TGF-β

expression in nasal mucosa were determined by real-time polymerase chain reaction. Flow cytometry of $CD4^+CD25^+Foxp3^+$ T cells in spleen were analyzed.

Results: Symptom scores, serum Derf-specific IgE and tissue eosinophil counts were decreased in both Pre-S and Pre-C groups (all, $P < 0.05$). Also, percentage of $CD4^+CD25^+Foxp3^+$ T cells were increased in Pre-S, Pre-C and steroid groups compared with those in Derf group (all, $P < 0.05$). AEBSF treatment reduced the proteolytic activity in NALF in Pre-S, Pre-C and steroid groups (all, $P < 0.05$).

Conclusion: Prophylactic and therapeutic treatment with serine protease inhibitor attenuates the airway inflammation in mouse model of AR and have potential for adjunct therapy. In both pre-S and pre-C groups, AEBSF suppressed the allergen-specific T helper 2 response and induced regulatory T cells in a murine model of AR.

Poster Session 32

Allergic rhinitis in clinical practice

941

Awareness of general practitioners of the current recommended management guidelines for allergic rhinitis

Okpala, NCE
Otolaryngology (ENT), Frimley Park Hospital, Camberley, United Kingdom

Background: Allergic rhinitis is reported to be a growing part of General Practitioners' (GPs) consultations. Recently, the international guidelines and consensus statements about management of allergic rhinitis was developed to enhance the effectiveness and quality in managing cases reported in patients. High level of dissatisfaction with the treatment of allergic rhinitis amongst patients as well as poor compliance with medications has been reported. The aim of this study is to investigate GPs' awareness of the current recommended management guidelines for allergic rhinitis.
Method: Cross-sectional questionnaire approach was used. The questionnaires were piloted prior to the main survey to ensure appropriateness of questionnaire to the targeted audience. There was a focus group discussion involving GPs.
Results: The research is still on-going and almost completed. Results so far show that most GPs are not aware of the current recommended management guidelines. They are aware of some of the treatment. A substantial number of GPs are not aware of the link with asthma.
Conclusion: The results obtained will lead to better understanding of barriers to appropriate management of allergic rhinitis in general medical practice and facilitation of health planning. Continued education of GPs should be a priority in order to optimise successful management of patients.

942

Mono and poly-aeroallergen sensitisation in allergic rhinitis patients from a pre-Andes province in Argentine

Cuello, MN[1,2,3]; Teijeiro, A[1,2,3]; Jares, EJ[1,2,3]; Badelino, HA[1,2,3]; Baena-Cagnani, CE[1,2,3]
[1]Respiratory Departament, Pediatric Hospital, Cordoba, Argentina; [2]CIMER, Faculty of Medicine, Catholic University, Cordoba, Argentina; [3]Fundacion LIBRA, Buenos Aires, Argentina

Background: Poly-sensitisation is common in patients with allergic rhinitis (AR) and the frequency of mono-sensitisation is not yet known. There are few studies about sensitisation in AR patients in San Juan province, Argentine, close to the Andes mountain range with continental, dry climate. This work sought for the frequency of poly and mono-sensitised patient with AR.
Method: This observational cross-sectional study assessed the allergy sensitisation in a group of consecutive patients with persistent AR. Skin prick testing (SPT) with commercial extracts (Allergofarma Argentine) was performed. Total IgE was also measured (RIE).
Results: Three hundred and seventy patients with AR were evaluated, 57% of them were female, mean age 43 years. 10.3% of the patients were mono-sensitised. +(ve) SPT to grass allergens were the most prevalent in this group (81%). Other prevalent allergens were oriental plane 57%, blackbery 56% and olive 53%. In the group younger than 13 years old poli-sensitisation was found in 24.3%. In contrast, in the 13 or more years old group, 75.69% were poli-sensitised (OR: 1.81, $P = 0.09$). Increased IgE (+2 SD) was found in 71% of patients. This feature was found in 10.3% of mono-sensitised patients and 89.7% of poli-sensitised patients.
Conclusion: Persistent AR patients were sensitised mainly to grasses and Oriental plane. Poli-sensitisation is more frequent in all the age groups and is more prevalent in adult patients. The prevalence of mono-sensitisation is the double in children compared to adults. This can have a potential impact on the potential use of specific immunotherapy.

944

Profile of patients with severe respiratory allergies to house dust mite allergens: a survey in three European countries

Demoly, P[1]; Rossi, O[2]; Matucci, A[3]; David, M[4]; Polderman, C[4]; Vidal, C[5]
[1]University Hospital of Montpellier, Montpellier, France; [2]University of Florence, Policlinico di Careggi, Florence, Italy; [3]Immunoallergology Unit, Department of Biomedicine, Azienda Ospedaliero-Universitaria Careggi, Firenze, Italy; [4]Stallergenes, Antony, France; [5]Department of Allergy, Hospital Clinico Universitario, Santiago de Compostela, Spain

Background: The objective was to assess adult patients profiles of with severe, physician-diagnosed HDM respiratory allergy via a survey in three European countries.
Method: Three hundred and thirteen participants ≥18 years were recruited from the general population in France (FR $n = 92$), Italy (I $n = 114$) and Spain (SP $n = 107$). All participants had a specialist-diagnosed respiratory allergy to HDMs and severe symptoms not adequatly controlled by symptomatics and not treated by allergen immunotherapy (AIT). The participants filled out a questionnaire at inclusion. The survey complies with the EphMRA Pharmaceutical Market Research Code of Conduct and national guidelines.
Results: The participants had been suffering from HDM allergy for an average of 16.7 years. Overall, 73% of the participants had been diagnosed with respiratory allergies to other allergens (61–79%) – predominantly grass pollen. The patients consulted a general practitioner (GP) three times per year in average and a specialist at least twice. Strikingly, 40% had consulted a GP and two specialists or more, suggesting different attempts to find adequate relief. The most troublesome symptoms were sneezing (71%), blocked nose (71%) or runny nose (63.3%) and breathing difficulties (45%). Forty-nine percent stated that their symptoms bothered them generally during Spring (April–June) and Autumn (October–November). Twenty-seven percent reported being bothered year-round. Interestingly, the spring peak was also observed in the patients who were not codiagnosed to grasses. In France, the patients' disease state appeared to fluctuate less throughout the year. The main comorbidities were headache (67%) and low energy (71%). A high proportion of patients suffered from two or more concomitant comorbidities (>73%). The main impacts on QoL were reported on daily activities such as social activities, sports, housework (62%) and sleep (54%). Overall, 80% of the patient stated that they were resigned to coping with HDM allergy by adapting their behaviour to the disease. Seventy-five percent of patients regularly took combination therapies (two or more types of symptomatics). Year-round medication use was especially prominent for nasal corticoids (10%), bronchodilators (11%) and corticoid-bronchodilator combinations (6%).

Conclusion: Patients with HDM severe respiratory allergies experience troublesome symptoms and reduced quality of life throughout the year, with peaks in spring and early autumn. The majority are resigned to coping with the disease.

945
Symptom profiles of a population with grass-pollen-induced allergic rhinoconjunctivitis in France

Devillier, P[1]; Doussot, G[2]; Robin, B[3]
[1]UPRES EA 220, Hôpital Foch, Université Versailles Saint-Quentin, Suresnes, France; [2]Universal medica, Saint-Cloud, France; [3]Stallergenes, Antony, France

Background: Grass pollen allergy is the most frequent allergy in several European countries.
Method: We profiled 806 patients with grass pollen allergic rhinoconjunctivitis (AR) in a multicentre, observational study performed in France between April and July 2011. The study population consisted of 303 adults (aged ≥18), 250 adolescents (aged 12–17) and 253 children (aged 5–11).
Results: The mean ± SD time since disease onset was 12.5 ± 9.1 years in adults, 5.7 ± 3.1 years in adolescents and 3.4 ± 1.7 years in children. Three percent of the patients had a history of nasal polyposis and 10% had anosmia. AR was associated with atopic dermatitis, eczema and food allergies in respectively 18%, 14% and 10% of the study participants. Seventy-seven percent of the participants were polysensitised; the major allergens other than grass pollen were tree pollen (53%), house dust mite (HDM) (53%) and cat dander (28%). The most frequent co-sensitisations were grass pollen and tree pollen (19% of participants) and grass pollen with HDM (18%). Eighty-four percent of the patients had persistent, moderate-to-severe AR. By age class, the two most bothersome symptoms were rhinorrhoea and sneezing in adults (in 35% and 21% of cases, respectively), rhinorrhoea and nasal congestion in adolescents (30% and 24%, respectively) and itchy eyes and nasal congestion in children (35% and 25%, respectively). Baseline Rhinoconjunctivitis Quality of Life Questionnaire scores were 2.72 ± 1.0, 2.53 ± 1.0 and 4.9 ± 1.0 for adults, adolescents and children, respectively. The most frequently taken symptomatic treatments were oral antihistamines (77%), nasal corticoids (30%), cromone eye drops (20%) and local antihistamines (10%).
Conclusion: In a French study of mainly moderate-to-severe grass pollen AR, the most bothersome symptoms (and thus the ones most likely to contribute to a deterioration in quality of life) were rhinorrhoea in adults and adolescents and itchy eyes in children.

947
Nasal challenge test in assessing the significance of allergic factors in the group of patients diagnosed with allergic rhinitis

Krzych-Falta, E; Samolinski, B
Department of Prevention of Environmental Hazards and Allergology, Medical University, Warsaw, Poland

Background: Nasal challenge test in assessing the significance of allergic factors in the group of patients diagnosed with allergic rhinitis.
Method: The study was conducted within a group of 60 subjects, including 30 patients diagnosed with allergy to common environmental allergens. The method used in the study was a nasal provocation test with an allergen, with a standard dose of a control solution and an allergen. The reaction in the area of the activity of the allergen was assessed in two phases: the early phase and the late phase of the allergic reaction, using selected assessment techniques: VAS acoustic and optical rhinometry, the concentration of nitrogen oxide in the air exhaled from the upper airways and the concentration of tryptase and eosinophil cationic protein in nasal lavage fluid.
Results and conclusion: After contact with the mucous membrane of the nasal cavity, the allergen caused a number of events affecting the early and late phases of the allergic reaction. The beginning of the reaction was recorded in the 3.15th minute of the test on an optical rhinometry curve, accompanied by nasal itching ($P = 0.042$) with an increased number of sneezes. In the 10th minute of the test, nasal secretion increased and nasal obstruction was increasingly intense and remained at a high level during the early phase of the allergic reaction. Increased reactivity of the mucous membrane and a change, over time, of the minimal cross-sectional area of the nasal cavity in the head of the inferior nasal concha (MCA-2) were observed in the 15th minute within the groups of patients with allergic rhinitis. The intensifying nasal obstruction symptom was accompanied by a reduction in the concentration of nitrogen oxide in the air exhaled from the upper airways (from 1253.42 to 927.83 ppB) with an increase in the concentration of tryptase (2.39 μg/l) in nasal lavage fluid. As regards non-nasal symptoms, coughing was observed especially among the chronic rihinit patients in the 15th minute ($P = 0.044$) and the 20th minute ($P = 0.040$) of the test. The only objective measure of the late phase of the allergic reaction was the concentration of eosinophil cationic protein (ECP), with its fourfold increase to 8.0 μg/l observed within the group of patients with chronic rhinitis. In the late phase of the allergic reaction, increased concentration was observed of nitrogen oxide in the air exhaled from the upper airways to the threshold level of 25.17 ppB within the allergic rhinitis group.

949
Perceptions and experience of tree pollen allergy in the general public in four European countries

Brehler, R[1]; Tauleigne, L[2]; David, M[3]; Lemonnier, L[3]
[1]University Clinic of Dermatology, Münster, Germany; [2]LT Insight, Paris, France; [3]Stallergenes, Antony, France

Background: We aimed at describing the general public's perceptions and experience of tree pollen allergy via an online survey in four European countries.
Method: In Austria, France, Germany, and Spain, a sample of approximately 1250 people aged 18 and older was surveyed online in March or April 2012. Hence, a total of 5016 people answered an anonymous, 29-question online questionnaire in their local language on their perceptions and personal experience of tree pollen allergy. The survey procedures complied with the ESOMAR International Code of Marketing and Social Research Practice.
Results: Overall, 54% of the respondees stated that they experienced repetitive sneezing, nasal discharge, stuffy nose, eye irritation and breathing difficulties (i.e. symptoms potentially compatible with tree pollen allergy) for some or all of the year. Although 55% of the respondents considered that these symptoms were primarily associated with low and changing temperatures, 31% felt that tree pollen allergy could indeed be responsible. Even though 84% of the respondees stated that tree pollen allergy should be diagnosed by a specialist, only 15% had been diagnosed. Twenty-five percent of the respondents considered that tree pollen was the main cause of AR; indeed, tree pollen was ranked third after house dust mites (36%) and grass pollen (26%). Among this study, 78% of people allergic to tree pollens are pollyallergic. Even though 82% of the respondents believed that tree pollen allergy has a strongly negative impact on health status and quality of life, 69% considered that sufferers had to resign themselves to coping with the disease in everyday life. We also noted that 81% of respondents agreed that tree pollen allergy penalizes children at school. Sixty percent

of the respondents considered that they were very or fairly well informed about the symptoms of tree pollen allergy and 58% about the allergy's seasonality. However, the survey results revealed poor knowledge of available treatments for tree pollen allergy; even though 84% of the respondents considered that tree pollen allergy can lead to asthma and requires a consultation with a specialist.

Conclusion: The survey results clearly revealed a lack of accurate information on AR among the general public. Most respondents were not aware of existing therapies. More than half of the respondents used over-the-counter medication to treat their disease and thus may not be receiving optimal treatment for a condition that can severely impair quality of life.

950
Efficacy of saline nasal irrigation in children with allergic rhinitis

Chairattananon, C; Pensrichon, R; Veskitkul, J; Pacharn, P; Piboonpocanun, O; Visitsunthorn, N; Vichyanond, P
Division of Allergy & Immunology, Department of Pediatrics, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Background: Saline nasal irrigation is commonly used in treating allergic rhinitis in children. Its efficacy in such condition is not clear. We aim for comparing the use of saline nasal irrigation in the treatment of allergic rhinitis in children.

Methods: Thirty children with allergic rhinitis (age 5–15 year) with inclusion criteria positive skin prick test to aeroallergens, combined daily total nasal symptom score and medication score (CSM) between 3 and 9 per day, absence of use of intranasal steroids or nasal irrigation for at least 1 month prior to entry, were enrolled. They were randomly allocated into saline nasal irrigation (90 cc/side twice daily) and control groups. Irrigation was done via a new device (Hashi Nasal Rinser). Patients were prescribed cetirizine and pseudoephedrine to be used as needed. The duration of study was 8 weeks. Primary outcome was the comparison of CSM. The Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) was evaluated at week 2, 4, 8. Visual analog scale (VAS) was recorded weekly by the patients.

Result: The patients were enrolled in two groups (saline 17, control 13). Their mean ages were 9.4 ± 2.8 years. Demographic data and baseline CSM were comparable between groups. Both groups showed decrease CSM after enrollment, but significant decrease from baseline was observed only the saline group at week 4, 5, 7 ($P < 0.05$). Between group comparison of CSM showed significant difference at week 3 (saline better than control group, $P < 0.05$). No difference in VAS and RQLQ were observed between groups ($P > 0.05$). However, in saline group, VAS improved significantly at weeks 4, 5, 7 while RQLQ were better at weeks 2, 4, 8 when compared to baseline.

Conclusion: Saline nasal irrigation had some benefit in the reduction of CSM, RQLQ and improvement of VAS during this 8 weeks study.

951
Assessment of nasal responses is more accurate than skin testing in allergic rhinitis

Campo, P[1]; Rondón, C[1]; Salas, M[1]; Blanca-Lopez, N[2]; Guerrero, MA[1]; Herrera, R[3]; Blazquez, AB[3]; Galindo, L[3]; Osorio, M[3]; Canto, G[2]; Blanca, M[1]
[1]Allergy Unit, Complejo Hospitalario de Malaga, Malaga, Spain; [2]Allergy Service, Infanta Leonor Hospital, Madrid, Spain; [3]Fundacion IMABIS-Complejo Hospitalario de Malaga, Malaga, Spain

Background: The main objective of this study was to evaluate the accuracy of nasal allergen provocation test (NAPT) in the detection of clinically relevant aeroallergens in perennial allergic rhinitis patients with skin prick test (SPT) positivity to seasonal pollens.

Method: Nineteen patients with clinical history of perennial rhinitis for more than 2 years and positive SPT to seasonal pollens were included. Clinical questionnaire, SPT, serum total and specific IgE to aeroallergens and NAPT were performed. Response to NAPT was monitorized by nasal-ocular symptoms, changes in nasal patency evaluated by acoustic rhinometry, and determination of specific IgE, and inflammatory mediators (tryptase and ECP) in nasal secretions. The study was approved by the local ethics committees. All participants were informed and signed the informed consent.

Results: Patients referred a worsening of symptoms during spring in 26% and after natural exposure to house dust in 52.6% of cases. SPT was positive to olive and/or grass pollen in 68% and 37% of patients respectively. SPT and serum specific IgE were negative to perennial allergens (house dust mite, molds, and dander epithelia) in all cases. However NAPT induced a nasal allergic response to D. Pteroyssinus in 94.7%, and to Alternaria alternata in 63.2% of patients.

Conclusion: SPT and serum specific IgE can not be sufficient to detect relevant aeroallergens implicated in perennial allergic rhinitis despite the positive skin responses to seasonal aeroallergens.

952
How diverse is the nasal patency in the Polish population in the light of ECAP (Epidemiology of Allergic Disorders in Poland) multi-centre study?

Krzych-Falta, E; Samolinski, B
Department of Prevention of Environmental Hazards and Allergology, Medical University, Warsaw, Poland

Background: The aim of the study was to evaluate the usefulness of PNIF in assessing nasal airway patency based on test results.

Method: The sample in the study was a group of 4674 subjects, including 1291 people aged 6–7 years (woman 643, men 648), 1293 people aged 13–14 years (woman 625, men 668) and 2090 adults (woman 1284, men 806). The research method employed in the study was the measurement of peak nasal inspiratory flow using a peak flow meter with a suitable mask as used in rhinomanometry tests and with a flow rate ranging from 20 to 350 l/min. The study was conducted in 2006–2008 at the following centres: Katowice, Wroclaw, Krakow, Lublin, Warszawa, Bydgoszcz, Gdansk and in the rural areas of the former province of Zamosc

Results: For the purposes of the study, the average values for the subjects were calculated for a number of criteria:

Subject age: The average PNIF value was 52.4 l/min for subjects aged 6–7 years ($n = 1291$), 94.7 l/min for subjects aged 13–14 ($n = 1293$) and 108.0 l/min for the adults ($n = 2090$). Indeed statistical dependences for all aged groups were observed on level $P < 0.0005$.

Diagnosis: The average PNIF value for healthy was 52.3 l/min, $P = 0.338$ for subjects aged 6–7 years ($n = 680$), 97.3 l/min, $P = 0.279$ for subjects aged 13–14 ($n = 640$) and 111.7 l/min $P = 0.438$ for the adults ($n = 1035$) and for allergic rhinitis PNIF value was 50.4 l/min $P = 0.028$ for subjects aged 6–7 years ($n = 310$), 93.3 l/min, $P = 0.299$ for subjects aged 13–14 ($n = 389$) and 107.7 l/min $P = 0.276$ for the adults ($n = 623$) and asthma PNIF value was 51.6/min for subjects aged 6–7 years ($n = 149$) 87.3 l/min $P = 0.062$ for subjects aged 13–14 ($n = 145$) $P = 0.097$ and 105.3 l/min $P = 0.13$ for the adults ($n = 198$)

Exposure to tobacco smoke (adults): passive smoking – 105.3 l/min ($n = 1202$) $P = 0.017$, active smoking-119.1 l/min ($n = 885$) $P = 0.108$.

Conclusion: PINF is important investigative tool thanks which we can: to differentiate in dependence the functional state of nose from: put the recognition (the patients with allergic rhinitis, the bronchial asthma), the age and the studied sexes.

953
Allergic rhinitis and otitis media with effusion in children

Manole, FI
Faculty of Medicine, University of Oradea, ENT, Oradea, Romania

Background: Otitis media with effusion appear frequently in early childhood related to disorder of ventilation and drainage of the middle ear and is mainly a disorder opening mechanism of the Eustachian tube. Allergic rhinitis contribute to this pathology in different period and still is controversial with no prospective studies to evaluate the atopy in otic disease. The purpose of the study is to evaluate the reccurence symptoms of otitis media with effusion in children with allergic rhinitis.
Method: The group study is made up of 76 patients aged between 4 and 7 years with allergic rhinitis and otitis media with effusion, examined and treated in the Pediatric Hospital of Oradea in period 2011 and 2012. From all of this we exclude the patients with gross septal deviation, cistic fybrosis and another abnormality. The objective endocavitary E.N.T. examination was systematically performed on all patients: anterior rhinoscopy, posterior rhinoscopy otoscopy. Evaluation criteria: degree of hearing loss, serous rhinorrhea, nasal obstruction and sneezing. Specific Ig E was measured and correlated with allergic syptoms. Children were treated according with standard medical care and followed up for 9 months for recurrence. The hearing loss was evaluated by audiometry and pfonic acumetry.
Results: The results were established by clinical examination of nasal mucoasa and otoscopic appearance of tympanic membraine. Decrease of rhinorrhea and nasal congestion was well corelated with increase hearing acuity demonstrated by phonic acumetry.
Conclusion: We conclude that allergic rhinitis can increase the risk for reccurent otitis media with effusion in chidrens because of the Eustachian tube oedema and waterry rhinorrhea. More accentuated hearing loss in period of allergic exacerbation demostrate clinically this observation. Another study will be necessary to establish a correlation.

954
Allergic and non allergic rhinitis in children: the role of nasal cytology

Macchi, A[1]; Provero, MC[2]; Antoniazza, S[2]; Antoniazza, M[2]; Nespoli, L[2]
[1]Università degli Studi dell'Insubria, Clinica Otorinolaringoiatria, Varese, Italy; [2]Otorhinolaringology Clinci University of Insubria, Varese, Italy

Background: Nasal cytology is a diagnostic tool currently used in rhinology to study either allergic and vasomotor rhinological disorders or infectious and inflammatory rhinitis. Until the development of this method, there was a significant diagnostic problem in patients with NAR (non-allergic rhinitis), now solved by nasal cytology examination. In particular, it provided an important contribute to the definition and understanding of the pathophysiologic mechanism of allergic and non-allergic rhinitis and to the identification of new pathological entities. The advantages of nasal cytology are different: the ease of performance, the non – invasiveness allowing repetition and the low cost. It is useful to follow the disease during medical treatment by periodic cytologic controls, showing, for example, a significant reduction of inflammatory cells or the disappearance of bacteria. It's a simple and safe technique, which can be performed in outpatient setting and it is particularly feasible for application in children.
Method and results: We evaluated 100 children from 2 to 15 years old. Only 59/100 subjects were classified as affected by AR after skin prick test or RAST.

According to ARIA guidelines, the 59 children with AR were divided in 56 with persistent AR and just three with an intermittent form. 26/56 children had mostly seasonal symptoms associated to the prevailing allergy to grass pollen, while 30/56 children were allergic to housedust mites and molds and showed perennial symptoms.

17/59 children were monosensitised to perennial allergens, 12/59 were sensitised to seasonal allergens and 2/59 children were allergic to Betula v. and Corylus a. The other 28/59 children were poliallergic patients.

10/59 children with AR were under sublingual-specific immunotherapy (SLIT).

9/59 children with AR had a significant number of neutrophilis and eosinophilis at the nasal citology, documenting the presence of 'minimal persistent inflammation'.

10/59 AR patients showed a positive swab for bacteria.

Children with NAR were 32/100. After nasal cytology, 16/32 children were classified as patients affected by NARES, 1/32 as a child with NARESMA (non-allergic rhinitis with eosinophils and mast cell) and another 1/32 as NARMA (non-allergic rhinitis with mast cell).

One child with X-linked agammaglobulinemia presented nasal eosinophilia.
Conclusion: In conclusion, nasal citology allowed us to correctly classify children with NAR, and was also was able to better assess the condition of children with AR.

955
Electronic patient acquisition tablet demonstrates a high level of user acceptability and accommodation by patients with allergic rhinitis studied in in environmental exposure chamber

Nandkeshore, H[1]; Recker, S[1]; Patel, P[1]; Salapatek, AM[2]
[1]Inflamax Research Inc., Mississauga, Canada; [2]Inflamax Research Inc., President & CSO, Mississauga, Canada

Background: Patient Reported Outcomes (PRO) such as Total Nasal Symptom Scores (TNSS) are accepted and primary outcome endpoints in many allergy trials. We developed an electronic patient acquisition tablet (ePDAT) system to collect TNSS during allergen challenges in an environmental exposure chamber (EEC). The goal of this study was to compare the user acceptability and ability to accommodate a switch between paper and electronic diary card formats.
Method: Eight ragweed allergic patients with positive skin prick test (3 mm greater than negative control) and medical history were exposed to airborne ragweed pollen at 3500 ± 500 grains/m^3 in an EEC for 4 h. To assess patients ability to accommodate ePDAT, half of the patients (4) were asked to complete their diary cards for the first 2 h on paper format and then between the 2 and 2.5 h were asked to switch to ePDAT, the other half of the patients used ePDAT first then switched to paper for the remaining 2 h. Patients were also asked questions on their acceptability of ePDAT compared to paper.
Results: Patients who were assigned paper first then switched to ePDAT showed the same or better accommodation of the change in format than patients provided ePDAT first. In either case, there was little change in the TNSS level in the first time point post-switch and most patients returned to their pre-switch levels. Post study, the majority of patients (5/8) reported that ePDAT was easier

1 to use,
2 to select an answer, and
3 to read.

With regard to ePDAT Graphical User Interface (GUI) and physical characteristics, 100% reported:

1 Print size was good,
2 Background screen lighting did not interfere with reading,
3 Tablet size and weight was convenient for completing diary cards.

Conclusion: This pilot study indicates that patients accommodate a switch to ePDAT well. Subjects also indicate a high level of usability of the ePDAT GUI. Further studies are required to assess the usability preferences of the ePDAT system for at-home use. These data indicate that the ePDAT is suitable for TNSS collection in allergy trials performed in an EEC.

956
Nasal blockage induced by oral administration of non-steroidal antiinflammatory drugs in a guinea-pig model of allergic rhinitis

Terada, T[1]; Nabe, T[2]; Kawata, R[1]; NA-TEAM Study Group
[1]Otolaryngology, Osaka Medical College, Takatsuki, Japan; [2]Pharmacology, Kyoto Pharmaceutical, Yamashina, Japan

Background: When a certain population of asthmatic patients took aspirin, nasal blockage and hypersecretion were induced within 1 h after the medication, followed by the development of asthmatic airway obstruction. This drug-induced, harmful phenomenon is termed aspirin-induced asthma/rhinitis (AIAR) or aspirin hypersensitivity, in which airway obstructive responses are caused in the lower and upper airways after oral dosing with non-steroidal antiinflammatory drugs (NSAIDs) such as aspirin and indomethacin.
Method: To elucidate the mechanisms underlying nasal symptoms in patients with aspirin hypersensitivity, we evaluated the effects of orally administered non-steroidal anti-inflammatory drugs (NSAIDs) on the nasal patency of guinea pigs with cedar pollen-induced chronic allergic rhinitis.
Results: Indomethacin (10 mg/kg) administered 1 h before a pollen challenge amplified the antigen-induced nasal blockage. More interestingly, even in the absence of the pollen challenge, indomethacin induced nasal blockage at 30 min at 4 h after administration. However, indomethacin-induced nasal blockage was not provoked in non-sensitised animals. Another NSAID, diclofenac (30 mg/kg), also evoked nasal blockage, but unexpectedly, aspirin (500 mg/kg) did not affect nasal patency. Indomethacin-induced nasal blockage was unaffected by a cysteinyl leukotriene receptor (CysLT1 receptor) antagonist, pranlukast (30 mg/kg, p.o.), or by prostaglandin E2 (10^{-3} M, intranasal), suggesting that the nasal blockage may not be due to hyperproduction of cysteinyl leukotrienes or inhibition of prostaglandin E2 production.
Conclusion: In conclusion, both indomethacin and diclofenac induced nasal blockage in the sensitised, repeatedly challenged guinea pigs after oral administration, whereas aspirin did not affect the nasal patency. Because the indomethacin-induced nasal blockage was not induced in non-sensitised guinea pigs, chronic inflammation of the nasal mucosa may be required for the nasal blockage. In addition, the indomethacin-induced nasal blockage was not mediated by CysLTs. Studies of NSAID-induced nasal blockage may help to elucidate the mechanisms responsible for AIAR.

957
Effects of treated and untreated allergic rhinitis on actual driving and memory: an experimental patient study

Vuurman, E[1]; Vuurman, L[2]; Lutgens, I[3]; Kremer, B[4]
[1]Neuropsychology & Psychopharmacology, Maastricht University, Maastricht, The Netherlands; [2]Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands; [3]Otorhinolaryngology, Orbis Medical Centre, Sittard, The Netherlands; [4]Otorhinolaryngology, Maastricht University Medical Centre, Maastricht, The Netherlands

Background: Allergic rhinitis (AR), also known as hay fever, affects up to 30% of the adult population. While symptomatic, patients usually continue to engage in daily life activities, including car driving. Previous studies have shown that AR can impair cognitive functions, especially during longer lasting tasks and it has been identified as the cause of at least one fatal car accident. Treatment of AR is often done with drugs that might also induce somnolence and impair driving performance. This impairment is however exclusively based on studies with healthy volunteers. Not much is known on the (combined) effects of AR and drug treatment on driving performance of patients.
Aims: Primary objective was to determine the effect of AR per se on actual driving performance and compare it to the effects of AR treated with two types of drugs. Secondary objective was to determine the effects of treated and untreated AR on memory performance.
Methods: Nineteen patients with documented AR history engaged in a 1-h on the road driving test outside the pollen season. In a 4-leg repeated measures design patients underwent a nasal provocation test with a pollen mixture to provoke AR symptoms or a placebo sham provocation. In the three conditions with pollen provocation patients were pre-treated with either cetirizine 10 mg, fluticasone furoate 27.5 μg or placebo to alleviate the provoked AR symptoms.
Results: The driving performance of patients when symptomatic and not treated with medication was significantly impaired compared to their non-symptomatic performance in he placebo condition. When engaging in a secondary memory task during the driving task their performance deteriorated further and impairment was comparable to that seen at a blood alcohol level of 0.05%, the legal limit for driving in most countries. Treatment of the AR symptoms with drugs only partially counteracted the effect of AR on driving.
Conclusions: Untreated allergic rhinitis can seriously impair driving performance and put AR patients at increased risk, especially when engaging in other activities requiring attention during driving. Patients with AR should be cautioned that their condition can cause impairment. They should be advised that should therefore always be treated if possible.

Poster Session 33

Treatment of asthma and related diseases: from inhaled corticosteroids to monoclonal antibodies

964
Combination of highly potent ICS and effective device improve asthma control in persistent asthma patients

Iashyna, L; Feshchenko, Y; Polianska, M; Moskalenko, S; Ishchuk, S; Ignatieva, V
State Organization 'National Institute of Physiology and Pulmonology named after F.G. Yanovsky National Academy of Medical Sciences of Ukraine', Kiev, Ukraine

Aim of the investigation: To study effectiveness of budesonide (Novolizer) in monotherapy in persistent bronchial asthma patients. Study was carried out due to budget.

Materials and methods: Forty BA patients, FEV1 (83.1 ± 3.1)% pred.: 20 'steroid-naïve' (mean ACT 15.7), 20 – received ICS at the moment of enrolment ('steroid') (mean ACT 16.1); female 28, male 12 in the age (50.7 ± 2.4) years, duration of the disease (9.5 ± 0.9) years received budesonide (Tafen Novolizer) in daily doses 400–800 μg (according current control) – in steroid-naïve – 200 μg BID, in steroid patients – eight patients 400 μg BID and 12 patients – 200 μg BID in basic monotherapy during 3 months.

ACT was investigated at baseline, after 1 and 3 months; ACQ at baseline, after 1, 2, 4 weeks and after 3 months.

Results: Asthma control (ACT) significantly ($P < 0.05$) improved after 4 weeks and after 3 months there were no patients with uncontrolled asthma. In 'steriod-naïve' after 4 weeks 45% had score 20–24, after 3 months – 80%. In 'steroid' group after 4 weeks 55% had score 20–24, after 3 months – 70% and 15% fully controled BA.

ACQ score initially was 2 – in 'steriod-naïve', 2.25 – in 'steriod' patients. After 4 weeks it was 1.25 and 1.2 accordingly and after 3 months in all parients −1.

Conclusion: Budesonide Novolizer in monotherapy in persistent bronchial asthma patients improves asthma control (because of better delivery of the highly potent drug).

965
Asthma and steroid allergy

Pestana, ML; Aguiar, R; Mendes, AM; Pedro, E; Barbosa, MP
Serviço de Imunoalergologia, Centro Hospitalar Lisboa Norte, Hospital Santa Maria – CHLN, Lisboa, Portugal

Background: Patients with severe asthma often represent a challenge for physicians and a patient with severe asthma and steroid allergy can be a real problem.

Method: The authors present the case of a female patient with 32 years old with severe asthma and steroid allergy successfully treated with omalizumab.

Results: Since childhood she had rhinitis, severe asthma and allergy to house dust mites. At the age of 14 she was started on specific immunotherapy but it had to be suspended due to worsening of dyspnoea. Despite medical therapy with inhaled and topical corticosteroids, cetirizine and salbutamol as rescue medication, she had clinical worsening with >5 visits/year to the Emergency Department (ER). In one of the visits, at the age of 19, about 15 min after i.v. hydrocortisone she had hypotension, dyspnoea and glottis edema. A few months later she had an identical reaction after i.v. methylprednisolone. At the age of 25 she has also noted dyspnoea with inhaled corticosteroids (fluticasone, beclomethasone and budesonide) 10–30 min after the administration. She was then treated with formoterol bid, theophylline, montelukast and hydroxyzine. At age 29 she began clinical evaluation in our Immunoallergology Day Care Unit. She maintained wheezing on pulmonary auscultation and the ACT (Asthma Control Test) had a score of 7 (controlled asthma >20). Spirometric parameters revealed a FEV1 53% of the predicted which improved 33% after salbutamol. Intradermal tests with hydrocortisone, methylprednisolone, prednisolone and dexamethasone were positive. She underwent provocation test with inhaled fluticasone and budesonide with a decreased of FEV1 15 min after administration (>15%). The provocation test with beclomethasone was negative and this inhaled steroid was added to the previous medication. Despite the optimised therapy the patient showed no clinical improvement and monoclonal anti-IgE (omalizumab) 300 mg every 2 weeks was then proposed.

The clinical assessment after 16 weeks showed a decrease of the exacerbations and of rescue medication and the ACT score increased to 14. After 2 years of treatment she had an important improvement of nasal and bronchial complaints, no visits to the ER department, an ACT score of 21 and no adverse reactions due to omalizumab.

Conclusion: Omalizumab proved to be a safe and effective alternative therapy in this case of severe asthma and allergy to steroids.

966
Efficacy of omalizumab treatment in allergic bronchopulmonary aspergillosis

Martorell, C; Morales, C; Raducan, I; Cadavid, S; Sastre, A; Peláez, A
Allergy Service, Hospital Clínico Universitario, Valencia, Spain

Background: Here we present our experience of applying omalizumab to a patient with allergic bronchopulmonary aspergillosis (ABPA).

Method: A male 31-year-old patient with rhinoconjunctivitis and allergic bronchial asthma since childhood. The patient was first visited in 2004 for coughing episodes, high temperature, thick expectoration and wheezing dyspnoea attacks which had been treated with parenteral corticoids and antibiotics. After an allergy study he was diagnosed with ABPA (*Aspergillus fumigatus* skin prick test: positive, serum specific IgE to *A. fumigatus* 8 kU/l, serum specific IgG to *A. fumigatus* >200 mgA/l, serum total IgE 3500 kU/l, *A. fumigatus precipitins*: positive, *Aspergillus* detection in sputum, thoracic CT scan: mucus plugs, bronchiectases and middle lobe collapse). We initiated treatment with oral corticoids and inhaled corticoids + long-acting bronchodilators achieving good response and normalisation of the pulmonary function. From January, 2004 to June, 2011 he attended periodic check-ups at our allergy department during which symptoms,

pulmonary function, total serum IgE (UniCAP, ThermoScientific) along with other parameters were evaluated. Throughout these years the patient followed daily treatment with oral prednisone (average dose: 20 mg/day) manifesting flare-ups whenever we tried to reduce corticoids. In 2005 treatment with Intraconazol was associated unsuccessfully. After the steroid treatment side effects appeared (glaucoma, cataracts, osteoporosis). Treatment with omalizumab was initiated in June, 2011. A monthly dose was calculated on the basis of patient's weight and serum total IgE levels (0.016 mg/kU/UI): 450 mg/month. We proceeded to a progressive reduction in oral corticoids (2.5 mg/month), evaluating during periodic check-ups clinical condition, need for medication, pulmonary function and FeNO.

Results: The medication has been well tolerated. Prednisone was discontinued on the 1st of January, 2012. Patient's condition has been well controlled and showing no changes in pulmonary function (that is, mantained within normal limits) or in FeNO (range 26–30 ppb). Treatment with fluticasone 500 μg/day is maintained.

Conclusion: The treatment with omalizumab has allowed the patient's condition to be kept under control and enabled discontinuing oral corticoids administration for the time being.

968
Omalizumab treatment in brittle asthma

Skiepko, R; Zietkowski, Z; Skiepko, U; Bodzenta-Lukaszyk, A
Department of Allergology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland

Brittle asthma is one of clinical phenotypes of asthma. Two types of brittle asthma can be identified. Type I brittle asthma is characterised by more than 40% diurnal variation in PEF for more than 50% of time maintained over a period of at least 150 days despite considerable medical therapy. Type II brittle astma is characterised by sudden acute attacks occurring in <3 h on a background of apparent normal airway function or well controlled asthma.

Case 1: We describe a 46-year-old female with 30 years history of type 1 brittle asthma. Patient suffered from death-threatening asthma exacerbations 4–5 times per year. These events usually begun with fast progressive paroxysmal dyspnoea with low effect of SABA, wheezes in the chest and severe decrease in PEF. During several minutes patient percepted progressive dyspnoea, tachycardia, disturbances of consciousness and finally she lost her consciousness.

After few months of omalizumab treatment the patient achieved significant improvement in asthma control test score and symptoms, reduction in rescue medication use, significant decrease in acute asthma episodes and hospitalisations. In the following 60 months of omalizumab treatment patient did not experience severe exacerbations accompanied with loss of consciousness.

Case 2: We report the case of a 26 years old female, with 10 years history of asthma. Despite treatment with high dose of ICS and montelukast she suffered from frequent, sudden asthma attacks (2–3/month) with decrease of PEF >40%. These episodes required treatment with high doses of SABA and sometimes systemic corticosteroids. After several hours symptoms used to disappear and PEF increased.

After 16 weeks of omalizumab treatment the patient achieved significant improvement in asthma control. Over the next 16 months of treatment with omalizumab she did not suffer from asthma exacerbation episodes with PEF decrease >40% and administration of systemic corticosteroids.

As to our knowledge, it is the first publication on efficacy of omalizumab in patients with brittle asthma. Analysis of described cases suggests that omalizumab, despite so far unclear pathogenic mechanisms, could be useful in the treatment of brittle allergic asthma.

969
A case of recurrent anaphylaxis with asthma treated with anti-IgE monoclonal antibody omalizumab

Ernesti, I; Indinnimeo, L; Schiavi, L; De Vitori, V; Melengu, T; Bardanzellu, F; Duse, M
Sapienza University of Rome, Rome, Italy

Background: Anaphylaxis is a serious and life threating systemic reaction which occurs suddenly after exposure to an allergen. The most severe causes are usually IgE mediated. Omalizumab is a humanized monoclonal anti-IgE antibody that is currently FDA-approved for allergic asthma and exerts its action by binding to circulating IgE, reducing IgE receptor expression, and decreasing mediator release from mast cells and basophils. Given its mechanism of action, recent reports have suggested its possible clinical use for food allergy and some forms of anaphylaxis. Omalizumab has been reported to be effective in a few patients with idiopathic anaphylaxis and mast cell disorders.

Case presentation: We report a 18-year-old female adolescent with allergic asthma who presented frequent episodes of idiopatic anaphylaxis with abdominal pain, generalised urticaria and unconsciousness. From early childhood she showed a mild to moderate respiratory symptoms, above all during the spring, and sensitisation to Lolium. She has been treated with bronchodilators, antihistamine and corticosteroids as needed.

Past History: When 16 years old she presented the first of numerous episodes of anaphylaxis after exercise. She was hospitalised and treated with corticosteroids, bronchodilators and epinephrine. After, she started an alternative treatment option with desensitisation therapy to grass pollen, stopped, after 3 days, due to anaphylaxis.

Despite a complete antiasthmatic therapy with inhalation of corticosteroid, long-acting β(2)-aderenergic agonist (LABA) and regularly taking oral steroids, her asthma was poorly controlled and she continued to presents severe anaphylaxis episodes (mean 1–2 monthly attacks), needing PS access. Meantime was diagnosed suspected 'vocal cord dysfunction syndrome'. At admission, routine analyses were in the normal range, except for the increased total serum IgE level (594 IU/ml) and an isolated presence of specific IgE to Lolium (2.37 IU/ml). We looked for an alternative treatment option such as the anti-immunoglobulin (Ig) E monoclonal antibody omalizumab. Due to the high frequency of anaphilaxis episodes, we decided to start omalizumab at a dose of 300 mg once every 4 weeks. Currently, she has completed 6 months of treatment without any further attacks. This case suggests that omalizumab may be indicated not only to severe allergic asthma but also to prevent anaphylaxis.

971
Adherence and effectiveness of omalizumab treatment in severe allergic asthma patients

Ozseker, ZF[1]; Bulut, İ[1]; Erdenen, F[2]; Erdoğdu, D[3]; Aslı, G[3]; Ersoy, R[4]; Kutlu, A[5]; Öztürk, S[5]
[1]Immunology and Allergy, Sureyyapasa Chest Diseases and Chest Surgery Training Hospital, Istanbul, Turkey; [2]İnternal Medicine, İstanbul Training Hospital, İstanbul, Turkey; [3]Istanbul Faculty of Medicine, Immunology and Allergy, Istanbul University, Istanbul, Turkey; [4]İmmunology and Allergy, Yedikule Chest Diseases and Chest Surgery Training Hospital, İstanbul, Turkey; [5]GATA H.PASA Training Hospital, İstanbul, Turkey

Background: Asthma control requires adherence with treatment. Especially severe allergic asthma is life threatening disease if the patient is not adhere and persistent to the treatment. The purpose of this study was to evaluate the adherence to omalizumab treatment in severe allergic asthmatic patients.

Method: In this retrospectively designed study 118 adult patients' treated with omalizumab data was evaluated.
Results: 73.7% of the patients were women (mean age: 46.2 ± 12.5 years). Mean adherence rate was 86.4% (n = 102). Of all the patients were 73.5% under totally or partially control. When was compared pretreatment and after treatment asthma control test scores, annually asthma attack rates, emergency room admittances, annually hospitalisation rates, intensive care unit hospitalisations rate were significantly decreased (P = 0.000).
Conclusion: Our data shows that adherence of omalizumab treatment is high and patient's asthma attacks significantly decreased with this treatment.

972
Evaluation of omalizumab prescription for asthma patients. Adequacy to the recommendations of the regulatory agencies and asthma guidelines

de Rojas, HF[1]; González-Gregori, R[2]; Díaz-Palacios, M[1]; Pérez-Gómez, A[1]; López-Salgueiro, R[3]; Gimeno Genovés, E[3]; Nieto García, A[4]
[1]Department of Allergy, Hospital Universitari y Politecnic La Fe, Valencia, Spain; [2]Research Institute La Fe, Valencia, Spain; [3]Department of Allergy, Research Institute La Fe, Valencia, Spain; [4]Department of Children Allergy and Respiratory Diseases, Hospital Universitari y Politecnic La Fe, Valencia, Spain

Background: Omalizumab is indicated in patients with severe allergic asthma not controlled by high-dose inhaled glucocorticoids (ICS). We analyzed the adequacy of the prescription of omalizumab to the official recommendations given by the Summary of Product Characteristics (SPC) and to the most common asthma therapy guidelines.
Method: A retrospective study was performed including all cases of asthma patients (≥12 years) receiving therapy with omalizumab since its commercialization in a tertiary hospital. Data were obtained from the health record or, in case of absence of the information, by request to the responsible doctor. The parameters analyzed included: pulmonary function tests, day and night symptoms, limitation of physical activity, use of concomitant anti-asthma treatment before starting omalizumab, frequency and severity of asthma exacerbations, asthma control tests, levels of total IgE and sensitisation to perennial allergens (by skin prick test and/or specific IgE).
Results: Omalizumab was prescribed to 31 asthma patients (12 males/19 females), mean age 31.93 years (13–71). All of them showed sensitisation to perennial allergens (mites 77.42%, pollens 70.97%, epithelia 64.52% and moulds 32.26%). Mean total IgE was 634.97 kU/l (24–5000). Asthma was severe and persistent in 26 patients (83.87%). FEV1 was <80% in 19 patients (61.29%). Day symptoms were reported by 24 patients (77.41%) and night symptoms in 23 (74.19%). Before omalizumab initiation 13 patients were receiving low or moderate doses of inhaled corticosteroids. Adherence to therapy was good in 30 patients (96.77%). The number of exacerbations per year varied from 1 to 25 (average 9.63), with 27 (87.10%) patients receiving intermittent therapy with systemic corticosteroids (2–24 courses/year). The absolute adequacy to the recommendations of the SPC was 19.35%. Absolute adequacy to the NICE-2007 guidelines was present in nine cases (29.03%), to the local GEMA-2009 guidelines in 14 patients (45.16%) and to GINA-2001 in 21 patients (67.74%).
Conclusion: The profile of asthmatic patients receiving omalizumab in our center predominantly agrees with the recommendations given by the SPC. Nevertheless the absolute adequacy to the SPC and guidelines recommendations was low. Identifying and analyzing the unfulfilled recommendations may lead to improve prescription quality.

973
Anti-IgE monoclonal antibody (omalizumab) treatment increases blood glucose levels in severe persistent allergic asthma patients with type 2 diabetes

Yalcin, AD[1,2,3]; Cilli, A[4]; Gorczynski, R[5]; Bisgin, A[2,3]; Strauss, L[6]
[1]Department of Internal Medicine, Clinical Immunology and Allergy Unit, Antalya Training and Research Hospital, Antalya, Turkey; [2]Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden; [3]Department of Medical Genetics, Faculty of Medicine, Cukurova University, Adana, Turkey; [4]Akdeniz University School of Medicine, Antalya, Turkey; [5]Division of Cellular and Molecular Biology, Toronto Hospital, University Health Network, Toronto, ON, Canada; [6]Clinical Cooperation Unit Nuclear Medicine, German Cancer Research Center, Heidelberg, Germany

Background: The clinical experience during the patient follow-ups of omalizumab treated severe persistent allergic asthma patients with type-2 diabetes mellitus is introduced. Omalizumab is considered generally safe. The most common adverse reaction from omalizumab is injection- site pain and bruising but the package insert contains warnings regarding malignancies, geohelminth infections and a 'black box' warning about anaphylaxis.
Results: Moreover, additional clinical data from our experiences, we had two male patients of severe allergic asthma with type-2 diabetes mellitus at the ages of 57 and 52 and who had suffered a side-effect of increased blood glucose level that caused a need for an extra insulin injection to under control the hyperglycemia. Both patients were on the 20–25 week of omalizumab treatment within the dosage of 375 and 300 mg when they had the adverse reaction we reported here also without any other complaints. Diabetes is currently being treated with insulin lispro preparation (Humalog), 30 units before breakfast and 4 units before supper. They said they 'take a more' insulin when they note high blood glucose readings, but they have not been instructed on the use of an insulin adjustment algorithm. Their chief complaint was increasing blood glucose levels and the need for insulin after the anti-IgE treatment had started. They reported that during their round of omalizumab therapy, their postprandial blood glucose readings increased to the range of 13.9–16.7 mM despite large decreases in their carbohydrate intake. This increase occurred during the first 6 h of the omalizumab injection and never happened until the next round of omalizumab therapy and blood glucose level is stable as 5.6–6.7 mM.
Conclusion: As a conclusion the prescribing information might have been revised based on post marketing surveillance data and reported such cases indicating that different side effects may occur beyond 2 h of the injection. Patients with diabetes mellitus should be informed that such a need of insulin dose should be increased due to the possible omalizumab effect on blood glucose level.

974
Omalizumab and pregnancy: a case report

Cortese, S; Di Lizia, M; Di Bartolo, M; Cavallucci, E; Di Tommaso, L; Malandra, A; Pini, C; Di Gioacchino, M
G. d'Annunzio University, Specialization Course in Allergology and Clinical Immunology, Chieti, Italy

Background: Asthma exacerbation during pregnancy represents a risk factor for both poor maternal and fetal outcomes. Data from a large meta-analysis showed that maternal asthma was associated with increased risk of congenital malformations, low birth weight infants, intrauterine growth restriction, preterm delivery and preeclampsia. Although it is unclear whether these adverse consequences are an effect directly ascribable to asthma or secondary to its treatment, better maternal and fetal outcomes are observed with better asthma control. In general, the classical asthma medications are regarded as safe, on the contrary limited experiences are

available for new drugs, as omalizumab, despite being classified as category B by the FDA.

Methods: A female with a history of 10-year severe allergic asthma (weekly exacerbations despite medications and frequent hospitalisations for asthma) referred to our outpatient clinic. She reported in the last 7 years repeated failed attempts to get pregnant, even in the absence of gynecological diseases and genetic conditions. At present, the patient was treated by inhaled high doses of steroids and long acting beta agonists, montelukast and frequent cycles of oral steroids. Spirometry with reversibility test, total IgE, specific IgE for perennial allergens were executed.

Results: Spirometry showed a FEV1 of 68% of the predicted with 15% increase after Fenoterol 200 μg. Specific IgE were positive for mites and total IgE equal to 254 AU/ml. We added Omalizumab to the previous treatment with a marked improvement of the clinical conditions with a drastic reduction of the exacerbations and systemic steroids associated with the recovery of the quality of life, as assessed by asthma control test. During the treatment she became pregnant and after 15th weeks of pregnancy omalizumab was discontinued being asthma well controlled. The patients experienced two asthma exacerbations, requiring oral steroids, during the eighth month of pregnancy and in agreement with the gynecologist gave birth by Cesarean section. The baby was born without fetal distress and now after 16 month is in good health.

Conclusion: The described case report confirms that omalizumab is effective in treating severe asthma and its complications. The patient gave a pregnancy never achieved before. Omalizumab, administered until the 15th week, resulted in no teratogenic or other dangerous effects for the fetus.

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

Poster Session 34

The mechanisms of asthma

979
The role of molds in asthma exacerbation in desert climate

Arifhodzic, N[1]; Al Ahmad, M[1]; Alenizi, AA[1]; Paniker, R[1]; Alenezi, AA[2]
[1]Allergy Department, Ministry of Health, Al Rashed Allergy Centre, Kuwait, Kuwait; [2]Sabah Al Nasser Polyclinic, Kuwait, Kuwait

Background: Asthma exacerbations (AE) present major concern in asthmatic patients and may be related to different triggers.
Objective: To assess AE in patients sensitised to molds found in the desert environment.
Method: One thousand four hundred and fifteen randomly selected adult asthmatics, mean age 42.8 + 12.9, who visited tertiary Allergy Centre in Kuwait (January–December 2012) due to AE, were analyzed to assess the role of molds in sensitised patients. Patients with physician diagnosis of asthma on follow up in our Centre for more than 2 years, with AE that required ER treatment and who had been previously skin tested by inhalant allergens have been included in this study. Skin prick test was done with a battery of inhalant allergens, including local pollens and the most common molds (Cladosporium, Aspergilus, and/or Alternaria). In mold sensitised patients, AE were correlated with particular climate data in Kuwait, important for mold thriving.
Results: From a total of 1415 patients, 979 had a positive skin test to one or more of inhalant allergens, of which 7.3% have been sensitised to molds only. Local patients were more prone to mold sensitisation (76.6% vs 23.4%, $P < 0.001$), whereas sensitisation in females prevailed in both Kuwaitis and expatriates (64.9% vs 35.1%, $P < 0.01$). The occurrence of AE in mold sensitised patients was most frequent in January (rainy and humid weather) and October (high humidity). During extremely hot summer, there were no significant AE in this group of asthmatics.
Conclusion: Although allergic asthma is the most common asthma phenotype in Kuwait, the role of molds is less known. Sensitisation to mold is relatively low in our environment. This may be due to the fact that combination of humid and warm weather is not common and occurs usually at the end of long, dry summer (October, when the extremely high temperatures gradually subside), as well as in January, when there are some rainy days. Sensitisation to molds, together with other non-specific factors, may contribute to asthma exacerbation in allergic asthmatics.

980
Change of mean platelet volume values in patient with asthma

Park, JY[1]; Kim, YH[2]; Choi, CW[2]; Park, MJ[1]; Yoo, J-H[2]; Kang, HM[1]
[1]Kyung Hee University Hospital, Seoul, Korea; [2]Kyung Hee University Hospital at Gangdong, Seoul, Korea

Background: Mean platelet volume (MPV) is a indicator that reflects platelet function and size, and it is known that MPV is associated with inflammatory or prothrombotic condition as a marker of disease acitivity. Asthma is a kind of systemic inflammatory disease, many predictors of systemic inflammation in asthma have been investigated. The objective of the study is to evaluate MPV values in asthma and to find out the differences to disease activity.
Method: A retrospective review of medical records was performed on 130 patients who had a complete blood count (CBC) results and who were consistent with diagnosis of asthma using provocation test (methacholine or mannitol) in Kyung Hee university hospital from January, 2011 to June, 2012. MPV values were also collected from 130 age-matched healthy subjects of health screening center who had medical examinations in the same hospital during same period. We recorded their MPV values measured by automatic system using CBC samples.
Results: MPV values were 8.0 ± 0.7 and 8.2 ± 0.7 fl in patients with asthma and in healthy subjects, respectively. MPV values were significantly lower in patients with asthma than in healthy subjects ($P = 0.02$). There were no statistically significant differences of MPV values depending on the severity of asthma. The disease severity was evaluated according to PD15 (mannitol) or PC20 (methacholine).
Conclusion: We could find out the MPV values in patient with asthma were higher than healthy controls, but the exact mechanism of the differences were unclear. MPV can be affected by a variety of factors, like underlying diseases, obesity or smoking. Asthma has a variety of comorbidities, so the clear evidences about the reliability and the reproducibility of MPV for predicting disease activity in asthma are insufficient. We think further large population-based prospective studies about relationship between MPV and asthma are needed.

981
Effect of *N*-acetylcysteine on oxidative stress in human bronchial epithelial cell line (BEAS-2B)

Jang, G-C[1]; Lee, H-H[2]
[1]NHIC Ilsan Hospital, Goyang, Korea; [2]Kwandong University College of Medicine, Goyang, Korea

Background: The increasing number of epidemiologic associations between oxidant pollutant exposures and asthma exacerbation. Oxidative stress plays an important role in the pathogenesis of airway hyperresponsiveness and allergic inflammation of bronchial epithelial cells. *N*-acetylcysteine (NAC) is antioxidant which have antiinflammatory effect and anti-hyperreactivity. We evaluate the effect of NAC on oxidative stress in human lung epithelial cell line.
Method: BEAS-2B cell was cultured and stressed by H_2O_2 (1 mM/ml), ozone (2 ppm, 1 h), and Lipopolysaccharide (1 μg/ml). NAC (1 mM/ml) was pretreated 1 h before oxidant exposures. Oxidative stress was measured by DCF staining and GSH/GSSG ratio. IL-8 was measured in ozone exposed cells.
Results: NAC was protective effect in BEAS-2B cells, which was stressed by H_2O_2, ozone, and LPS. After ozone exposure, the GSH/GSSG ratio (8.3 ± 1.1) was decreased to 5.6 ± 0.1 significantly but, the cells pretreated with 1 mM NAC show the increase in GSH/GSSG ratio (9.9 ± 0.7). NAC suppressed ozone-induced IL-8 production in BEAS-2B cells also (277.8 ± 11.0 vs 157.0 ± 14.8).
Conclusion: NAC was expected to the treatment for the purpose of asthma exacerbation and airway inflammation by oxidant exposures. And further study was needed.

983
Silent gastroesophageal reflux disease may associate bronchial asthma

Xhixha, F[1]; Bakiri, A[2]; Malaj, V[1]; Hitaj, M[3]; Elezi, K[1]; Denizi, V[1]; Mingomataj, E[4]
[1]Specialties Policlinic No 3, Tirana, Albania; [2]Hygeia Hospital Tirana, Tirana, Albania; [3]Specialties Policlinic No 1, Tirana, Albania; [4]Department of Allergology & Clinical Immunology, Mother Theresa School of Medicine, Tirana, Albania

Background: Different studies report the association of bronchial asthma (BA) with gastroesophageal reflux disease (GERD). The aim of this study was the assessment of GERD symptoms and clinically silent GERD in asthmatic patients.
Method: Seventy-eight consecutive patients with moderate to severe BA were examined about GERD. Patients were und appropriate regular treatment and the asthma control test scored over 19. Patients without clinical GERD were asked to undergo gastroesophageal fibroscopy for silent GERD.
Results: Fifty-seven out of 78 patients reported about GERD symptoms. Fifty-four of them reported about pyrosis, 46 of them about regurgitation, and 33 of them non-cardiovascular retrosternal discomfort (pain). Of 21 remained patients, 16 accepted the undergoing of fibroscopy. In 11 patients was revealed sphincter incontinence and in other three patients the incontinence was associated with mild esophagitis.
Conclusion: Moderate and severe BA is generally associated with GERD, even if respective symptoms are absent. It is not excluded that anti-GERD treatment may be helpful even in asthmatics with silent GERD.

987
Relation between the forced expiratory volume in 1 s and gastro-oesophageal reflux symptoms in asthmatics

Goseva, Z; Gjorcev, A; Arsovski, Z; Arbutina, S; Janeva, E
Clinic of Pulmology and Allergology, Skopje, Macedonia

Background: It is known that there is some relation between airways obstruction and the gastro-oesophageal reflux (GER) symptoms in asthmatics. Some authors found an increased prevalence of GER symptoms in patients with asthma bronchiale.
Method: In our investigation we have examined 58 patients with asthma bronchiale divided in groups as follows: 1 g: 20 patients with mild obstruction, with forced expiratory volume FEV1 > 65%; 2 gr: 18 patients with moderate obstruction with FEV1 between 65% and 45%; 3 gr: 20 patients with severe obstruction with FEV1 < 45%. We have also a control group of 12 healthy subjects. All subjects completed their self-reported questionnaire about symptoms like: acid-regurgitation, heartburn, dysphagia, dyspnoae and chronic cough.
Results: Asthmatics with airways obstruction and significant GER symptoms had their respiratory symptoms associated with reflux events. Grater proportion of asthmatics had significant GER symptoms defined as regurgitation and dysphagia once or more per week. Asthmatics with forced expiratory volume in 1 s (FEV1) <45% showed more prevalent GER symptoms compared to those with values of FEV1 > 45%.
Conclusion: We could conclude that prevalence of gastro-oesophageal reflux symptoms is high in asthmatics. GER symptoms are more prevalent in asthmatics with severe airways obstruction when compared to less airways obstructed group and controls. We could suggest an association between the degree of airways obstruction in asthmatics and the increased rate of GER symptoms.

988
Effect of arginine metabolites on airway inflammation and bronchial hyperresponsiveness in allergic airway diseases

Lee, JY[1]; Paeng, JW[2]; Oh, MJ[3]; Lee, BJ[4]; Choi, DC[4]
[1]Center for Health Promotion, Samsung Medical Center, Sunkyunkwan University School of Medicine, Seoul, Korea; [2]Samsung Biomedical Research Institute, Seoul, Korea; [3]Bundang Jesaeng Hospital, Bundang, Korea; [4]Department of Medicine, Samsung Medical Center, Sunkyunkwan University School of Medicine, Seoul, Korea

Background: Nitric oxide (NO) imbalance plays an important role in the pathogenesis of asthma. NO is produced from L-arginine by NO synthase (NOS) competing with arginase. S-nitrosoglutathione reductase (GSNOR) is an important regulator for *S*-nitrosoglutathione (GSNO), the main source of bioavailable NO. Recent evidence supports upregulation of the NOS-2 isoform in the asthmatic airway, leading to increased production of NO. However, there is a controversy whether the upregulation of NOS, arginase, and GSNOR go bad on airway inflammation and hyperresponsiveness. The purpose of this study was to evaluate the role of enzymes involving arginine metabolites such as iNOS, arginase, and GSNOR in allergic asthma mouse model.
Methods: To evaluate the role of iNOS and arginase in allergic airway disease, we compared airway inflammation and hyperresponsiveness, arginase expression using iNOS or arginase inhibitors in 6 week-old female C57BL/6 and NOS2-knockout mice. To investigate the effect of GSNOR on airway inflammation and hyperresponsiveness in diverse mouse strains, we compared airway inflammation and hyperresponsiveness, GSNOR expression and activity in 6 week-old female A/J, BALB/c, and C57BL/6 mice. To evaluate GSNO could ameliorate airway inflammation and hyperresponsiveness, 6 week-old ovalbumin-sensitised and challenged (OVA) BALB/c mice were administered 0.3 cc of aerosol delivery of 0–10 mM GSNO.
Results: Airway inflammation and the expression of arginase I increased in NOS2-deficient mice than in wild-type mice. The inhibition of arginase attenuated airway inflammation in wild-type and NOS2-deficient mice. A/J mice showed increased airway hyperresponsiveness and GSNOR activity compared with BALB/c and C57BL/6 mice strains. GSNO inhalation significantly decreased airway hyperresponsiveness but did not affect airway inflammation in allergic asthma model.
Conclusion: We concluded that NOS2 knock-out mice are more sensitive to ovalbumin-induced airway inflammation associated with increased expression of arginase I and GSNO/GSNOR activity are important factors to control airway hyperresponsiveness.

989
Increased epidermal growth factor and vascular endothelial growth factor in nasal secretion of young children with recurrent wheeze

Chung, HL; Lee, EJ
Pediatrics, Catholic University of Taegu, Taegu, Korea

Background: Many epidemiologic studies have indicated that most children with asthma experience their first episode of wheezing during early life. A dose-response relationship has been demonstrated between the severity of virus-induced wheezing in infancy and the increased odds of developing childhood asthma. Although data are limited, inflammatory changes and remodeling typical of asthma have been reported to develop in the airways of young children with recurrent wheeze. Epidermal growth factor (EGF) is well known as a key factor of bronchial epithelial repair or airway remodeling. In this study, we examined EGF and other mediators thought to be involved in airway remodeling both in the young children with recurrent wheeze and the older children with physician-diagnosed asthma.

Method: Forty-five patients admitted with exacerbation of wheezing or asthma were enrolled. The patients were divided into two groups: young children <2 years of age with recurrent wheeze ($N = 30$) and older children >6 years of age with physician-diagnosed asthma ($N = 15$). EGF, vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β1, and resistin-like molecule (RELM)-β were measured in nasal secretion samples from the two patient groups and two age-matched controls. The levels of these mediators were standardised with total protein concentration and analyzed in relation to the clinical status of the two patient groups.

Results: EGF levels were significantly higher in the young children with recurrent wheeze compared with controls ($P < 0.01$). EGF and VEGF were significantly higher in the children with a previous history of multiple hospitalisation for severe wheezing than in those without ($P < 0.05$). The levels showed no relationship with the severity of symptoms during present admission. EGF showed significant age-related difference between the two control groups with higher levels in older children than in younger ones ($P < 0.05$). VEGF, TGF-β1, and RELM-β showed no age-related difference. EGF levels showed negative correlation with age, duration of asthma, and FEV1 in the older asthmatic children ($P < 0.01$).

Conclusion: Our study seems to suggest that early structural change might be present in the airways of the young children with recurrent wheeze, especially in those of who had frequent severe episodes of wheezing before.

990
The association between GSTP1 gene and adult asthma was modified by smoking habit

Wang, T-N[1]; Hsiao, RL[1]; Lin, M-C[2]; Huang, M-S[3]; Wu, C-C[2]; Wang, C-C[2]
[1]Department of Public Health, College of Health Science, Kaohsiung Medical University, Kaohsiung, Taiwan; [2]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan; [3]Departments of Pulmonary, Critical Care Medicine and Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan

Background: *Asthma is a global public health issue*. Previous studies have reported that a single nucleotide polymorphism (SNP) of GSTP1 is a new marker for identifying bronchial hyperresponsiveness and asthma. The aim of the present study was to explore *the association between* polymorphisms of GSTP1 gene and asthma, and to determine whether the relationship between GSTP1 gene and asthma was modified by *smoking habit*. We also investigated the association between GSTP1 genotype, lung function and IgE concentrations in adult asthma.

Methods: We recruited hospital-based asthmatic adults and age and sex-matched community controls in the present case-control study, and the controls were free from asthma, pneumonia, tuberculosis, emphysema, COPD and cancers by self-report. We selected five tagSNPs which included GSTP1 rs6591256, GSTP1 Ile^{105}/Val^{105} (rs1695), rs749174, rs1871042 and rs947895 from HapMap website. All subjects completed a questionnaire and were genotyped by using real-time PCR.

Results: After controlling for confounding factors, the smokers carried major *homozygous allele* for above five SNPs had a significantly increase risk of asthma. The haplotype analyses showed an increasing risk for the major allele (AAC) of rs6591256-rs1695-rs1871042 on asthma in ever-smokers, but not in never smokers.

Conclusion: Among ever-smokers, *major homozygous carriers* and the haplotype (AAC) of GSTP1 tagSNPs are associated with an increasing risk of asthma. The results suggested that *cigarette smoking* and GSTP1 gene may play important roles in adult asthma.

991
Relationship between anti-oxidants status, passive smoking and inhaled glucocorticotherapy in children with persistent asthma

Slavkovic Jovanovic, MR[1]; Kostic, G[2]; Stankovic, I[3]; Stamenkovic, H[4]; Cosic, V[5]; Markovic, D[6]; Jovanovic, DL[6]; Pavlovic Tokin, V[7]
[1]Department of Pulmonology, Clinic of Pediatry, Clinical Centre Nis, Nis, Serbia; [2]Clinic of Pediatry, Clinical Centre Kragujevac, Kragujevac, Serbia; [3]Clinic for Lung Disease, Clinical Center Nis, Nis, Serbia; [4]Clinic of Pediatry, Clinical Centre Nis, Nis, Serbia; [5]Center for Medical Biochemistry, Clinical Center Nis, Nis, Serbia; [6]Clinic of Dermatology and Venereology, Clinical Centre Nis, Nis, Serbia; [7]General Hospital Vrsac, Vrsac, Serbia

Background: Asthma is chronic inflammation diseas where oxidative stress (increased production of oxidants and decreased antioxidants activity) like endogenous factor has got important role in her ethipathogenesis. However, besides of that, there are many other exogenous factors which may influence to her oxidative stress, like passive smoking or glucocorticotherapy and change the nature of the disease.

The aim of our study was to evaluate relationship between anti-oxidants status, passive smoking and inhaled glucocorticotherapy in children with persistent asthma.

Method: We have investigated 80 children with persistent asthma, divided in four groups according to passive smoking and inhaled glucocorticotherapy. The antioxidative status was estimated by antioxidative enzymes: superoxide dismutase (SOD) and glutathione peroxidase (GPx). For the confirmation of passive smoking we made qualitative screening test for cotinine in human serum. In patients, who had used different inhaled glucorticomedicines, doses were expressed in equivalent micrograms dose of Budesonide. All data were analysed by comparation between groups according to passive smoking and glucocorticotherapy.

Results: The equivalent dose of inhaled glucocorticosteroids expressed by Budesonide was near the same between groups on therapy. The serum cotinine quantitative ELISA test confirmed that all asthmatic children in passive smoking group were exposed to tobacco smoke (100%) and one (5%) group were no-passive smoking asthmatic children test. The activity of SOD statistical significantly lower level was found only in groups without inhaled glucocorticotherapy between passive and no-passive smokers: 1021.75 ± 142.60 vs 1121.22 ± 102.38 U/gHb ($P < 0.05$). For the GPx activity was statistical significantly lower only in asthmatics on glucocorticotherapy between passive and no-passive smokers: 36.22 ± 3.27 vs 41.06 ± 9.35 U/gHb ($P < 0.01$).

Conclusion: Our investigation showed reduced antioxidative status in children with peristent asthma. In asthmatics who are passive smokers when they are without glucocorticotherapy SOD may be better marker and when they are on therapy GPx may be better marker for anti-oxidants status.

992
Rural vs urban: does the place of living make a difference in asthma control among asthmatic children in Croatia?

Segulja, S; Volkmer Staresina, R; Buljevic, D; Ratkovic, D; Zvonarek Valkovic, M
Special Hospital Thalassotherapia Crikvenica, Crikvenica, Croatia

Background: Current and former studies show that there is a difference between asthmatic children living in rural and urban areas. The difference is found in asthma morbidity and onset of disease. Asthma control is important factor and end goal in treatment of asthmatic patients. Fraction of exhaled nitric oxide (FeNO) is used as diagnostic tool in asthma control. We wanted to investigate weather there is a difference in asthma

control between asthmatic children living in rural and urban areas using FeNO values as a tool.

Method: We took 80 samples of FeNO values from 80 asthmatic children that were treated at our hospital during 2012. FeNO samples were taken at arrival and departure since respiratory rehabilitation is 3 weeks long. Forty children were from rural and 40 from urban areas. Their place of living was the same for five or more years. Rural was defined as area with population <10 000 and absence of industries. Urban area had population more than 10 000 and presence of industries in environment. The population numbers were taken from official classification of settlements in Croatia.

Results: In comparison of medians for FeNO values at arrival no significant difference is found ($P = 0.5530$), as well at departure ($P = 0.6805$). Difference in values before and after treatment in our hospital between two groups is not found ($P = 0.7874$). Treatment efficiency analyzed in two groups trough FeNO is proved ($P < 0.0001$). In comparison of asthmatic children under inhaled corticosteroids therapy between two groups, no significant difference is found ($P = 1$), as well in number of asthmatic children with positive skin prick test between two groups ($P = 0.6363$).

Conclusion: We did not prove the difference in asthma control between asthmatic children living in rural and those in urban areas using FeNO as a diagnostic tool. However there is a difference between FeNO at arrival and departure within groups suggesting that environment does play a significant role in asthma control since treatment in our hospital lasts for 3 weeks and hospital itself is placed by the sea in environment with absence of industries.

993
Impact of anxiety and depression in asthma control

Tiotiu, A[1]; Clément-Duchêne, C[1]; Vaillant, P[1]; Tiotiu, D[2]; Martinet, Y[1]
[1]Pulmonology, CHU Nancy, Nancy, France; [2]Public Health, CHU Nancy, Nancy, France

Background: Despite effective treatment, asthma control remains suboptimal. This is partly due to the fact that anxiety and depression are particularly common in people with severe and difficult-to-control asthma.

Objective: The purpose of this study was to determine whether the level of mild persistent asthma control is associated with anxiety and depression.

Method: We included 42 subjects, divided into two groups: group 1 (21 patients with confirmed mild asthma, mean age 37 years) and group 2 (21 non-asthmatic control subjects, mean age 33 years). We collected demographic, clinical data, hospital anxiety/depression (HAD) score for all subjects, and spirometric data and asthma control scores (ACQ7) for asthmatics patients.

Results: The two groups are comparable in terms of demographical data. The sample was predominantly female. All patients have normal respiratory function (FEV1 = 98 ± 9%, FVC = 108 ± 11%, FEV1/FVC = 79 ± 8). Seventeen patients had an atopic background (81%). Sixteen patients had a partially controlled asthma and five were optimally controlled. Eight patients had an elevated anxiety score (score >10), and none of the 42 subjects presented depression. The level of anxiety and depression was significantly higher among patients compared to control group ($P = 0.0001$ and $P = 0.03$, respectively). In the asthma group, we did not find any correlation between the degree of anxiety or depression and the level of asthma control.

Conclusion: In our study, the anxiety was higher in the patients with mild asthma than in the control group. There is no correlation between anxiety or depression and asthma control in mild asthmatic patients.

994
Beyond asthma – pathogenic alteration of two alleles in CFTR gene

Al-Zahrani, DM[1]; Al-Shaikh, A[2]; Shahzad, A[3]; Dhabab, R[3]; Hasosah, M[4]; Shawli, A[5]
[1]Immunology and Allergy, Pediatrics, King Abdulaziz Medical City-WR, Jeddah, Saudi Arabia; [2]Pediatrics Endocrinology, King Abdulaziz Medical City-WR, Jeddah, Saudi Arabia; [3]Pediatrics, King Abdulaziz Medical City-WR, Jeddah, Saudi Arabia; [4]Pediatrics Gastroenterology, King Abdulaziz Medical City-WR, Jeedah, Saudi Arabia; [5]Pediatric Genetics, King Abdulaziz Medical City-WR, Jeddah, Saudi Arabia

Background: Asthma is common childhood disease, but cystic fibrosis is rare in Saudi Arabia. We confirmed pathogenic alterating mutations of two alleles in *CFTR* gene causing cystic fibrosis (CF).

Method: We describe 18-months old boy with frequent emergency room visits and five hospital admissions due to cough, shortness of breathing and wheezing. He has history of failure to thrive, rickets and GERD on treatments, then diagnosed as asthmatic and referred to allergy/immunology clinic for assessment.

Results: His growth parameters <5% ile. Chest radiograph showed hyperinflation of both lungs and chronic airspace disease. Milk scan showed moderate delay in gastric emptying. Upper GI study showed no reflux. Immunologic work up showed normal lymphocyte markers and function and immunoglobulin levels. Sweet Cl^- skin test was negative. Molecuar genetic analysis revealed mutation c.2657+5G>A (IVS16+5G>A) in the CFTR gene in heterozygous state that would not explain the disease. However, extended analysis revealed mutation c.3485G>A (p.Arg1162Gln) in the CFTR gene. Pancreatic elastase is 165 μg/g stool (indicates moderate to severe pancreas insufficiency). He was put on maximum treatment: nebulised bronchodilator q 6–8 hourly, budesonide q 12-hourly and intermittent steroid for reactive airway exacerbations, prophylactic oral antibiotics, pancreactic enzyme (pancrealipase 2000 IU/kg/meal) and vitamins supplementation. He dramatically improved with no hospital admission since the time of diagnosis and continue to gain weight (currently on 10% ile.

Conclusion: Asthma in common childhood disease but would not explain failure to thrive and persistent lung infiltrates that should rise other differential diagnosis, ie; CF even though it is rare in Arabian Peninsula. Alternating gene mutations in CFTR gene can cause moderate to severe pulmonary disease. Negative sweet Cl– skin test does not rule out CF.

995
The role of beta2 adrenergic receptor polymorphism in bronchial asthma

Cortez e Castro, MMTC[1]; Andreia, M[2]; Albuquerque, J[2]; Ferreira, J[2]; Bicho, M[2]
[1]Immunoallergy, CHLN-HSM, Lisbon, Portugal; [2]Genetic Department, Lisbon Medical School, Lisbon, Portugal

Background: It is known that beta2 adrenoreceptor polymorphisms could lead to a genotype-specific response to treatment and different asthma phenotypes. The purpose of this study is to analyze the association between Arg16Gly (rs1042713) and Gln27Glu (rs1042714) polymorphism with asthma severity.

Method: Asthmatic patients ($n = 49$) were studied for these polymorphisms by PCR-RFLP (Polymerase chain reaction- restriction fragment length polymorphism). Control of asthma assessed by validated instrument (ACQ7 and PAQLQ). Statistical analysis was performed with PASW version 18 establishing a significance level of $P < 0.05$.

Results: The mean age of the 49 asthmatics was 35.59 ± 20.519 (7–72 years); 24 females and 25 males; 41 atopics and eight non-atopics; 32 with controlled and 17 with uncontrolled asthma. In asthmatics for Arg16Gly polymorphism, the frequencies of the allele A is 66.3% and the allele

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

G is 33.7%; and for the Gln27Glu polymorphism, the frequencies were for allele C 30.6% and G 69.4%. Genotypes in the asthmatics for Arg16Gly polymorphism were: AA: 38.8%; AG: 55.1%; GG: 6.1% and for the Gln27Glu polymorphism were: CC: 10.2%; CG: 40.8%; GG: 49%. In asthmatics for Arg16Gly and Gln27Glu polymorphisms, there is no statistical difference ($P > 0.05$) in genotypes: between atopics and non atopics; controlled and uncontrolled asthma; and in the different age-groups. The frequencies of genotype Arg16Gly polymorphism were statistical different between males and females, being the genotype carrying the allele G more frequent among males ($P = 0.039$).

Conclusion: In this study sample we were not able to demonstrate an influence of Gln27Glu and Arg16Gly beta-2 receptor gene polymorphisms on asthma severity.

996
Relationship of polymorphisms of toll-like receptors 2 and 4 and T-regulatory cells in patients with atopic asthma

Kaidashev, IP[1]; Lyakhovskaya, N[1]; DuBuske, LM[2]
[1]Ukrainian Medical Stomatological Academy, Poltava, Ukraine; [2]Immunology Research Institute of New England, Gardner, United States

Background: Genetic aspects in the development of atopic asthma (AA) may involve gene polymorphisms. This study investigated the relationship of polymorphisms 22258 G/A gene TLR2 (rs5743708), 896 A/G gene TLR4 (rs4986790) and the level of allergic inflammation markers in patients with AA.

Method: Forty-five adult patients with atopic (IgE-dependent) asthma were examined during remission. The levels of the CD4/CD25/Foxp3, IgE, IL-4, IL-10 were assessed as were polymorphisms of TLR4 and TLR2 genes. The control group of 90 people with no symptoms of atopy were assessed to determine the prevalence of polymorphism of TLR 2 and TLR 4.

Results: Genotype AG of the gene for TLR4 was detected more frequently in patients with atopic asthma than in healthy controls. The patients who were carriers of the mutant allele of the gene 896G TLR 4 had symptoms of food allergy, more frequent exacerbation of AA, and significantly lower levels of T-regulatory cells (CD4/CD25/Foxp3) and IL-10 than carriers of the 'wild type'. Genotype GA of gene TLR2 is also more common in patients with asthma. The carriers of this allele were often ill with pneumonia in childhood.

Conclusion: No difference in the levels of IgE and IL-4 occurred in patients with AA who had the presence of polymorphism 22258 G/A in the TLR2 gene. The levels of CD4/CD25/Foxp3 and IL-10 were greater in patients without the mutant genotype.

Poster Session 35

Modern management of asthma

999
The 'Allergy Blog': an analysis of the variability of visits along the seasons

Emerson, MF; Rios, JL; Rios, JM; Pereira, NF; Cordeiro, NG; Arcanjo, LC; Martins, ER; Sardenberg, JL; Tavares, T; Carvalho, LP; Kuschnir, F; Filho, SL; Alergistas Coligados Study Group
Policlínica Geral do Rio de Janeiro – PGRJ, Alergologia – Faculdade de Medicina de Petrópolis, Rio de Janeiro, Brazil

Background: There are many gaps in patients information about allergic problems and concerns. The Allergy Clinic of Policlinica Geral do Rio de Janeiro edits the 'Allergy Blog' since 2006 (www.blogdalergia.com), an institutional and non-profit home page, created for information, dissemination and clarification of doubts of patients regarding medical topics in Allergy. The 'Allergy Blog' received to date a total of 2 948 159 unique visits and 4 125 503 page views, averaging 29 450 texts visited weekly.
Objectives: To report the interactive educational experience, through the internet on http://www.blogdalergia.com; to find out if there is any variability in the number of visits and page views through the different seasons of the year, and if the main asked topics change with the year seasons. Furthermore, we analyze the most popular topics in e-mails sent between January and December 2012.
Method: Analysis of traffic ranking of the site researching on Site Meter's home page, to know the number of hits on the site, and analysis of the audience's questions by e-mails sent during the year 2012.
Results: The 'Allergy Blog' answered 2126 e-mails and 2700 comments during the year of 2012, but the comments were excluded because they were not catalogued by date. The number of e-mails received was similar in all seasons, but the number of unique visits and the number of page views were quite higher in Winter than in Summer: Spring: 289 274 unique visits and 390 507 page views; Summer: 263 000 unique visits and 338 181 page views; Autumn: 300 474 unique visits and 396 517 page views; Winter: 318 695 unique visits and 420 809 page views. Despite this little difference, the most requested topics during all the year were that related to skin allergies.
Conclusion: The seasonal variability of allergic diseases has a slight reflection on the frequency of access in this educative Allergy Blog, although this variability may not be significant.

1000
Content validity of three asthma-specific quality of life questionnaires: the patients' perspective

Apfelbacher, C[1,2]; Jones, C[1]; Frew, A[3]; Smith, H[1]
[1]Division of Public Health and Primary Care, Brighton and Sussex Medical School, Brighton, United Kingdom; [2]Medical Sociology, Institute of Epidemiology and Preventive Medicine, University of Regensburg, Regensburg, Germany; [3]Department of Respiratory Medicine, Brighton & Sussex University Hospitals NHS Trust, Brighton, United Kingdom

Background: Several asthma-specific quality of life questionnaires exist and it is unclear which of these best captures the lived experience of people with asthma. The purpose of this study was to explore patients' views in relation to three common asthma-specific quality of life questionnaires.
Method: Thirty adult individuals with asthma recruited in Brighton, UK were asked to complete three asthma-specific quality of life questionnaires (Juniper Asthma Quality of Life Questionnaire (AQLQ-J), Sydney Asthma Quality of Life Questionnaire (AQLQ-S) and the Living with Asthma Questionnaire (LWAQ)). Following completion, semi-structured interviews were conducted to elicit patients' views on the content validity of the questionnaires. Interviews were transcribed verbatim and thematic content analysis performed.
Results: The data revealed a tension between missing content (e.g. asthma management) and irrelevant content (e.g. smoky restaurants). Several patients felt that the dynamic nature of asthma was not captured by the questionnaires. There was uncertainty whether to report the effects of controlled or uncontrolled asthma and how to take into account co-morbidities such as allergies or infections.

The AQLQ-J was perceived as a 'narrow' 'medical' questionnaire with a focus on symptoms, the environment and activities. The choice of activities which the AQLQ-J allows was perceived to be positive by some and difficult by others. In contrast both the LWAQ and the AQLQ-S were perceived to be 'non-medical'. The emotional focus of the LWAQ and its use of positive and negative items were perceived as irritating by some participants. It was perceived as burdensome to complete and described as a 'test' or 'quiz'. A recurrent theme, however, was that the LWAQ was a wide-ranging, embracing and holistic questionnaire. Finally, the AQLQ-S was described as a simple, quick and easy questionnaire although there was also a perception that it lacked depth. Overall, the AQLQ-S was considered to sit midway between the AQLQ-J as a 'medical' questionnaire and the LWAQ as an 'emotional' questionnaire.
Conclusion: Patient involvement highlights shortcomings and strengths of various asthma-specific questionnaires in terms of their content validity. The AQLQ-S seemed to have an acceptable length but also sufficient coverage of medical, social and emotional aspect of health-related quality of life in asthma.

1002
Improvement in asthma after Bariatric surgery in severe asthma patients in Singapore

Tan, LL; Koh, MS
Respiratory and Critical Care Medicine, Singapore General Hospital, Singapore, Singapore

Obesity has become an increasing problem even in Asian countries, including Singapore. Asthma in the obese is more severe and often resistant to standard asthma therapies. Although weight loss can improve asthma control, significant and sustained weight loss through diet and exercise is often difficult and without much success. Bariatric surgery offers a 'new form of treatment' for these severe asthmatics as results seem to be encouraging. We describe two such cases amongst a group of 275 patients with severe asthma in our database.
Case 1: A 40-year- old Malay female patient with Body Mass Index (BMI) of 35.5 kg/m(2) who had poorly controlled atopic asthma despite adherence to Salmeterol/fluticasone 25/125 two puffs twice a day and Montelukast 10 mg every night. Despite weight loss program, there was not significant reduction in weight. Bariatric

surgery was performed and after 3 months, her BMI was reduced to 29.4 kg/m(2) and Asthma control test (ACT) improved from 10/25 (pre-op) to 25/25 (post-op). Her maintenance Salmeterol/fluticasone was decreased to one puff twice a day and Montelukast was stopped. Six months post-surgery, Salmeterol/fluticasone was further reduced to 25/50 one puff two times a day and asthma control remained very good.

Case 2: A 37-year- old Malay female patient with BMI 42.4 kg/m(2) with severe atopic asthma on Formoterol/budesonide 160/4.5 four puffs twice a day, Tiotropium 18 μg daily, Montelukast 10 mg daily, theophylline 125 mg daily, long term prednisolone between 10 and 15 mg daily and Ciclosporin 200 mg twice a day. She was previously given Omalizumab for 1 year without much improvement in exacerbation rates and hospitalisations. She had daily symptoms with ACT of 14/25 and three admissions in 2012 despite adherence to her multiple medications. Weight loss program was unsuccessful. She underwent Bariatric surgery and 1 month after surgery, her BMI reduced to 36.6 kg/m(2) and her prednisolone dose was reduced to 8 mg daily and ciclosporin has been gradually reduced to 50 mg daily. Her ACT was 20/25.

Conclusion: These two cases illustrate the promising results of Bariatric surgery on our Severe Asthmatics. In addition to weight loss, both patients have demonstrated significant improvement in asthma control – both symptom control and medication requirement. Although we only have short term results, we are hopeful that the results will be sustained.

1004
Level of vitamin D is decreased in patients with asthma despite their allergic status

Zilinskaite, L; Lavinskiene, S; Sakalauskas, R; Sitkauskiene, B
Department of Pulmonology and Immunology, Lithuanian University of Health Sciences, Kaunas, Lithuania

Background: Previous studies have shown that vitamin D is important not only in production of minerals, but also in regulation of immune response. It is thought that deficiency of vitamin D induces allergic reactions. We aimed to assess levels of vitamin D in patients with asthma according to their allergic status.

Methods: Eighty-five patients with asthma and 72 healthy persons as a control group were investigated. Subjects were selected from register of international project 'Proteasomal gene alleles as risk factors for bronchial asthma in Latvian, Lithuanian and Taiwanese populations'. Patients with asthma (diagnosed using GINA recommendations) were divided into groups by asthma phenotypes. Allergic status was assessed using skin prick test and measuring amount of eosinophils and total IgE in peripheral blood. Allergic asthmatics were divided into three subgroups according to the results of skin prick test: sensitivity to one inhaled allergen; to more than one inhaled allergen; and to mixed allergen (inhaled and food). Environmental smoking and smoking history were also assessed. Lung function was evaluated by spirometry (FEV1, FVC and FEV1/FVC). Concentration of vitamin D (25(OH)D3) was measured by ELISA using standardised method.

Results: Vitamin D level was found lower in patients with asthma than in control group (14.36 ± 5.22 vs 22.04 ± 7.18 ng/ml, $P < 0.001$). There were no significant differences between vitamin D level in groups with allergic and non-allergic asthma (14.36 ± 5.79 vs 14.35 ± 3.99 ng/ml, $P > 0.05$). Vitamin D levels did not correlate with lung function (FEV1, FVC, FEV1/FVC), amount of eosinophils and total IgE level in asthmatics. However, vitamin D level negatively correlated with amount of eosinophils ($rs = -0.79$, $P < 0.05$) in subgroup of sensitivity to mixed allergen. The same tendency was noticed between vitamin D level and amount of eosinophils in asthmatics with smoking history ($rs = -0.74$, $P < 0.05$). Positive correlation was estimated between vitamin D level and FEV1/FVC ($rs = 0.7$, $P < 0.05$) in smokers.

Conclusions: Lower vitamin D level in asthmatics than in healthy subjects is not related with their allergic status, but smoking may play a role. It let us to suggest that vitamin D is important in asthma pathogenesis despite allergic hypersensitivity.

1006
Asthma management among different specialists: results from a national Italian survey

Caminati, M[1]; Magnoni, MS[2]; Rizzi, A[2]; Senna, G[1]
[1]Allergy Unit, Verona University Hospital, Verona, Italy; [2]Medical and Scientific Department, GlaxoSmithKline, Verona, Italy

Background: In Europe more than 50% of asthmatic treated patients have not well-controlled asthma. According to GINA guidelines different specialists should be involved in the achievement and maintenance of optimal asthma control. Our survey aims at investigating how Allergists, Pneumologists and General Practitioners (GPs) approach asthmatic patients, in order to highlight pitfalls and unmet needs concerning real-life asthma management.

Method: A web anonymous questionnaire was randomly administered to 675 GPs, 180 Pneumologists and 90 Allergists between December 2010 and January 2011. The 24 questions concerned: epidemiology, diagnostic work-up, follow-up and risk factors, treatment and future risk.

Results: Sixty-four percent of GPs declare that among their patients at least 20 have asthma. Concerning Specialists report, one out of three visits more than 20 asthmatic patients per week. A general agreement emerges about the importance of spirometry, reversibility test, metacholine challenge and evaluation of atopic status in assessment and diagnosis. Inflammation assessment (FeNO) is important for Pneumologists and Allergists, but not for GPs. All categories are aware of the impact of comorbidities on asthma and take it into account in treatment and follow-up planning. The main shared goal of treatment is reducing asthma exacerbations using a regular therapy. All categories consider long acting beta2 agonist/inhaled steroids combination the first choice treatment for both seasonal and perennial asthma. Pneumologists prescribe more leukotriene antagonists, Allergists more immunotherapy. Surprisingly depot steroids and beta2 agonists alone are still prescribed by GPs. Concerning follow-up, Allergists rely on inflammation biomarkers whereas reduction of rescue medication is more relevant for GPs. Asthma Control Test is considered time consuming by more than 50% of all physicians and is not known by most of GPs. Adherence is considered a crucial problem in asthma management, especially by specialists.

Conclusion: All categories seem to have a good knowledge about asthma. The cultural background may account for mild differences concerning asthma monitoring tools and treatment options. Since patients are often evaluated in primary care setting as a first line approach, GPs have a pivotal role in detecting patients who need specific assessment by specialists and are involved in their follow-up. It is thus important that GPs and specialists share common strategies in asthma management.

1008
Characteristics of difficult to treat asthma in Korea; pilot study

Park, YB[1]; Yoo, KH[2]; Lee, W-Y[3]; Korean Asthma Study Group
[1]Hallym Sacred Heart Hospital, Hallym University College of Medicine, Seoul, Korea; [2]Internal Medicine, Konkuk University School of Medicine, Seoul, Korea; [3]Internal Medicine, Yonsei University Wonju College of Medicine, Seoul, Korea

Background: Difficult-to-treat asthma afflicts a small percentage of the asthma population. However, these patients remain refractory to treat, and account for 40–50% of the health costs of asthma treatment, incurring significant morbidity. We conducted a multi-center cross-sectional study to characterise difficult-to-treat asthma in Korea.
Methods: Subjects with difficult-to-treat asthma and subjects with controlled asthma were recruited from 5 outpatient clinics of referral hospitals. We reviewed medical records of previous 6 months and obtained patient-reported questionnaires composed of treatment compliance, asthma control, and instruments for stress, anxiety, and depression.
Results: We recruited 21 subjects with difficult-to-treat asthma and 110 subjects with controlled asthma into the study. The subjects with difficult-to-treat asthma were associated with longer treatment periods, more increased health care utilisation, more medication (oral corticosteroids, number of medication), and more anxiety disorder compared to those of well-controlled asthmatics. There was no difference in age, gender, history of allergy, serum IgE, blood eosinophil count, or body mass index between the two groups. The proportion of FEV_1 improvement is larger in subjects with well-controlled asthma compared to those with difficult-to-treat asthma during the treatment despite severity of initial FEV_1 at diagnosis of asthma is similar between two groups.
Conclusion: Difficult-to-treat asthma is characterised by increased health care utilisation and more co-morbidity of anxiety. Therefore, there is a need to better understanding the mechanism and pathophysiology of difficult-to-treat asthma so more effective therapies can emerge.

1012
Long-term follow-up outcomes of pulmonary function as evaluated by forced oscillometry technique, eNO, and spirometry for early intervention anti-inflammatory therapy for asthma in under 2-year-olds

Ikeda, M; Sekimoto, K; Tsutimoto, H; Fujiwara, K; Okamura, T; Ogasawara, H; Sakamoto, T; Kitada, K; Hosogi, M; Nojima, I; Takahashi, N; Araki, T
Pediatrics, National Hospital Organization, Fukuyama Medical Center, Fukuyama, Japan

Background: The final goal of childhood asthma treatment is to outgrow the disease via remission. After pediatric guidelines for the management of asthma were devised and after antiinflammatory treatment became widespread, the quality of life (QoL) improved for many asthmatic children. However, the long-term outcomes for early intervention antiinflammatory treatment for asthma in young children have been unclear.
Method: We investigated a total of 63 children <2 years old who were diagnosed with asthma with recurrent wheezing between October 1998 and September 2000 and who had allergenic sensitisation and a family history of asthma. Early intervention antiinflammatory treatment with inhaled corticosteroid/leukotriene receptor antagonist/disodium cromoglycate was commenced to induce active remission before 2 years of age in accordance with Japanese Pediatric Guidelines for The Treatment and Management of Asthma 2000–2005. In 2011, subjects were evaluated by forced oscillometry technique (FOT), eNO, and spirometry, and their clinical symptoms were assessed.
Results: The long-term remission (≥5 years without treatment) rate was 79.2% (intermittent asthma, 100%; mild persistent asthma, 73.9%; moderate persistent asthma 78.6%; and severe persistent asthma 75.0%) 11 years after initiating early intervention therapy. The long-term remission rate improved in comparison with past asthmatic convalescence surveys (mild asthma, 50.0–69.2%; severe asthma, 5.0–30.8%). eNO level was 55.0 ± 48.1 ppb, and 61.0% of subjects showed ≥35 ppb. Complications involving allergic rhinitis (AR) were seen in 78.6% of subjects, and the eNO level was significantly higher in AR(+) subjects than in AR(−) subjects (66.4 ± 49.7 vs 18.5 ± 10.0 ppb, respectively). Asthma recurrence was also higher in AR(+) subjects. FOT revealed small airway resistance (R5-R20) of 0.079 ± 0.087 kPa/l/s, and 23.8% of subjects exceeded +2 standard deviations, but total resistance (R5) was 0.375 ± 0.129 kPa/l/s, and none exceeded this value. $\%V_{50}$ and $\%V_{25}$ were $86.2 \pm 16.9\%$ and $89.2 \pm 26.8\%$, respectively, and were normal in 90% of subjects. $\%FEV_1$ was $94.6 \pm 11.9\%$, and all subjects were within normal range.
Conclusion: Early intervention antiinflammatory therapy and maintaining long-term total control of asthma may not only prevent progression of disease severity and improve the QoL of patients, but also increase the outgrow rate and provide normal development of pulmonary function in young children with asthma.

1014
International Guidelines introduction and free medicines for asthmatic patients in Kazakhstan

Nurpeissov, TT; Nurpeissov, TN; Abdushukurova, G; Akpeissova, RB; Nurpeissov, T
SRI of Cardiology and Internal Diseases, Republican Allergological Center, Almaty, Kazakhstan

Background: Bronchial asthma (BA) is now proved as a treatable and controllable disease due to the use of modern medicines and international evidence based guidelines. Combined inhalers (ICS+LABA) and montelucast are the unchallengeable part of basic treatment of persistent BA. Only these preparations are able to reliably prolong the life of patients and improve its quality. The lack of modern medicines is relatively high price, especially for developing countries.
Aim: To analyze the situation about providing with free-of-charge modern medicines for asthmatic patients in Kazakhstan and their interaction with newest International guidelines.
Method: GINA and other international guidelines for asthma management, Kazakh Laws, Minister of Public Health's Orders, the State statistical reporting of the Republic of Kazakhstan.
Results: In Kazakhstan according the national laws BA became so called 'the socially significant disease' and patients should be provided with free-of-charge medicines including ICS + LABA (budesonid + formoterol or fluticasone + salmeterol) from 2008. Montelucast was included in this list of free medicines in 2012, which provided the possibility of full match of national schemes of BA management and GINA even for poorest part of population. But there are many remaining problems. One of them is that only registered asthmatics can get free medicines, while the condition of a large number of patients underestimated by regional practitioners. Diagnosis 'bronchitis' instead of BA is still a common mistake. Kazakhstan is 9th country in the world by its territory. Many of inhabitants, especially in rural regions,

cannot reach the outpatient hospitals in time for appropriate examination and for getting their free medicines. Besides this the cheapness and high availability of system steroids in tablets leads to the growth of side effects and the rate of BA complications.

Conclusion: At this moment Kazakhstan allergists have all possibilities for providing good BA management with free-of-charge medicines according the newest international guidelines. The biggest challenge now is to improve early detection of BA patients on a national scale, to ensure appropriate knowledge of practitioners in rural regions and to bring free medicines getting points as close as possible even to the most remote settlements. That should make further positive effect in BA management in Kazakhstan.

1015
The frequency and risk factors of oropharnygeal candidiasis in patients with asthma using inhaled corticosteroids

Erdogan, T; Karakaya, G; Kalyoncu, AF
Pulmonary Medicine Clinic Adult Allergy Unit, Hacettepe University Faculty of Medicine, Ankara, Turkey

Introduction: Inhaled corticosteroids are the mainstay of asthma treatment. The incidence of asthma, which is a major health problem, is rising all over the world. High dose and long term use of corticosteroids may induce side effects. In this study, we planned to investigate frequency and risk factors of oral and systemic candidiasis infections in adult asthmatic patients.

Method: Randomly selected 186 asthma patients who were admitted to adult allergy clinic between May 2011-September 2012 were evaluated. Asthma beginning age, additonal atopic diseases, comorbid diseaeses, oral hygiene (mouth rinse, dry mouth), lifelong all fungal infections (foot, nail, vaginal, skin, etc.), and atopy were recorded.

Results: Most of the patients were female (79%). Lifelong incidence of oral candidiasis was 19.4% ($n = 36$), which was also detected in 10 (5.38%) patients at the study time period. Incidence of lifelong any fungal infection anywhere in body was 59.7% ($n = 111$), which was also detected in 12 (6.4%) patients at the study time period. There were no significant differences, when the groups were compared in terms of sex, age, asthma beginnning age, dry mouth condition, the presence of decayed teeth, mouth rinsing rates, atopy and comorbid diseases. Among 26 patients who have had history of vaginal candididasis after antibiotic use only seven had oral candidiasis history. There were no statistically significant differences.

Persistant rhinitis history, use of leukotriene receptor antogonist together with any inhaled corticosteroid and use of ciclesonide were associated with increased frequency of oropharnygeal candidiasis.

Conclusion: Results of this study indicate that frequency of orophargngeal candidiasis in adult asthmatic patients is quite high (19.4%) and there is no definitive risk factor to explain this. Frequent oropharnygeal candidiasis infection was related with use of ciclesonide which might be due to suggestion of ciclesonide by the doctors to patients who already have candidasis. Further studies are needed to distinguish these individual differences.

Poster Session 36

Combining education, medical treatment and supportive measures to improve asthma control

1019
Asthma control is reached in a large majority of severe asthmatics treated with omalizumab. An Italian observational study

Novelli, F; Lenzini, G; Latorre, M; Paggiaro, P; Italian Xolair Study Group
University of Pisa, Pisa, Italy

Background: The efficacy of omalizumab in reducing exacerbations and emergency room visits is largely demonstrated. Less data are available on the proportion of asthmatics achieving a good asthma control (according to GINA guidelines) during omalizumab treatment. The aim of this study is to assess the level of control achieved in a group of severe asthmatics under treatment with omalizumab, to characterise the factors that influence the lack of control and to correlate the level of control with the treatment duration.
Method: We studied 178 patients with asthma under treatment with with omalizumab from a median of 34 months (range 4–95). All patients underwent spirometry. Asthma control was evaluated according to GINA guidelines and also by ACT questionnaire.
Results: The majority of patients (76.3%) showed a good or partial control of asthma (37.4% of these showed a well control without any exacerbation in the last year). The median value of ACT was 20.4; 66.3% had an ACT score ≥ 20. The comparison between patients with poorly controlled asthma and patients with partially or well controlled asthma showed a higher prevalence in the first group of some comorbidities [obesity (39% vs 16.7%, $P = 0.04$), gastro-oesophageal reflux (51.2% vs 33.3%, $P = 0.004$), aspirin intolerance (33.6% vs 13.6%, $P = 0.001$)] and a lower FEV1 (68.1% vs 76.9% of pred, $P = 0.01$). Similarly, asthmatics with at least one exacerbation in the last years showed a higher prevalence of comorbidities [obesity (35.3% vs 13.7%, $P = 0.001$), chronic rhinosynusitis (51.5% vs 26.5%, $P = 0.001$) and nasal polyps (35.3% vs 16.7%, $P = 0.006$)] and a lower FEV1 (67.3% vs 78.9% of pred, $P < 0.001$) than patients without exacerbations in the last year. Subdividing the patients into quartiles of the duration of omalizumab treatment, the percentage of poorly controlled asthmatics remained similar in all groups (from 22.2% to 25%).
Conclusion: The treatment with omalizumab allows a high percentage of asthmatics to obtain a well or partial control of asthma, even after several months of therapy. Among patients who remain poorly controlled or continue to have exacerbations, there is a higher prevalence of comorbidities, such as obesity, gastro-oesophageal reflux and pathology of upper airway diseases, which are probably not optimally treated.

1020
Real life experience with omalizumab in patients with poorly controlled moderate to severe asthma

Ricciardi, L; Arena, E; Loschiavo, G; D'Angelo, AG; Sinagra, F; Paffumi, I; Saitta, S; Marotta, G
Clinical and Experimental Medicine, Allergy and Clinical Immunology Unit, University Hospital, Messina, Italy

Background: A considerable proportion of patients with allergic asthma, particularly those with severe disease, have poorly controlled symptoms and are at increased risk of exacerbations. In some patients inadequately controlled asthma is due to poor adherence with treatment, untreated comorbidities, dysfunctional breathing or psychological problems. For other there is a need for additional or new therapies.
Method: Between June 2011 and June 2012, 18 patients, aged 14–70 years, with perennial allergen asthma and a history of exacerbations and poor symptom treatment with high dose inhaled corticosteroids ($\geq$500 μg of fluticasone inhaler twice daily or equivalent), inhaled long-lasting β_2 agonist bronchodilators, with other controllers such as anti-leukotrienes (montelukast 10 mg daily) and oral or parenteral corticosteroids (betamethasone 1–4 mg daily) underwent treatement with omalizumab. Patients evaluation was carried out after a 6-months treatment period with omalizumab. The dose of omalizumab for each patient and the dose frequency was based on the serum total IgE level (IU/ml) and the patient's body weight (kg). Based on this calculation omalizumab was given at a dose of 225–600 mg by subcutaneous injection every 2 or 4 weeks. Statistical evaluation was carried out using the Mc Neman test. Results were considered statistically significant if $P < 0.05$
Results: Omalizumab was well tolerated and no local or systemic adverse effects occurred during the treatment period. A statistically significant reduction in drug consumption occurred after 6-months treatment as far as systemic corticosteroids were concerned (18 vs 5; $P = 0.004$), inhaled corticosteroids (18 vs 7; $P = 0.031$), inhaled long-lasting β_2 agonist bronchodilators (18 vs 7; $P = 0.031$) and a reduction of treatment with anti-leukotrienes even if not statistically significant (18 vs 10). A significant increase in the mean asthma quality of life questionnaire (AQLQ) score was also observed between base line and after 6 months treatment.
Conclusion: Taken together the results obtained in our patients treated with Omalizumab because of poorly controlled allergic asthma suggest that this kind of treatment is effective and that efficacy outcomes in a 'real life' setting are similar to those reported in clinical trials.

1021
Efficacy and safety of omalizumab in real-world clinical practice in Indian patients with allergic (IgE-mediated) asthma in India: interim analysis at 28 weeks

Rajput, A[1]; Singh, S[2]; Kotnis, M[3]; Vora, A[4]; EASE Study Group
[1]Department of Respiratory Medicine, Army Hospital, Delhi, India; [2]Department of Respiratory Medicine, Army College of Medical Sciences, Delhi, India; [3]Medical Affairs, Novartis Pharmaceuticals India, Mumbai, India; [4]Chest Medicine, Suchak Hospital, Malad, India

Background: Omalizumab is a recombinant humanized anti-IgE monoclonal antibody, indicated as add-on therapy for moderate-to-severe persistent allergic (IgE-mediated) asthma. Here, we report the 28-week interim results of a 52-week observational study of omalizumab in

patients with allergic asthma in India, focusing on lung function and asthma control in different age groups.

Methods: In this open-label, non-comparative, non-interventional study, patients (age ≥ 12 years) with moderate-to-severe persistent allergic asthma (GINA step 4), inadequately controlled despite regular treatment with ICS + LABA, were recruited. All patients were receiving omalizumab at baseline. Efficacy outcomes were assessed every 4 weeks, and included mean change in (D) FEV_1, ACQ5 score, ACT score and ICS dose vs baseline. Adverse events were also recorded. Patients were stratified for subgroup analysis into three groups defined by age: group 1 (≥12–<35 years), group 2 (≥35–<50 years) and group 3 (>50 years). Qualitative and quantitative variables were analyzed using the chi-squared and paired t-tests, respectively. All parameters were compared between baseline and Week 28, post-omalizumab treatment.

Results: To date, 100 patients have completed 28 weeks of follow-up (11 patients in group 1, 35 in group 2 and 54 in group 3). Compared to baseline, significant improvements in lung function were seen at week 28. Mean DFEV1 was +1.0 L ($P = 0.000$) in group 1 and +0.9 L ($P = 0.000$) for both groups 2 and 3. Between baseline and Week 28, mean composite ACQ5 score declined significantly by −8.0 ($P = 0.001$) in group 1, and by −7.4 ($P = 0.000$) and −6.7 ($P = 0.000$) in groups 2 and 3, respectively. Similarly, mean ACQ5 score decreased significantly, by −1.6 ($P = 0.001$) in group 1, −1.4 ($P = 0.003$) in group 2, and −3.4 ($P = 0.044$) in group 3. Mean ACT scores increased significantly, by 10 ($P = 0.002$), 8.6 ($P = 0.000$) and 9.3 ($P = 0.000$) in groups 1, 2 and 3, respectively. Mean ICS dose decreased in all three groups. The decrease was statistically significant in group 3 (−274.3 μg; $P = 0.000$). One gastrointestinal adverse event of moderate intensity, which resolved with treatment, was suspected to be related to study medication.

Conclusion: Twenty-eight weeks' treatment with omalizumab was associated with improved lung function and asthma control in a real-world clinical setting in India.

1022
Omalizumab in patients with Churg-Strauss syndrome: a 18 month follow-up

Varricchi, G; Detoraki, A; Gravante, C; Spadaro, G; de Paulis, A; Marone, G
Department of Clinical Immunology and Allergy and Center for Basic and Clinical Immunology Research, University of Naples Federico II, Naples, Italy

Background: Churg-Strauss Syndrome (CSS) is a systemic vasculitis characterised by bronchial asthma and eosinophilia, with the lung being most commonly involved. The mainstay of therapy of CSS are oral glucocorticoids and the response is usually favourable. Preliminary reports suggest that patients with CSS can be treated with anti-IgE (omalizumab) in addition to conventional therapy to achieve asthma control. Here we report the efficacy of a 18-month treatment with omalizumab in five patients with CSS characterised by asthma, rhinosinusitis, and eosinophilia.

Method: We evaluated five CSS patients (two females, three males), age: 41–64 years. Average CSS characteristics at time of evaluation (T0) were: moderate-severe allergic asthma [mean value: FEV1 68%, mean value Asthma Control Test score (ACT): 11], eosinophilia (mean value: 1100/mm^3) and rhinosinusitis. Mononeuropathy/polyneuropathy and/or histological evidence of tissue eosinophil infiltration were also present. Patients were treated with omalizumab (300–600 mg s.c. every 2–4 weeks) as add-on therapy to prednisone (T0 mean value 25 mg/die), inhaled steroids and bronchodilators. The dose of omalizumab was done accordingly to total IgE and weight parameters following the drug information leaflet. We evaluated during treatment dose of omalizumab, ACT score, spirometry testing (FEV1), eosinophilia and prednisone tapering.

Results: After 18 months of treatment with Omalizumab patients reported a significant improvement in asthma control as indicated by ACT score (mean value T0: 11; T18: 22) and spirometry (mean value FEV1 T0: 68%; T18: 88%). Eosinophil count was reduced from 1100/mm^3 (T0), to 550 mm^3 (T18). Oral prednisone was also reduced (mean value T0: 25 mg/die; T18: 7.5 mg/die). Prednisone was withdrawn after 24 weeks of treatment in three patients. No adverse events have been registered.

Conclusion: Here we report that omalizumab can be beneficial and safe for treatment and control of asthma in CSS patients exerting an important steroid sparing effect. The efficacy of omalizumab in CSS might attributed to the possible inhibition of IgE-dependent mechanisms of eosinophil proliferation and lung infiltration.

1023
Once-daily add-on tiotropium: a dose-finding trial in adult patients with moderate persistent asthma

Beeh, K-M[1]; Ablinger, O[2]; Moroni-Zentgraf, P[3]; Hollaenderova, Z[4]; Pivovarova, A[5]; Engel, M[3]; Korn, S[6]
[1]Insaf Respiratory Research Institute GmbH, Wiesbaden, Germany; [2]Traunufer Arkade, Thalheim bei Wels, Austria; [3]Boehringer Ingelheim Pharma GmbH & Co KG, Ingelheim am Rhein, Germany; [4]Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria; [5]Boehringer Ingelheim Pharma GmbH & Co KG, Biberach an der Riss, Germany; [6]Pulmonary Department, University Medical Center, Johannes Gutenberg University, Mainz, Germany

Background: The once-daily long-acting anticholinergic bronchodilator tiotropium (5 μg administered via Respimat® Soft Mist™ Inhaler) has been shown to significantly reduce the risk of severe exacerbations and improve lung function in patients with severe persistent asthma symptomatic despite the use of inhaled corticosteroids (ICS) and long-acting β_2-agonists. This exploratory dose-finding trial evaluated the efficacy and safety of three doses of tiotropium on top of medium-dose ICS in patients with moderate persistent asthma.

Methods: In this Phase II, double-blind, 4-way crossover study with no washouts, patients with a pre-bronchodilator forced expiratory volume in 1 s (FEV_1) of 60–90% of predicted normal were randomised to 4 × 4-week treatment sequences: tiotropium (1.25, 2.5 and 5 μg) and placebo administered once daily in the evening via Respimat® inhaler. Primary efficacy end point was FEV_1 $peak_{(0–3\ h)}$. Secondary end points included forced vital capacity (FVC) $peak_{(0–3\ h)}$, trough FVC (defined as measurement at end of the 24-h dosing interval), trough FEV_1 and area under the curve for FEV_1 (FEV_1 $AUC_{(0–3\ h)}$) and FVC (FVC $AUC_{(0–3\ h)}$). Safety and tolerability were assessed for all patients.

Results: Of 149 randomised patients who received treatment, 141 completed the study. Mean age was 49.3 years; mean pre-bronchodilator FEV_1 was 71.3% of predicted normal. FEV_1 $peak_{(0–3\ h)}$ was statistically significantly different ($P < 0.0001$) for all three doses of tiotropium compared with placebo. The largest response was achieved with 5 μg tiotropium. Secondary end point analyses of lung function tests were consistent with the primary end point findings. All tiotropium doses improved the mean evening FVC $AUC_{(0–3\ h)}$ response vs placebo. A greater increase in trough FVC response was observed with tiotropium vs placebo; 5 and 2.5 μg tiotropium led to the largest response. Overall frequency of adverse events (AEs) was comparable across all treatment periods, with no apparent dose-dependency in terms of AEs. All treatments

were well tolerated, with no observed treatment-related serious AEs.

Conclusions: Tiotropium is an effective bronchodilator as add-on therapy to ICS in symptomatic patients with moderate persistent asthma. The most effective dose of tiotropium was 5 μg.

1024
Phase III trials to investigate tiotropium as add-on therapy to inhaled corticosteroids for patients with symptomatic asthma: trial design and planned statistical analyses

Casale, T[1]; Bleecker, E[2]; Meltzer, E[3]; Pizzichini, E[4]; Schmidt, O[5]; Bateman, E[6]; Engel, M[7]; Bour, L[8]; Verkleij, C[9]; Moroni-Zentgraf, P[7]; Kerstjens, HAM[10]
[1]Division of Allergy and Immunology, Creighton University, Omaha, NE, United States; [2]Center for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Winston-Salem, NC, United States; [3]Allergy & Asthma Medical Group & Research Center, San Diego, CA, United States; [4]NUPAIVA (Asthma Research Centre), Universidade Federal de Santa Catarina, Santa Catarina, Brazil; [5]KPPK GmbH, Koblenz, Germany; [6]Department of Medicine, University of Cape Town, Cape Town, South Africa; [7]TA Respiratory Diseases, Boehringer Ingelheim Pharma GmbH & Co KG, Ingelheim am Rhein, Germany; [8]Biostatistics, Boehringer Ingelheim Pharma GmbH & Co KG, Biberach an der Riss, Germany; [9]Medical Department, Boehringer Ingelheim bv, Alkmaar, The Netherlands; [10]Department of Pulmonary Medicine and Tuberculosis, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Background: Once-daily long-acting anticholinergic bronchodilator tiotropium improves lung function and reduces the risk of severe exacerbation when used as add-on therapy for patients with symptomatic asthma despite using ICS/LABAs. We designed two identical Phase III trials (NCT01172808, NCT01172821) to assess the efficacy and safety of tiotropium via Respimat® Soft Mist™ Inhaler (Respimat® SMI) in patients with symptomatic moderate asthma using at least ICS.

Methods: Two international, randomised, double-blind, double-dummy, placebo-controlled, parallel-group trials (n = 1070; n = 1030).

Primary objective: To test the efficacy of tiotropium 5 and 2.5 μg after 24 weeks vs placebo in two pre-specified co-primary end points: peak $FEV_{1(0–3\ h)}$ response (difference from baseline) and trough FEV_1 response. A blinded salmeterol HFA-MDI arm will serve as an active comparator without inferential analysis. To control for a type I error, stepwise testing of the null hypothesis will be used to first test the efficacy of tiotropium 5 μg, then of 2.5 μg, over placebo. If both co-primary end points reach statistical significance in each trial, pooling of data from both trials is planned per protocol for analysis of the 3rd pre-specified co-primary end point, responder rate assessed by ACQ.

Key inclusion criteria: Age 18–75 years; asthma diagnosis before the age of 40 and ≥3 months before inclusion; FEV_1 increase ≥12% and ≥200 ml 15–30 mins after 400 μg salbutamol; pre-bronchodilator FEV_1 60–90% of predicted; ACQ score ≥1.5; life-long non-smokers or ex-smokers ≥1 year prior with total of ≤10 pack-years; ≥4 weeks of stable medium-dose ICS (alone or in fixed combination with a β-agonist). Patients diagnosed with COPD were excluded.

Treatment: Patients were randomised to tiotropium 2.5 or 5 μg (both Respimat® SMI), salmeterol HFA-MDI or placebo. During screening and treatment all patients received medium-dose ICS. LABAs, other anticholinergics, cromone, methylxanthines and anti-IgE were not permitted. Continuation of other pre-study maintenance therapy, and rescue salbutamol, was permitted to mimic normal clinical management and better assess the actual role of tiotropium in asthma care.

Conclusion: The hierarchical statistical design controls the probability of a type I error, while allowing for three co-primary end points. Concomitant medications closely resemble real-life practice so results should provide a practical assessment of the role of tiotropium in asthma management.

1025
The written action plan in childhood asthma can reduce unscheduled physician visits

Oishi, T[1]; Narita, M[2]; Morisawa, Y[3]; Watanabe, H[4]; Fukuie, T[5]; Akashi, M[6]; Futamura, M[2]; Nomura, I[2]; Syoda, T[2]; Horimukai, K[2]; Doi, M[2]; Yoshida, M[2]; Fujieda, M[1]; Ohya, Y[2]
[1]Pediatrics, Kochi Medical School, Kochi University, Nankoku, Japan; [2]Allergy, National Center for Child Health and Development, Setagaya, Japan; [3]Kera Pediatric Clinic, Kochi, Japan; [4]Pediatrics, National Hospital Organization, Kanagawa Hospital, Hadano, Japan; [5]Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan; [6]Pediatrics, Saitama City Hospital, Saitama, Japan

Background: Self-management is essential for asthmatics to control their symptoms and a written action plan (WAP) is recommended to be used in many asthma guidelines all over the world. Especially self-management capability, to cope with acute asthma attack properly is expected to reduce unscheduled visits to emergency units in hospitals, and a new WAP was developed for the Japanese Pediatric Guideline for the Treatment and Management of Asthma (JPGL 2012). The purpose of this study is to verify the effectiveness of the newly developed WAP in JPGL 2012.

Method: To measure the outcome of WAP use during 24 weeks, we conducted a multicenter randomised controlled trial using a computer-generated random sequence method. Only caregivers of intervention group were given WAP. All participants were prescribed based on JPGL 2012 and asked to keep an asthma diary, comprised of acute asthma events, unscheduled visits to doctors, and hospital admissions. The health related 24-item QOL questionnaire which was developed for a caregiver of an asthmatic child, was also applied at the point of the trial entry and after 24 weeks. Statistical analyses were done by using SPSS statistics 17.0.

Results: Six hundred fifty-five asthmatic patients aged from 0 to 19 years old participated to this study. The data of 575 participants were completed for analysis (WAP users were 272 and non WAP users were 303). No significant difference was found in basic characteristics between the two groups except their age (mean age ± standard deviation: 5.9 ± 3.2 in WAP users, 6.5 ± 3.5 in non-users). Although there were no differences in acute asthma events and hospital admissions (P = 0.74 and P = 0.40 respectively) between both groups, WAP users experienced fewer unscheduled physician visits than non WAP users (P = 0.014). No significant difference was seen between the QOL scores of both groups at the entry point and after 24 weeks.

Conclusion: This newly developed WAP in JPGL 2012 was effective to reduce unscheduled visits of asthmatic children to doctors in Japan, where shortage of pediatric workforce in emergency units is serious.

1026
The radiological findings guide to the effect of tiotropium bromide on lung function in patients with severe asthma: a real-life study

Abadoglu, O[1]; Gumus, C[2]
[1]Immunology and Allergy Diseases Department, Cumhuriyet University Medical Faculty, Sivas, Turkey; [2]Radiology, Cumhuriyet University Medical Faculty, Sivas, Turkey

Background: In patients with poorly controlled asthma despite of treatment with inhaled glucocorticoids and LABAs, adding tiotropium bromide (TB) significantly improves asthma control. The aim of this study was to evaluate the radiological characteristics of the patients with severe asthma capable of predicting the effect of (TB) on lung function.

Method: We retrospectively assessed 633 patients with asthma followed in Allergic Diseases clinic between 2003 and 2011 and 64 severe asthmatic patients with TB had

added at least for 3 months to their treatment with inhaled glucocorticoids and LABAs were evaluated. One year after add-on TB treatment, the efficacy of TB on FEV1 and FVC percentages was compared by high resolution computed tomography (HRCT) characteristics of patients. The HRCT findings were categorised emphysema/bronchiectasis, fibrosis and ground glass appearance.

Results: At baseline, all patients had a mean FEV1 of 57.5 ± 15.4% and FVC 74.3 ± 15.7% before TB. Among patients with emphysema/bronchiectasis (18. 28.1%), there was significant improvement in FEV1 and FVC percentages between before and after TB treatment (FEV1 51.2 ± 13.4% vs 68.0 ± 14.5% and FVC 71.7 ± 13.9% vs 89.1 ± 13.4%, respectively, $P < 0.05$) However, there was no significant change in patients with fibrosis (34, 53.1%) and ground glass appearance (16.25%).

Conclusion: Combined treatment with TB may be useful in severe asthmatic patients with emphysema/bronchiectasis HRCT findings.

1027
Homemade spacers as an aid for persistent asthma control: a randomised pragmatic clinical trial

Rizzo, JA[1]; Schor, D[1]; Peixoto, DM[1]; Silva, AM[1]; Nunes, C[2]; De Almeida, MM[3]; Sarinho, ES[1]
[1]Serviço de Alergia e Imunologia Clínica – HC, Universidade Federal de Pernambuco, Recife, Brazil; [2]Centro de Alergia de Algarve, Algarve, Portugal; [3]Centro de Alergialmunoalergologia doHospital CUF Descobertas, Lisboa, Portugal

Background: Valved spacers are effective in reducing the need for hand-lung coordination and oropharyngeal deposition of inhaled medications administered by pressurized metered-dose inhalers (pMDI) Previous studies showed good clinical response with the use of home-made spacers coupled to beta-agonists pMDI in the treatment of acute asthma, however, there are no studies regarding the use of homemade spacers coupled to steroid pMDI for persistent asthma treatment.

Objective: To compare the efficacy of HFA-beclomethasone dipropionate (HFA/BDP) administered through a pMDI coupled with homemade or valved commercial spacers in the control of persistent asthma.

Method: A pragmatic parallel randomised clinical trial was conducted with 63 poorly controlled persistent asthma patients that were randomised to use an HFA/BDP pMDI coupled to homemade spacers made of plastic 500 ml bottles (Group HS, $n = 32$) or to commercial valved spacers (Group CS $n = 31$). Treatment responses were compared assessing the variations in asthma control test (ACT) scores and FEV_1 values measured before, 30 and 60 days after treatment.

Results: Both groups had basal comparable ACT scores and FEV1 values ($P > 0.05$) but showed statistically significant improvement in ACT scores after 30 and 60 days (7 and 7.8 points for the HS group and 5.9 and 7 points for the CS group, respectively $P < 0.001$). There was no statistically significant difference between groups at any observation time. The same behavior was observed for FEV1.

Conclusion: Asthma control achieved with HFA/BDP administered through a pMDI was similar when either coupled to a homemade or commercial spacers. These results may be valuable when planning asthma treatment for low-income communities.

1028
Significance of yoga in asthma management to improve the status of quality of life

Agnihotri, S; Kant, S
Department of Pulmonary Medicine, King George's Medical University, Lucknow, India

Background: Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation causes an associated increase in airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing at night or in the early morning. About 300 million people are suffering from asthma globally and about 10% of it about 30 million asthmatics belong to India. To best of our knowledge there is only one of the studies related to quality of life of asthmatics. The present study is attempted to show the effect of yoga on quality of life of asthmatics.

Method: In the present study 276 subjects with mild to moderate asthma were recruited from the Department of Pulmonary Medicine, King George's Medical University, U.P., Lucknow, India. They were randomly divided into two groups' yoga/intervention group ($n = 121$) and control group ($n = 120$), 35 subjects were dropout in completion of the study. The yoga group received an intervention based on yoga (asanas, pranayama and meditation), in addition to standard medical treatment and the control group received only standard medical treatment, then both groups were assessed at 0th, 3rd and 6th month with the help of Asthma Quality of Life Questionnaire (Elizabeth Juniper, England).

Results: There was statistically significant improvement found at 3rd month in yoga group in total quality of life as well as the four sub domains of Asthma Quality of Life Questionnaire (symptom score, activity limitation score, emotional function and environmental stimuli score) while the significant improvement was seen in control group by 3rd month only in total quality of life and environmental stimuli score.

Conclusion: In this present randomised controlled trial, there was a significant improvement found in Asthma Quality of Life scores in both groups over the period of 6 months. But the improvement was achieved earlier by yoga group in comparison to control group. Thus yoga can be used as an adjuvant therapy in the management of asthma to improve the status of quality of life.

1029
Control of allergic rhinitis and asthma test: an evaluation of administration by telephone interview

Pereira, AM[1,2]; Nogueira-Silva, L[3]; Morais-Almeida, M[4]; Sá e Sousa, A[5]; Jacinto, T[2,5,6]; Azevedo, LF[2,5]; Bugalho de Almeida, A[7]; Fonseca, JA[1,2,5]
[1]Allergy Department, Centro Hospitalar São João, E.P.E., Porto, Portugal; [2]Department of Health Information and Decision Sciences, Faculty of Medicine of the University of Porto, Porto, Portugal; [3]Internal Medicine Department, Centro Hospitalar S. João, E.P.E., Porto, Portugal; [4]Immunoallergy Department, Hospital CUF-Descobertas, Lisbon, Portugal; [5]Faculty of Medicine of the University of Porto, CINTESIS – Centre for Research in Health Technologies and Information Systems, Porto, Portugal; [6]Allergy Unit, Instituto and Hospital CUF Porto, Porto, Portugal; [7]Faculty of Medicine of the University of Lisbon, Pulmonology University Clinic, Lisbon, Portugal

Background: The use of CARAT (Control of Allergic Rhinitis and Asthma Test) questionnaire in individuals with asthma and rhinitis (AR) is validated (Fonseca JA, Allergy 2010 and Clin Transl All 2012); however, its telephonic application and the use in healthy individuals were not studied. We aimed to evaluate the telephonic application of CARAT and to describe its scores in individuals with neither asthma nor rhinitis.

Methods: We analyzed data from the Portuguese Survey on Asthma Control (INCA. This was a cross-sectional, population-based telephone survey including a sample of 2232 Portuguese inhabitants. Participants classified in four groups: Asthma (AST), Rhinitis (RHI), Asthma and rhinitis (AR) and Healthy (H). Healthy individuals were defined as those without AST, RHI, self-reported heart disease and without chronic medication (considering pathologies that might interfere

with disease classification) in the 4 weeks previous to the questionnaire. Subjects that could not be included in one of the predefined groups ($n = 613$), those younger than18 years old ($n = 351$), and those that didn't complete CARAT ($n = 295$) were excluded from the analysis. Total CARAT score (CARAT-T) (range: 0, worst, to 30, best) was divided in upper airways (CARAT-UA) and lower airways (CARAT-LA) scores; lack of control was defined as CARAT-T£24. Correlations with Mini-Asthma Quality of Live Questionnaire (miniAQLQ) and mini-Rhinitis Quality of Life Questionnaire (miniRQLQ) were computed.

Results: Overall, 973 individuals (57% female) were included; the mean (SD) age was 50(18) years; 159 were classified in AST group, 229 in RHI, 111 in AR and 474 in H. The mean (SD) score of CARAT-T was 29.6(1.6) in H, 24.1 (5.5) in AST, 24.4(5.6) in RHI and 18.9(7.7) in AR ($P < 0.001$). Ninety nine percent of the healthy ($n = 467$) had CARAT-T > 24 (vs 54% of those with AST, 61% with RHI and 30% with AR, $P < 0.001$); 431(86%) of the healthy had CARAT-T $\geq$ 29. In the AST group, the correlations between miniAQLQ and CARAT-T and CARAT-LA scores were 0.656 e 0.727, respectively ($P < 0.001$). In the RHI group, the correlations between miniRQLQ and CARAT-T and CARAT-UA were −0.756 and −0.683, respectively ($P < 0.001$).

Conclusions: CARAT, applied telephonically, had adequate correlations with miniAQLQ and miniRQLQ. Moreover, healthy individuals presented very high scores in CARAT, as expected in the absence of disease. These results provide preliminary support for the administration of this questionnaire by telephone.

1031
Difficulties in education of the asthmatic patients

Gruchała-Niedoszytko, M[1]; Niedoszytko, M[2]; Skrzypski, M[3]; Jassem, E[2]; Malgorzewicz, S[1]
[1]Clinical Nutrition, Medical University of Gdansk, Gdansk, Poland; [2]Allergology, Medical University of Gdansk, Gdansk, Poland; [3]Oncology, Medical University of Gdansk, Gdansk, Poland

Background: Education of the asthmatic patients is a crucial step of the treatment. The aim of the project was to create a program of asthma education targeted at the personal needs of the patients.

Method: Patients suffered from asthma were referred to the Asthma School near the Department of Allergology Medical University of Gdansk, Poland by their physicians. The educational program included initial meeting where pathology of the disease was discussed, the methods of treatment, the proper inhalation of the drug, the peak flow meter usage, the self management in the asthma exacerbation. Patients were asked about the most interesting topics which could be discussed at the following meetings. All patients were examined by the allergologist, spirometry and in check examination was performed. The inhalation technique of the drug was evaluated.

Results: The group of consecutive 217 patients was analyzed including 159 (73%) women and 58 (27%) men. All patients declared the participation in the following meeting, however only 30% of the patients continued the educational program. The most interesting topics declared by the patients included the allergen avoidance (34%), the self management of the life threatening conditions (28%). Among the patients who participated in the following parts of the education 96% declared that the education has helped them to achieve asthma control. The improper inhalation of the drugs was fond in at least 40% of the patients.

Conclusion: Asthma education is a difficult process, which could help adherent patient to achieve better asthma control.

1032
Achieving better control of asthma by a self education program in Asian immigrants with low socioeconomic status and complete ignorance of the Greek language

Fevranoglou, C; Tsaroucha, A; Petras, P; Ralli, M; Konstantopoulou, G; Dimitriadou, E; Pavlakou, G
8th Pulmonary Department, Sotiria General Hospital of Chest Diseases, Athens, Greece

Background: Asthma, a chronic lung disease that affects people of all ages, races and ethnic groups, is a growing concern throughout the world. In our country where the arrival of immigrants is continuous and the recent economic crisis rises, we tried to integrate immigrant asthmatic patients in a training program in order to achieve the best possible control and minimise the need of hospital admission.

Method: We scheduled a low cost self education program conducted by a single pulmonologist for 23 Pakistan and Bangladesh patients admitted to the emergency room in 2011. They spoke little English and no Greek. The majority was unemployed. 17/23 had no inhalers. Only 3/23 were submitted to follow up. We supported groups of four patients every month for 3 months. With an 1-h educational session in simple English and with the aid of global sign language and designs, we explained them about asthma and its potential triggers, the proper inhaler techniques and the self- management of disease. Our instructions were aided by FEV1 and PEF measurements at baseline and at 3 months. Finally they were given therapy with inhalers and followed up for 1 year by telephone counseling and scheduled visits. After a year, they were reassessed by the number of hospitalisations, unscheduled visits and oral steroids used.

Results: 3/23 patients abandoned program due to return home and relocation. Totally, 20 patients attended the program. At the end of 3 months a questionnaire was given to assess their asthma knowledge. All patients answered correct about the course of disease and were well educated to the use of inhalers. Their self-management plan was re-examined by the doctor. 19/20 patients demonstrated significant improvements in lung function at 3 months and 1 year. Only 3/20 admitted to unscheduled emergency room visit whereas the majority of patients was completely covered by telephone advice. Completing 1 year follow up period only 2/20 received oral steroids and only one was hospitalised due to severe exacerbation.

Conclusion: Asian asthmatic immigrants could be very well educated to recognise and manage their asthma by a simple and low cost program. All we need is doctors with spare time and an appetite to offer. Further studies using more patients are necessary to improve our approach.

1033
The impact of sensitisation to aeroallergens on asthma control

Qutub, M; Koshak, E; Tayeb, M
King Abdul Aziz University, Jeddah, Saudi Arabia

Background: Sensitisation to aeroallergens has a significant role in the aetiology and exacerbation of allergic asthma. For optimal asthma management, current guidelines recommend the assessment of both control level and sensitisation to aeroallergens.

Objective: To examine the relationship between patterns of sensitisation to aeroallergens and levels of asthma control.

Method: This is a cross-sectional study over 1 year period starting from January 2011. Asthmatics were sequentially selected from the allergy clinic of King Abdul Aziz University Hospital at Jeddah, Saudi Arabia. Asthma control level was based on Global Initiative for Asthma (GINA) guideline. The degree of sensitisation to aeroallergens was measured *in-vivo* by the wheal size and the number of positive reactions on standard skin prick test (SPT).

SPSS was used to analyse any statistical correlation.

Results: One hundred and eighteen asthmatics with a mean age of 34 ± 14 years were included, of which 63.6% being females. Asthma was controlled in 16 cases (14.5%), partially controlled in 26 cases (23.6%) and predominantly uncontrolled in 68 cases (61.8%). SPT to common aeroallergens was positive in 81 asthmatics (77.9%): in 54 uncontrolled asthmatics (55.1%), 13 partially controlled asthmatics (13.3%) and nine controlled asthmatics (9.2%). The predominant inhalant allergens were to *Dermatophagoides pteronyssinus* in 57 cases (54.8%), *Dermatophagoides farinae* in 49 cases (47.1%), cat epithelia in 35 cases (33.7%) and cockroach in 23 cases (22.1%). Asthmatics with positive SPT were significantly correlated with poor control level ($P = 0.038$).

Conclusion: This is one of the early studies that explore the impact of sensitisation to aeroallergens on asthma control. Sensitisation to indoor aeroallergens predominate in outpatient asthmatics. This promotes the search for sensitisation to aeroallergens by SPT if asthma is not controlled.

Poster Session 37

Management of drug allergy: desensitisation and beyond

1034
A successful desensitisation protocol for horse derived anti thymocyte globulin (h-ATG) in children with severe aplastic anemia

Demir, E[1]; Cigerci Gunaydin, N[1]; Karadas, N[2]; Gulen, F[1]; Tanac, R[1]; Esen, S[2]; Atasever, M[1]; Yilmaz, D[2]
[1]Pediatric Allergy and Immunology, Ege University Faculty of Medicine, Izmir, Turkey; [2]Pediatric Hematology, Ege University Faculty of Medicine, Izmir, Turkey

Background: Horse derived anti thymocyte globulin (h-ATG) is accepted as the first treatment option in patients with acquired aplastic anemia (AA) without suitable sibling donor. Since h-ATG is a product of heterologous sera, it has the potential to produce anaphylaxis. It is recommended that patients' hypersensitivity status should be determined with skin test prior to h-ATG administration. In patients who demonstrate hypersensitivity to ATG alternative treatment options are suggested to be searched. Although desensitisation has severe risks for allergy and anaphylaxis, in patients who lack alternative treatment option desensitisation should be considered.
Method: Patients with severe AA who were treated at Ege University Medical School, Children's Hospital, were screened with skin prick test with undiluted h-ATG solution before administration of h-ATG. Patients who had a negative result with this test underwent intradermal test with h-ATG at the dilution rate of 1/1000, 1/100, 1/10 and then with undiluted solution. The patients who had an enduration >10 mm at any dilution rates were taken into the h-ATG desensitisation. Desensitisation was started with intravenous 0.1 ml (0.0025 mg), 1/1000 diluted h-ATG, every 30 min the dose given to the patients was doubled until the planned total dose was achieved. During desensitisation, all patients were monitorized to see blood pressure, cardiac pulse, respiratory rate, oxygen saturation every 15 min. The whole period was observed and managed by an attending allergist.
Results: A total of five patients, four boys and 1 girl with severe AA, aged between 7 and 16.5 years (mean 11.2 years) had an enduration >10 mm after intradermal test with h-ATG. They all were given the same desensitisation protocol. No adverse event was seen in any of the patients during or after desensitisation protocol. One patient experienced arthralgia 7 days after the desensitisation. All of these patients completed the planned h-ATG treatment (h-ATG 20 mg/kg/day for 8 days, with prednisolon, cyclosporine A and Granulocyte Colony Stimulating Factor).
Conclusion: Our protocol revealed a successful desensitisation to h-ATG in children with AA. Although it may cause severe allergic reactions, the expected benefit from the treatment is very high. Therefore, we recommend our protocol as a safe and effective protocol to desensitise children who require h-ATG, of course, under optimum conditions and with skilled and experienced team.

1035
Successful chemotherapy desensitisation in a Portuguese immunoallergology department, based on Brigham and Women's Hospital Model

Caiado, J[1]; Mendes, A[1]; Elisa, P[1]; Pereira-Barbosa, M[1]; Castells, M[2]
[1]Immunoallergology Department, Santa Maria Hospital, Lisbon, Portugal; [2]Division of Rheumatology, Immunology, and Allergy, Department of Medicine, Brigham and Women's Hospital, Boston, MA, United States

Background: Hypersensitivity reactions (HSR) to chemotherapy agents are increasingly frequent and lead to the discontinuation of potentially curative drugs. Rapid desensitisation is the only strategy to maintain first line therapy and to protect against anaphylaxis. The protocol developed by the Allergy Department at Brigham and Women's Hospital (BWH) in Boston has been successfully applied in hundreds of patients and thousands of desensitisations. The aim of this review is to validate the BWH 12–16 steps protocol in a Portuguese cancer population referred to the Immunoallergology Department in Lisbon's Santa Maria Hospital.
Method: A retrospective review between July 2008 and December 2012 of patients referred for evaluation of mild to severe chemotherapy HSR and desensitisation was done. Aspirin and montelukast were added as pre-medications in addition to the standard H1 and H2 anti-histamines for desensitisations. Skin testing was done for platin sensitive patients. The desensitisation protocol mimicked the BWH three bags with different concentrations (1:100, 1:10 and 1:1 of the final target concentration) infused in 12 consecutive steps at increasing concentration and infusion rates, over 5.8 h. Breakthrough reactions during the desensitisation procedures were assessed.
Results: We desensitised 114 patients to 117 chemotherapy agents (three double desensitisations), with a total of 502 desensitisations. The desensitised drugs included platins (carboplatin = 24; oxaliplatin = 33; cisplatin = 3), taxanes (docetaxel = 30, paclitaxel = 22), monoclonal antibodies (Trastuzumab = 3; Cetuximab = 1) and Liposomal Doxorubicin (n = 1). Skin tests with platins were positive in 57 out of 60 patients. Forty-nine (9.7%) HSRs occurred (mild = 34; moderate = 11; severe = 4), of which 88% were induced by platins. Most HRS occurred on the first desensitisation (28–57%) and on the last step of the desensitisation protocol (33–67%). Epinephrine was used in four patients. Only one patient did not receive the target dose due to recurrent anaphylaxis.
Conclusion: The majority of patients (487–97%) tolerated the desensitisations with only mild or no HSR, with no deaths, confirming the efficacy and safety of the BWH protocol. This review highlights the importance of developing a Desensitisation Program for all allergic cancer patient populations in need of first line therapy with well trained allergists and nurses.

1036
Non allergic rhinitis associated with angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists

Montoro de Francisco, AM[1]; Garcia Luque, A[2]; Burgos Pimentel, AM[1]; Fonseca Avendaño, JA[1]; De Vicente Jiménez, T[1]; Puerro Vicente, M[2]; Mateos Galván, JM[1]
[1]Allergy Service, Hospital Central de la Defensa Gómez-Ulla, Madrid, Spain; [2]Pharmacology, Hospital Central de la Defensa Gómez- Ulla, Madrid, Spain

Background: Angiotensin-Converting Enzyme Inhibitors (ACEI) and Angiotensin II Receptor Antagonists (ARA II) are widely used. Although there are few references in the literature of rhinitis associated

to these drugs, it is not a well known adverse drug reaction (ADR) and it is not included in the summary of product characteristics.

Method/design: A case series of 50 patients with rhinitis associated with ACEI/ARAII.

Scope: Allergy service, Hospital Central de la Defensa, Madrid.

Period: March 2009–December 2012.

Main variables assessed: demographic and clinical variables, diagnostic test, treatment, evolution, causal realtionship between drugs and rhinitis according to the modified Karch Lasagna algorithm.

Results: Fifty patients with persistent rhinitis. Five severe rhinitis with anosmia and 45 moderate, all of them associated with ACEI/ARA II. In addition to rhinitis, the patients showed cough, conjunctivitis and angioedema. Age 68.1 years (37–85), 36 females and 14 males. Ten drugs were involved: ACEI (enalapril, captopril and lisinopril), ARA II (valsartan, losartan, olmesartan, ibersartan, candesartan, eprosartan and telmisartan). More frequently involved drugs were enalapril (30 cases), valsartan (15 cases) and losartan (nine cases). Re-exposure to drug ($n = 22$ patients 44%). Treatment was drug withdrawal, achieving complete remision in all cases, in an average time of 7.2 weeks (3–12 weeks).

The causal relationship between drugs and rhinitis were defined as 22/50 and probable as 28/50.

Conclusion: Antihypertensive drugs are widely used and they should be taken into account in moderate/severe persistent rhinitis. ADR caused by these drugs is resource-consuming and has a direct effect in patients'quality of life. Postmarketing Surveillance Systems should inform health professionals of this ADR.

1037
How does drug hypersensitivity affect the patients' mood?

Ozseker, ZF[1]; Bulut, i[1]; Angın, U[2]; Gürgen, E[2]; Gelincik, A[3]; Demirturk, M[3]
[1]Immunology and Allergy, Sureyyapasa Chest Diseases and Chest Surgery Training Hospital, Istanbul, Turkey; [2]Liason Psychiatry, Sureyyapasa Chest Diseases and Chest Surgery Training Hospital, Istanbul, Turkey; [3]Istanbul Faculty of Medicine, Immunology and Allergy, Istanbul University, Istanbul, Turkey

Background: Drug hypersensitivity (DH) accounts for approximately 5–10% of all adverse drug reactions. DH may cause various clinical disorders ranging from mild skin rush to anaphylaxis or even death. Patients having had a severe reaction with drugs may worry about their future drug exposures. The objective of this study was to evaluate the effects of past drug reactions on patients' mood.

Method: In this cross sectional study 51 consecutive patients with DH history (42 female, mean age 38.6 ± 11.9) who were referred to our hospital for a challenge test for alternative drug and 51 healthy volunteers were included. After their clinical evaluations, all subjects underwent the following psychodiagnostic tests: Beck depression inventory, Beck anxiety inventory and WHO quality of life questionnaire (WHO-QoL -brief). The scores of these tests were compared between DH and control groups.

Results: Thirty one of the patients had experienced anaphylaxis with one or more drug (of which eight had anaphylactic shock). Other common symptoms caused by DH were urticaria and/or angioedema, maculopapular rush and fixed drug eruption respectively. Non-steroidal antiinflammatory drugs ($n = 28$) were the most common culprit drugs, followed by antibiotics as the second. In 20 patients doctors had prescribed the culprit drug despite the patient's warning about his or her past history. DH patients had higher anxiety scores ($P = 0.000$), while depression and OQoL scores were not different from the healthy subjects ($P > 0.05$). There wasn't any correlation between the number and severity of drug reactions with anxiety, depression and QoL scores.

Conclusion: The results of this study show that drug hypersensitivity causes a significant degree of anxiety in the patients. It is between the responsibilities of an allergist to give enough psychological support and education to DH patients besides the allergological tests necessary to find the safe alternatives. In addition, to increase the awareness about drug allergy between the practioners is an utmost requirement.

1038
Immediate type allergic drug reactions with first line antituberculosis drugs: how to manage?

Koycu, G[1]; Keren, M[1]; Dursun, AB[1]; Güler, M[2]; Dulkar, G[2]; Kalaç, N[2]; Özkara, Ş[2]; Öner Erkekol, F[1]
[1]Immunology and Allergy Department, Atatürk Chest Disease and Thoracic Surgery Training and Research Hospital, Ankara, Turkey; [2]Chest Diseases Department, Atatürk Chest Disease and Thoracic Surgery Training and Research Hospital, Ankara, Turkey

Aim: The aim of the study was to determine the frequency, risk factors and the characteristics of immediate type allergic reactions due to first line antituberculosis drugs and to evaluate the usefulness of graded challenge protocols in this group of patients.

Method: Patients referred to our allergy clinic with allergic symptoms in 2 h after the consumption of antituberculosis drugs were included in the study. Demographics, disease and treatment characteristics and the allergic symptoms of the patients were recorded. Antituberculosis drugs were re-administered gradually according to a defined protocol. Demographics, disease and treatment characteristics of other tuberculosis patients, treated in our hospital in the same time interval, were also screened from their files. The two groups were compared in terms of these traits.

Results: In 1 year period 563 tuberculosis patients were treated in our hospital. The patients using second line tuberculosis drugs, those with insufficient file information and the ones with mycobacterial infection rather than tuberculosis were excluded. Three hundred and seventy-nine patients were included in the study. Eighteen allergic reactions were detected in 13 patients. The severity of the reaction was grade 1 in 72%, grade 2 in 17% and grade 3 in 11% of the patients. The only risk factor for allergic reactions was female gender. (OR: 4.085 95% CI: 1.234–13.522) In 11 patients HRZE, in two patients R were given with graded challenge protocols. Only in two patients allergic reactions with R were detected. In these patients, we stepped down to the previous dose and than restarted to increase the dose according to the protocol. All of the patients managed to use the drugs.

Conclusion: Immediate type allergic reactions due to antituberculosis drugs are not related to the disease or treatment characteristics. Graded challenge protocols used in this study are safe and efficient ways to re-administer the drugs to the patients.

1039
The Irish experience of rapid desensitisation to chemotherapeutic agents: a case series

Mahabir, S[1]; Reynolds, C[1]; Keane, M[2]; Tormey, V[1]
[1]Immunology Department, Galway University Hospital, Galway, Ireland; [2]Oncology Department, Galway University Hospital, Galway, Ireland

Background: A hypersensitivity reaction (HSR) to a chemotherapeutic agent can severely limit a patient's options for treatment of malignancy. Rapid desensitisation protocols for chemotherapeutic agents have been published. These are potentially lifesaving treatments when no suitable alternative is available. Seven patients have undergone rapid desensitisation in our centre and are the first to do so within the Republic of Ireland to our knowledge.

Method: We conducted a retrospective chart review of seven oncology patients who had a documented HSR to either oxaliplatin or rituximab, followed by rapid desensitisation in University College Hospital, Galway, between 2008 and 2012. All patients received desensitisation as per protocol by Castells et al.
Results: Initial HSRs were usually moderate, with one severe. While patients with HSRs to oxaliplatin were exposed to the agent on multiple occasions prior to the initial HSR, those with HSRs to rituximab reacted on first exposure. The time required for the initial protocol varied from 4.5 to 8 h, and most had minor or moderate reactions during the procedure. Skin-prick testing was positive to oxaliplatin at a concentration of 5 mg/ml in one patient and intradermal testing was positive for rituximab at a concentration of 0.1 mg/ml in one patient. Desensitisation was successful for six out of seven patients. These six patients were able to complete their treatment course of chemotherapy using the rapid desensitisation protocol on the Oncology ward.
Conclusion: In summary, we present the first patient cohort who underwent rapid desensitisation to chemotherapeutic agents in the Republic of Ireland. These seven patients completed a total of 32 desensitisation procedures, with most of these proving successful. These procedures require multidisciplinary input, including pharmacists, nursing staff, oncologists and immunologists, and are very labour intensive. The initial desensitisation requires a high-dependency bed, which is not always readily available on an elective basis. These procedures are likely to remain uncommon and used only in carefully selected cases.

1040
Negative predictive value of typing safe local anesthetics

Specjalski, K; Kita-Milczarska, K; Jassem, E
Department of Allergology, Medical University of Gdansk, Gdansk, Poland

Background: Although allergy to local anesthetics (LA) is rare, patients often report unwanted reactions after their administration. History of anaphylaxis or untypical reaction related to LA is an indication for typing safe anesthetic for future surgical or dental procedures. The aim of the study was to determine negative predictive value of typing safe local anesthetics.
Method: One hundred and fifty-four patients with the history of unwanted reaction to local anesthetic were enrolled into the study. Stepwise typing of safe anesthetic included skin prick tests and intracutaneous tests with three or four of the following LA: lidocaine, bupivacaine, mepivacaine, articaine. Skin tests were followed by provocations with one or two of LA. Telephone follow-up visits were performed 4–12 months after drug typing. On the basis of follow-up questionnaire results negative predictive value (NPV) of protocol was calculated.
Results: The full protocol was performed in 148 patients. Positive results of SPT were found in two, ICT-19 and provocations – in 11 cases. Lidocaine was assessed as safe in 44, bupivacaine -in 14, mepivacaine- in 34 and articaine – in 61 patients. Drug typed at clinical visit was administered in 78 patients and 76 reported no reactions (NPV = 97%).
Conclusion: Stepwise approach including skin prick tests, intracutaneous tests and provocations is safe and allows typing safe anesthetic in vast majority of patients.

1041
Usefulness of skin testing in preventing hypersensitivity to iodinated contrast media

Berti, A; Della Torre, E; Barbara, G; Yacoub, M-R; Sabbadini, MG; Colombo, G
Department of Allergy and Clinical Immunology, San Raffaele Scientific Institute IRCCS, Milano, Italy

Background: Anaphylaxis to iodinated contrast media (ICM) represents a major problem in clinical settings where repeated radiological examinations are required. In particular, after a first hypersensitivity reaction has occurred, the extent to which steroid premedication is protective towards a second ICM exposure has to be established. Recently, skin tests demonstrated a significant reliability in diagnosing ICM allergy when performed within the correct timing, as proposed by EAACI recommendations. However, no data are presently available regarding their usefulness in preventing hypersensitivity reactions before ICM re-exposure.
Methods: Of 33 patients tested in our department between 2010 and 2012 because of a previous ICM reaction, 16 (13 females, three males; mean age 55 years) underwent to a second radiological examination after the skin tests with ICM, and were interrogated after the procedure. Skin prick tests (SPTs), intradermal tests (IDTs) and patch tests were performed with iohexol, iomeprol, iopromide and iodixanol according to standardised protocols in order to identify a culprit and an alternative ICM. The median time interval between the first reaction and skin testing was 14.5 (3–23) weeks. The second radiological examination was preceded by premedication for all the patients and was performed with an alternative ICM selected on the basis of skin test results.
Results: Of the 16 patients tested, previous hypersensitivity manifestations consisted of immediate reactions in nine patients (56%), and non-immediate reactions in seven patients (44%). With respect to diagnostic skin testing, none of the patients had positive SPTs, whereas seven patients had positive IDTs: three at immediate reading IDTs, two at 48 h and two at 72 h. Patch tests were negative. Only one patient presented a mild non-immediate reaction (urticaria) 48 h after alternative ICM re-injection.
Conclusion: In our patients series, skin testing for ICM hypersensitivity demonstrated a negative predictive value of 93.7%. Recently, Caimmi and al. reported similar results, however, in their study, the great majority of patients was tested more than 6 months after the occurrence of the hypersensitivity reaction, when sensitisation is known to significantly decrease. Our results support standardised skin testing as a useful tool for selecting safe alternative ICM and introduce additional arguments of debate concerning the role of premedication for re-exposure to ICM.

1042
Aspirin hypersensitivity: long term results of ASA desensitisation therapy

Muehlmeier, G; Hausch, R; Maier, H
ENT, German Federal Armed Hospital Ulm, Ulm, Germany

Background: Relapsing nasal polyps are frequently coincident with a hypersensitivity to aspirin or other NSAIDs. To date there exist only weak data of the long term courses of ASA desensitisation therapy.
Method: One hundred and thirty-eight patients with a positive reaction to a intravenous ASA provocation testing were desensitised with a dosage of 100–500 mg daily according to the thresholds of reaction in their provocation followed by observation and examination over 24 months.
Results: Seveenty-seven percent of the desensitised patients showed improvement, while 16% observed no changes and 7% had signs of progression of the disease. Improvement resulted mainly in reduction of persisted sniffing, nasal polyps and obstruction as well as bronchial asthma symptoms. 4.2% had to undergo re-surgery due to relapsing polyps. Sixty-nine percent adhered to desensitisation after 1 year and 51% after 2 years.
Conclusion: ASA desensitisation therapy is an effective instrument of prophylaxis

against nasal polyps carried out without relevant complications, well tolerated and with promising therapeutic results. Adherence rates are similar to specific allergen immunotherapy or medication regimens in arterial hypertonic disease.

1043
Delayed type drug reactions due to chemotherapy agents: re-administration, is it possible?

Keren, M; Köycü, G; Öner Erkekol, F; Dursun, AB
Immunology and Allergy Department, Atatürk Chest Disease and Thoracic Surgery Training and Research Hospital, Ankara, Turkey

Background: Delayed- type drug reactions due to chemotherapeutic agents isn't a well-defined topic. Limited number of drug options in cancer patients raises the question, weather these drugs can be applied safely again.
Method: Patients referred to our allergy clinic with delayed type hypersensitivity reactions due to chemotherapy agents between January 2009 and August 2012 were included in this study. Demographics, disease and chemotherapy treatment characteristics and the allergic symptoms of the patients were recorded. Culprit drugs were re-administered with a prolonged premedication protocol.
Results: A total of 24 cases (20 non-small cell lung cancer, two small cell lung cancer, two mesothelioma) were evaluated in our clinic. The mean age of the patients was 59.88 ± 7.02. Twenty-two (91.7%) cases were male, 2 (8.3%) cases were female. The reactions developed after the use of gemcitabine, pemeterexed, docetaxel in 11, 4, two patients, respectively and after concomitant therapy with cisplatine-gemcitabine, cisplatine-paclitaxel, carboplatine-docetaxel and cisplatine-pemeterexet in 4, 1, 1, 1 patients, respectively. Fourteen (45.2%) of the reactions developed in the first applications. Seven (29.2%) patients had only erythema, 17 (70.8%) patients had additional lesions. Four patients received only antihistamines, 18 patients were treated with corticosteroids and antihistamines. In 14(58.3%) patients culprit chemotherapy agent/agents were re-administered with a prolonged premedication (methylprednisolone applied 13, 7, 1 h before chemotherapy and antihistamine applied 1 h before chemotherapy). All of these patients tolerated the culprit agents well. Ten (41.7%) patients did not receive the culprit drug for the second time in this study group.
Conclusion: Delayed-type drug reactions due to chemotherapeutic agents could be seen in the first application. These reactions respond well to therapy. Prolonged premedication protocol can be an effective and safe regimen in lung cancer to allow continuation of the treatments to which patients have presented with delayed type hypersensitivity reactions.

1044
Desensitisation to allopurinol in delayed hypersensitivity reactions

Ribeiro, F; Faria, E; Gomes, R; Calado, G; Almeida, E; Carrapatoso, I; Segorbe-Luís, A
Immunoallergology, Coimbra University Hospital, Coimbra, Portugal

Background: Allopurinol is the most frequently prescribed drug for the treatment of hyperuricemia. There are very limited alternatives in the case of hypersensitivity (HS) to allopurinol and a desensitisation therapy must be considered. The aim of this study was to perform a retrospective analysis of patients who underwent desensitisation to allopurinol.
Method: We analyzed, retrospectively, the medical files of patients with delayed allopurinol HS reaction undergoing desensitisation, observed in a 6 year period (2007–2012). The demographic data, pathology that motivated prescription of allopurinol and concomitant diseases/medications were evaluated. Moreover, the desensitisation protocol followed and the adverse reactions were analyzed.
Results: Six out of seven patients were male, with ages between 37 and 79 years (mean age of 64 ± 14 years) at the time of desensitisation. The type of reaction was fixed erythema in three patients, urticaria with/without angioedema in two, anaphylaxis in one and maculopapular rash in one. Six patients had gouty joint pathology and one had hyperuricemia with chronic renal failure. Six patients had associated cardiovascular pathology and all were polymedicated. The desensitisation protocol used was adapted from Vervloet (1999), with an initial dose of 10 μg up to 300 mg/day, adjusted in case of an adverse reaction. There were no complications in the progression of the protocol in three patients and the remaining patients had mild/moderate skin reactions. In patients with HS reaction during desensitisation, only one did not required dose adjustment, while in the other three it was necessary to decrease the dose. The maintenance dose therapy was achieved extending the duration of desensitisation. The time of desensitisation varied between 16 and 22 days.
Conclusion: In this study the majority of patients had HS reactions during protocol and dose adjustment was necessary. We emphasize the effectiveness of a slower protocol desensitisation in the therapy of patients with allopurinol HS delayed reactions, such as in patients with fixed erythema.

1045
COX-2 inhibitors are better tolerated than paracetamol and meloxicam by patients with intolerance to non-steroidal antiinflammatory drugs

Escobar Bolaños, C; Peralta Filpo, G; Cimarra, M; González, ML; Robledo, T; Rodríguez, M; Fernandez Rivas, M
Allergy, Hospital Clinico San Carlos, Madrid, Spain

Background: The non- steroidal antiinflammatory drugs (NSAIDs) are the most widely used drugs worldwide given its antiinflammatory, analgesic and antipyretic properties. As a result they are frequently involved in adverse reactions.
Method: We conducted a retrospective study in which we reviewed the medical records of patients diagnosed of NSAID intolerance in our Department over a period of 18 months (from May 2011 to November 2012). All the patients had been challenged, following a single-blind placebo-controlled procedure, with progressive increasing doses of paracetamol (up to 1 or 2 g cumulated dose), meloxicam (up to 15 mg), and the selective COX-2 inhibitors celecoxib (up to 200 mg) and etoricoxib (up to 90 mg). All patients had given written consent to assess the tolerance of these alternative drugs.
Results: A total of 57 patients, 37 females (65%) and 20 males (35%), with a mean age of 48.5 years were included. According to the previous reactions to NSAIDs they were classified in three phenotypes: 41 patients (72%) had cutaneous reactions, eight subjects (14%) had respiratory symptoms, and eight patients (14%) had both skin and respiratory involvement. Four patients (7%) reacted to paracetamol, and two subjects to meloxicam (3.5%), whereas celecoxib and etoricoxib were tolerated by all subjects. The patients who reacted to meloxicam were one female with respiratory reactions to NSAIDs who tolerated 1 g of paracetamol; and one male with a cutaneous phenotype who tolerated 2 g of paracetamol. The four patients who reacted to paracetamol included two females and one male with cutaneous reactions, and one male with respiratory reactions to NSAIDs. All the patients who tolerated paracetamol and meloxicam also had good tolerance to celecoxib and etoricoxib. We have not found an association between sex and phenotype of reactivity to NSAIDs with the challenge outcome of the four alternative drugs tested.

Conclusion: Our results suggest that coxibs are better tolerated than paracetamol and meloxicam by patients with NSAIDs intolerance, and that all the subjects who tolerate paracetamol and meloxicam also tolerate coxibs. If the latter is confirmed in a larger group of patients, it will not be necessary to assess the tolerance of the coxibs in the hospital setting in patients who have previously tolerated paracetamol and/or meloxicam.

1046
Outcomes of the management for patients with antituberculosis drug-induced cutaneous adverse drug reactions

Thongraung, W[1]; Rerkpattanapipat, T[2]; Pungrassami, P[3]
[1]Clinical Pharmacy Department, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla, Thailand; [2]Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [3]Zonal TB Center 12, Yala, Thailand

Background: Cutaneous adverse drug reactions (CADRs) have been reported during tuberculosis (TB) treatment but antituberculosis (anti-TB) drugs should not be ceased without necessary. TB guidelines, 2010 by World Health Organization recommended that a patient who developed itching without a rash should try symptomatic treatment with antihistamines and continued TB treatment and a patient who developed a skin rash, all anti-TB drugs must be stopped. In contrast to another TB guideline, a treatment should be based on CADR severity. If there was no sign of severe CADR such as fever and/or mucous membrane involvement, anti-TB drugs can be continued with closely monitor. Previous studies had limited data on these recommendations. This study aimed to investigate the outcomes of the management for TB patients with mild to moderate CADRs.
Method: During August 2011–September 2012, 576 adult TB patients from 13 hospitals of southern Thailand were included, 108 patients (18.8%) developed CADRs. The decision to stop or continue antituberculosis drugs was depended on their attending physicians. The outcomes of the management for CADRs classified by severity were recorded. Severity of CADRs was classified into three types. Mild was itching without a rash. Moderate was any rash without a sign of severe CADRs. Severe included a rash such as a petichial rash, exfoliative dermatitis, Stevens-Johnson Syndromes and toxic epidermal necrolysis (SJS-TEN), and a rash with fever, mucous membrane and/or internal organ involvement.
Results: Of the 108 patients, 63 (58.3%) were mild, 39 (36.1%) were moderate (maculopapular rash, $n = 20$; erythematous rash, $n = 14$; erythematous papule, $n = 2$; urticaria, $n = 3$), and 6 (5.6%) were severe CADRs (SJS-TEN, $n = 4$ and maculopapular rash with fever, $n = 2$). Of 63 patients with mild CADRs, 61 patients continued TB treatment with or without antihistamines and two patients stopped TB regimens. CADRs were improved among all patients with mild CADRs. Of 34 patients with moderate CADRs who continued their TB treatment with oral antihistamines and/or topical steroids, 33 patients (97.1%) resolved their symptoms and only one patient (2.9%) had to further cease the treatment.
Conclusion: Almost mild to moderate CADRs can be resolved by oral antihistamines and/or topical steroids without treatment interruption.

1047
Immediate-type allergic reactions due to chemotherapy agents: what have we learned in 3.5 years period?

Öner Erkekol, F[1]; Yılmaz, Ü[2]; Keren, M[1]; Yılmaz, N[2]; Köycü, G[1]; Bayız, H[2]; Dursun, AB[1]
[1]Immunology and Allergy Department, Atatürk Chest Disease and Thoracic Surgery Training and Research Hospital, Ankara, Turkey; [2]Chest Diseases Department, Atatürk Chest Disease and Thoracic Surgery Training and Research Hospital, Ankara, Turkey

Background and aim: Hypersensitivity reactions due to chemotherapy agents limit the use of these drugs. Since replacement with an alternative chemotherapy regimen is often limited by tumour sensitivity, approaches to treat patients with cancer who have potential allergies are needed. Immediate-type allergic reaction rates due to chemotherapy agents, reaction types, risk factors and approach to treat these reactions will be discussed.
Method: Demographics, disease and treatment characteristics of the patients with immediate-type allergic reaction were compared with the patients without allergic reaction. Allergic reaction characteristics and desensitisations in these patients were discussed in detail.
Results: About 30 542 days of chemotherapy was applied to a total of 3049 patients between January 2009 and June 2012. Seventy-two immediate type allergic reactions have been identified in 61 patients (2.0%) The rates of drug-induced allergic reactions were 1.21%, 0.55%, 0.14%, 0.05%, 0.26% reaction/dose for docetaxel, pactitaxel, cisplatin, carboplatin, and etoposide, respectively. We did not observe any immediate allergic reaction with gemcitabine, vinorelbine, pemetrexed, irinotecan, vincristine, adriamycin, cyclophosphamide, topotecan. The patients with allergic reactions were younger (56.61 ± 9.66 vs 60.84 ± 9.51, $P = 0.003$). Female gender was the other risk factor. (OR: 2.57 95% CI: 1.39–4.75) The severity of the allergic reactions was grade 1 in 33.3%, grade 2 in 38.9% and grade 3 in 27.8% of the reactions. In vast majority of the reactions, antihistamines (83.3%) and systemic corticosteroids (94.4%) were applied. Adrenalin was used in seven allergic reactions (9.7%). A total of 76 desensitisations were performed in 27 patients (three cisplatin, 34 docetaxel, 20 paclitaxel, 19 etoposide). Only two patients developed allergic reactions during desensitisation, still desensitisations were completed successfully in all patients.
Conclusion: Substantial level of allergic reactions due to chemotherapeutic agents has been detected in lung cancer patients. Rapid desensitisation is an effective and safe regimen to allow continuation of the treatments in these patients.

1049
Efficacy of a desensitisation protocol in a patient with a hypersensitivity reaction to denosumab

Gutierrez Fernandez, D[1]; Foncubierta Fernández, A[2]; Medina Varo, F[3]; Fernandez Melendez, S[4]; Fernandez Anguita, MJ[5]; Anguita Carazo, JL[6]
[1]Hospital Universitario Puerta del Mar, UGC Neumologia-Alergia, Cadiz, Spain; [2]Distrito Sanitario Bahia de Cadiz-La Janda, UGC Joaquin Pece, San Fernando, Spain; [3]Hospital Universitario Puerta del Mar, UGC Reumatologia, Cadiz, Spain; [4]Hospital Universitario Carlos Haya, UGC Alergia, Malaga, Spain; [5]Hospital Universitario Puerta del Mar, UGC Farmacia, Cadiz, Spain; [6]Complejo Hospitatalario de Jaen, UGC Alergia, Jaen, Spain

Background: Denosumab is a human monoclonal antibody with a high affinity and specificity for RANKL (Receptor activator of nuclear factor Kappa B.), preventing it from activating its receptor, RANK, on the surface of osteoclasts and their precursors. The recommended dose is 60 mg subcutaneously every 6 months. We report the case of a 65-year-old woman with a history of generalised osteoporosis, intense bone pain and risk of fracture, referred from the Orthopaedic department after failed treatments for osteoporosis with bisphosphonate therapy (alendronic acid and risedronic acid), who experienced an adverse reaction to Denosumab 1 week after the first dose of the drug, resulting in generalised urticaria over thighs, abdomen, breasts and back, bilateral eyelid angioedema and a pruriginous lesion at the injection site in the abdomen, all of which resolved within 15 days of treatment with H1-antihistamine and oral and local corticosteroids.

Method: We performed skin prick tests for neumoallergens and latex, and further prick tests with undiluted and 1:10 and 1:100 dilutions of Denosumab, which were repeated on 10 control subjects who had had no prior contact with the drug; patch test responses to Denosumab were measured, total and specific IgE levels determined, and an 8-step desensitisation protocol was implemented.

Results: The skin prick tests for neumoallergens and latex were negative in the patient with an allergy to Denosumab and in the control group of healthy subjects. Total IgE was below 100 kU/l and specific IgE tests were negative. The patch test responses were negative. The 8-step desensitisation protocol, shown in Table 1, was completed in 2 h, and, to date, continues to be well tolerated.

Conclusion: Denosumab desensitisation has proved a safe and effective alternative therapy for patients who are unable to tolerate other treatments, but have experienced a hypersensitive reaction to the drug.

1050
Diagnosing hypersensitivity reactions to radio contrast media

Gomez, F[1]; Ariza, A[2]; Salas, M[1]; Sanchez-Rivas, MI[2]; Doña, I[1]; Blanca-López, N[3]; Aranda, A[2]; Mayorga, C[2]; Blanca, M[1]; Torres, MJ[1]
[1]H.R.U Carlos Haya Malaga, Malaga, Spain; [2]Fundación IMABIS, Malaga, Spain; [3]Hospital Infanta Leonor, Madrid, Spain

Background: Immediate hypersensitivity reactions (IHR) to radio contrast media (RCM) are those appearing within 1 h after RCM administration and are usually mild, although fatal reactions have been reported. Currently, there is not a consensus on the diagnostic approach in patients with IHR to RCM. The aim of this study was to analyse the diagnostic value of skin test, drug provocation test (DPT) and basophil activation test (BAT) in patients with symptoms compatible with IHR to RCM.

Method: We studied patients referred over a 5-year period (2006–2011) to our Allergy Service because of a clinical history suggestive of an IHR after RCM administration. Prick and intradermal (IDT) skin tests with a panel of RCM were done, and if negative, DPT was carried out with the culprit RCM. Also, in cases with skin test or DPT positive, tolerance was assessed with an alternative RCM. In those confirmed as allergic, BAT was done with a panel of RCM.

Results: Eight out of 90 subjects evaluated (8.9%) were confirmed as allergic: 5 (62.5%) by skin testing and 3 (37.5%) by DPT. Regarding skin testing, three cases were prick-test positive (one to Iodixanol, one to Iomeprol and one to Iohexol) and five IDT positive (four to Iohexol, three Iodixanol and two to Iomeprol). DPT was positive in three cases to Iodixanol, two to Iomeprol and one to Iobitridol and to Iohexol. BAT was positive in five cases (62.5%): four to Iohexol, four to Iodixanol and two to Iomeprol, and negative in 20 controls with good tolerance to RCM.

Conclusion: Allergy to RCM was confirmed in 9% of the patients, being both skin tests and DPT necessary for establishing the diagnosis. Moreover, the BAT has been shown as a valuable method for diagnosis.

1051
Value of tryptase measurement in 111 cases of suspected anaphylaxis during general anaesthesia

Dua, S; Ewan, PW
Allergy Department, Addenbrooke's Hospital, Cambridge, United Kingdom

Background: Tryptase rises in acute allergic reactions and is useful in confirming anaphylaxis. However there is limited data on its use in general anaesthesia (GA), value in less severe reactions, time course and optimal sample timing.

Method: We studied tryptase in 111 patients age range 7–89 years, (aetiology investigated in 106 in our clinic) with suspected anaphylaxis during GA. Anaesthetic charts, drug charts and tryptase results were pre-obtained and aetiology determined, using skin prick, intradermal tests and challenge. Reactions were graded 1–4 by severity. Baseline tryptase was measured (ImmunoCAP). Tryptase data was analysed using six time periods. Time course and true baseline were determined and peak levels were related to severity and aetiology.

Results: In paired samples, levels at 0–1 and 1–2 h were statistically similar (mean 75 and 67 ng/ml), but fell by 20% from 2 to 4 h. A correlation was found between peak tryptase and severity ($P = 0.01$). In grade 4 reactions mean peak levels were 97 ng/ml or 1500% of baseline and grade 1 + 2 reactions 218% of baseline. Levels were elevated when allergy was the cause (92%); similar for antibiotics, gelofusine, patent blue and non-steroidals and slightly higher for neuromuscular blocking agents ($P = 0.064$). Values >200 ng/ml were found in seven patients (persisting at 4 h in 4), five with profound hypotension or cardiorespiratory arrest. In two of three physiological reactions tryptase was not raised. Twenty-four hour tryptase was higher than the clinic baseline (mean 12.6 and 7.6 ng/ml respectively, $P = 0.003$).

Conclusions: Tryptase is of value in GA reactions. Elevated values were found in allergic reactions of all causes. Levels correlated with severity and were increased even in milder reactions. Similar peak values occurred at 0–1 and 1–2 h; levels fell thereafter. Acute sampling at 0–2 h should suffice, but if missed a 4 h sample is still useful. Twenty-four hour levels are not always the true baseline.

Table 1 Denosumab desensitization protocol

Dose	Dose (mg)	Cumulative dose (mg)	Blood pressure (mmHg)	Arterial pulse	Adverse reactions
1	0.005	0.005	166/77	87	None
2	0.050	0.055	152/65	84	None
3	0.500	0.555	147/73	80	None
4	1.500	2.055	145/73	67	None
5	3.000	5.055	150/72	73	None
6	7.500	12.555	166/82	70	None
7	15.000	27.555	149/81	68	None
8	33.000	60.555	144/73	76	None

Poster Session 38

Epidemiology of drug allergy

1053
Non-steroidal antiinflammatory drug hypersensitivity in adults and risk factors for asthma

Karakaya, G; Celebioglu, E; Kalyoncu, AF
Adult Allergy Unit, Department of Chest Diseases, Hacettepe University School of Medicine, Ankara, Turkey

Background: Non-steroidal antiinflammatory drug (NSAID)-hypersensitivity (NH) in relation to self-reported reaction patterns with underlying/accompanying diseases has not been studied in Turkey. Risk factors for later development of asthma are not well known. We aimed to investigate the relation between NH and chronic urticaria, rhinitis/rhinosinusitis, and asthma. Our secondary aim was to define risk factors associated with asthma.
Method: Data of 1137 NSAID-hypersensitive patients were analyzed via the allergy clinic database. Patients were divided into four groups with respect to their accompanying diseases (chronic urticaria, asthma, rhinitis/rhinosinusitis). Asthmatic patients were compared to non-asthmatic group to define factors associated with asthma. A classification scheme for a relation between NH and astma was proposed.
Results: Reaction patterns, and patient characteristics in each group were different from the reference group (only NH). Asthma in patients with NH was associated with female gender, sinonasal polyposis/polyp surgery, rhinitis/rhinosinusitis, NSAID-induced rhinitis/asthma or blended reaction pattern, immediate reaction after NSAID intake, self-reported food allergy history, and family history of asthma.
Conclusion: Chronic urticaria, rhinitis/rhinosinusitis, and asthma are closely related with NH. They precede or later develop during the course. More than a quarter of astmatic patients with NH experience urticaria/angioedema type reactions, and there may be different phenotypes of Samter's syndrome which needs further investigation.

1054
Evaluation of drug allergy during childhood: 5 years' experience

Aslan, GD; Soyer, OU; Cavkaytar, O; Sekerel, BE; Kalayci, CO; Sackesen, C; Tuncer, A
Department of Pediatric Allergy, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Background: Drug allergy is an important issue in the concept of adverse drug reactions. The child, who presented with symptoms of drug allergy, should be evaluated carefully with a detailed history and appropriate tests before decision of avoidance of culprit drug or use of alternatives. In this study, we aimed to evaluate the prevalence of drug allergy in children who were prediagnosed as 'drug allergic' in our department and risk factors associated with drug allergy in this population.
Method: We enrolled children who attended Hacettepe University, Pediatric Allergy Department with a history of adverse drug reaction and prediagnosed as 'drug allergic' between 2005 and 2010, retrospectively. We noted demographic features and history of adverse drug reactions with ENDA questionnaire. The 'drug allergy' was diagnosed by skin and/or provocation tests with the culprit drug.
Results: Ninety-six children who attended to our department because of adverse drug reactions and prediagnosed as 'drug allergic' participated in the study. A total of 140 suspected drug hypersensitivity reactions were evaluated. Eleven children were diagnosed as 'drug allergic' with skin test and five children with drug provocation tests. A child had an anaphylaxis due to ondansetron and subsequent cardiac arrest in our hospital so that we did not perform any diagnostic tests. The prevalence of chronic disease was high in children with 'drug allergy' compared to children without 'drug allergy' [58.8% vs 26.5% ($P = 0.018$)]. Drug anaphylaxis history was associated with increased risk for 'drug allergy' [OR: 5.789, %95 CI: (1.880–17.554), $P = 0.002$]. The logistic regression analysis for diagnosis of 'drug allergy' showed that history of dyspnea [OR: 5.589, %95 CI (2.118–16.209), $P = 0.002$] and sweating [OR: 11.156, %95 CI (2.118–16.209), $P = 0.011$] during adverse drug reaction were risk factors.
Conclusion: The diagnosis of drug allergy was made in 17.7% of children with a prediagnosis of 'drug allergic' in our population. History of anaphylaxis in drug reactions is important for diagnosis of 'drug allergy'. This study will guide prospective studies in the field of drug allergy during childhood in Turkey.

1055
Drug-induced urticaria and angioedema in Latin America

Jares, EJ[1]; Sanchez Borges, M[2]; Ensina, LF[3]; Arias Cruz, A[4]; Gómez, M[5]; Diez-Zuluaga, S[6]; Ramírez-Giraldo, RH[6]; González Díaz, S[4]; Mimessi, G[5]; Macias Weinmann, A[7]; Sole, D[3]; Serrano, C[8]; Camelo Nunes, I[3]; De Falco, A[9]; Barayazarra, S[10]; Cherrez, I[11]; Schuhl, J[12]; Salvatierra, A[13]; Cuello, MN[14]; Toche Pinaud, P[15]; Mercovich, G[16]; Vinuessa, M[17]; Weisz, A[16]; Zanacchi, A[10]; Andrade, D[3]; Machado, L[3]; Cardona Villa, R[6]
[1]CMP, Buenos Aires, Argentina; [2]Centro Medico-Docente La Trinidad, Caracas, Venezuela; [3]Federal University of São Paulo, São Paulo, Brazil; [4]Hospital 'Dr. Jose Eleuterio Gonzalez' Universidad Autónoma de Nuevo León., Monterrey, Mexico; [5]Hospital San Bernardo, Salta, Argentina; [6]Universidad de Antioquia, Medellin, Colombia; [7]Hospital 'Dr. Jose Eleuterio Gonzalez' Universidad Autónoma de Nuevo León., Monterrey, Mexico; [8]Fundación Valle del Lili, Cali, Colombia; [9]Universidad Nacional de La Plata, La PLata, Argentina; [10]Nuevo Hospital San Roque, Córdoba, Argentina; [11]Hospital Kennedy, Guayaquil, Ecuador; [12]Hospital Británico, Montevideo, Uruguay; [13]Fundair, San Luis, Argentina; [14]Consultorios San Juan, San Juan, Argentina; [15]Clínica Las Condes, Santiago, Chile; [16]Hospital Municipal Prof. Dr. Bernardo A. Houssay, Vicente López, Argentina; [17]Universidad de Rosario, Rosario, Argentina

Background: Clinical features and management of urticaria-angioedema (U-A) triggered by drugs in Latin America have not been previously described. The present initiative focuses on putative etiologic agents, allergological work up and treatment.
Methods: A descriptive cross sectional study using a modified ENDA questionnaire was implemented in 19 allergology units from 11 different Latin American countries, reporting patients assisted in the last year because of a suspected hypersensitivity drug reaction. Patients presenting U-A were selected. Causal relationship was categorised into certain, probable, possible, unlikely, and conditional, according to WHO-UMC Causality Categories.
Results: From 564 patients evaluated, 403 (71%) presented U-A. Sixty-nine percent of them were female, and 53% had an atopic background. Nineteen percent had a

history of previous U-A, 33% of drug reactions, and 12% had presented previous reactions with the same drug. Certain and probable causal relationship were attributed to: NSAIDs in 63% of the patients, beta-lactams in 14%, non-beta-lactam antibiotics in 8%, local anesthetics in 3%, radio-contrast media, gastroenterological drugs and neurological drugs, in 2%, steroids, muscle relaxants, ACE inhibitors, other cardiological drugs, monoclonal antibodies, chemotherapy, opiates and antiparasitic drugs in 1% each. In cases with certain and probable causal relationship non-allergic hypersensibility was present in 51% of the patients, IgE-mediated reactions in 36%, and cell-mediated reactions in 9%. Thirty-two percent of the reactions were mild, 49% moderate and 19% severe. Skin prick tests were done in 13% of patients, and specific IgE in 7%. Drug provocation tests were performed in 140 patients (35%), being positive in 30% of the patients provoked.

Sixty-two percent were treated in the ED, 15% by an allergist, 8% by a general practitioner, 6% were self medicated, and 8% received no medication. Seventy-seven patients had severe reactions, but only 23 received epinephrine.

Conclusions: Female sex, previous history of atopy and drug allergy stand as the most prevalent features in patients showing drug induced U-A. NSAIDs, and antibiotics were the drugs implicated in more than 80% of patients. More than half of the reactions were treated in the ED but epinephrine was administered only in 30% of the patients with severe reactions. Medical education of general and ED physicians in Latin America should focus on this topic.

1056
Prevalence of drug allergy in healthcare workers, in Albania

Shytaj, KM[1]; Pistulli, E[1]; Hoxha, S[2]; Deda, L[3]; Shytaj, ZX[4]; Mingomataj, EÇ[5]
[1]Faculty of Medical Sciences, University of Tirana, Tirana, Albania; [2]Legal Medicine Institute, Tirana, Albania; [3]Faculty of Medicine, University of Tirana, Tirana, Albania; [4]Ambulatory Service, University of Hospital Centre 'Mother Tereza', Surgery Hospital, Tirana, Albania; [5]Clinic of Allergy, University of Hospital Centre 'Mother Tereza', Tirana, Albania

Background: Healthcare workers are reported to be in a higher risk for drug allergy, due to their professional exposure. This study intends to measure the prevalence of these allergies in healthcare workers in Albania.

Method: A cross-sectional study was conducted at the University Hospital Centre 'Mother Theresa' where 662 nurses were requested to fill up a questionnaire. Six hundred and thirty-nine of them accepted to answer the questionnaire. The questionnaire has detailed questions about the drug that caused the allergy, its nature, treatment, and diagnostics tests. The RR and the chi-square were calculated between three subgroups; 0–9, 10–19 and >20 years of work in health services, with 95% CI. The data were analyzed with MS Excel 2007 and PASW Statistics 18.

Results: The prevalence of drug allergy as reported by the subjects was 13.62% (87). Only one subject reported allergy of more than one chemically unrelated drug (penicillin and streptomycin). As analyzed by the therapeutic classes, antibiotics caused 72.41% (63) followed by non-steroid anti-inflammatory drugs that caused 10.33% (9) of all the drug allergies. As analyzed by the chemically related classes beta-lactams caused 35.63% (31) followed by the aminoglycosides 16.09% (14).

The two subgroups (10–19 and >20 years) had a significantly higher risk when compared with the subgroup that have 0–9 years of work in health services (respectively, RR = 3.11; 95% CI = 1.49–6.48, $P < 0.05$ and RR = 3.54; 95% CI = 1.78–7.01, $P < 0.05$).

Conclusion: This is the first study about drug allergies in Albanian healthcare workers. The most common drug allergies in our group are those caused by antibiotics, especially by beta-lactams followed by aminoglycosides and NSAIDs. The duration of professional engagement clearly impacts the risk for drug allergies.

1057
Acute allergic reactions – 1 year clinicoepidemiologic study

Radenkova-Saeva, J[1]; Petrunov, BN[2]
[1]Toxicology Department, Emergency Hospital 'Pirogov', Sofia, Bulgaria; [2]Laboratory of Allergy, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

Aim: The aim of the study is to present some clinicoepidemiologic data in patients with acute allergic reactions.

Methods: The records of the Toxicology Clinic, Department 'Toxicology-adults', Emergency Hospital 'Pirogov' were reviewed retrospectively for all hospitalised patients due to acute allergic reactions during 1 year period – from January 1, 2010 to December 21, 2–10.

Results: The number of patients hospitalised in the Department, due to acute allergic reactions was 629 during that period of time. There were 239 men (38%) and 390 women (62%), median age 53 (range 18–88) years. The number of allergic reactions du to drugs was 137 (21.78%), and allergic reactions with other or unknown etiology – 492 (78.22%). The main groups of drugs – induced adverse events are: antibiotics – in 73 patients (53.28%), ACE inhibitors – 26 patints (18.98%), NSAIDs – in 17 patients (12.41%), analgetics – in 15 patients (10.95%), other drugs – in six patients (4.38%). The proportion of patients with life-threatening anaphylactic reactions is relatively high – 7.5%.

Conclusion: According to statistics, antibiotics are the most common cause of acute drug allergy. Our results confirm this fact and therefore should not resort to the use of antibiotics withouit prescription – the choice of drug, dosage, duration of intake should be determined by a medical doctor.

1060
Recurrent angioedema by antihypertensive

Burgos Pimentel, AM[1]; Fonseca Avendaño, JA[1]; Montoro de Francisco, AM[1]; García Luque, A[2]; Núñez Hernandez, MÁ[1]; De Vicente Jiménez, T[1]
[1]Allergy, Hospital Central de la Defensa Gómez Ulla, Madrid, Spain; [2]Farmacología Clínica, Hospital Central de la Defensa 'Gómez Ulla', Madrid, Spain

Background: Angioedema is an adverse drug reaction (ADR) serious, rare but known of inhibitors of angiotensin converting enzyme (ACEI) inhibitors and less frequently to receptor antagonists of angiotensin II (ARB). We report three patients who developed angioedema important, two of them previously with enalapril, and more severe to replacing it with an ARB.

Method: Three patients admitted for angioedema located in oral cavity, including the tongue and pharynx, with involvement in two of them glottis, in their backgrounds featuring previous episodes of angioedema associated with enalapril in two of them (with admission), and current treatment with ARB (valsartan 2, losartan 1) respectively in the three. Were ruled out alternative causes of angioedema. Were performed [prick test with common aeroallergen and food battery, total and specific IgE, tryptase, complement fractions (C2, C3, C4), CH50, C1 inhibitor and CH100]. The causal connection between ARB and angioedema was established according to the Karch Lasagna modified algorithm, used by the Spanish Pharmacovigilance System (SEFV).

Results: The allergy study either *in vivo* or *in vitro* was negative, except for olive pollen sensitisation in a patient. The complement study was normal in all cases.

The ARB is replaced by a calcium channel blocker + diuretic. Following the withdrawal of the ARA involved angioedema was not observed in any of the patients. The causality applying SEFV algorithm was defined in all cases.

Conclusion:

1 Angioedema associated with ACE inhibitors and ARB is a serious RAM, not unusual by the widespread use of these drugs.
2 Although less prevalent their relationship with ARB, they must be used with caution, and not always the substitution of ACEI by ARB prevents the angioedema recurrence.

1061
Drug hypersensitivity: a case/non-case study from a Tunisian pharmacovigilance database

Chaabane, A; Ben Fadhl, N; Chadli, Z; Ben Fredj, N; Boughattas, NA; Aouam, K
Pharmacology, Medicine University, Monastir, Tunisia

Background: Beside the pharmacovigilance algorithms, the case/non-case is a recent pharmacoepidemilogic approach based on the measurement of the association between a drug intake and the occurrence of (adverse drug reactions) ADRs in a pharmacovigilance database. We carried a case non case study aiming to identify the association between drug hypersensitivity and drug classes.
Methods: The data were obtained from a Tunisian pharmacovigilance database. The ADRs reported with a causality assessment of certainly, probably or possibly drug related (according to the Naranjo score) were analyzed. The association between drugs and ADRs was assessed using the case/non-case method. The 'cases' were defined as reports of drug hypersensitivity (anaphylactic reactions and skin eruptions). The 'non-cases' were all the other ADRs reports. The frequency of the association between drug hypersensitivity and the suspected drug in comparison with the frequency of drug hypersensitivity associated to all the other drugs was calculated using the ADR reporting odds ratio (ROR) and their 95% confidence intervals.
Results: Overall 1144 reports of ADRs were analyzed; of which 623 were excluded because they were unclassifiable or unlikely in terms of causality assessment. Therefore, the analysis was carried out on 521 reports of which, 392 (75.2%) were drug hypersensitivity. These reactions were skin eruptions (88.5%) and anaphylactic reactions (11.5%). Drugs most frequently associated with drug hypersensitivity were antibacterial drugs (ROR = 2.8, 95% CI [1.8–4.3], $P < 10^{-3}$) and non steroidal anti inflammatory drugs (ROR = 2.3, 95% CI [1.2–4.2], $P = 0.006$). Among antibacterial agents, betalactams were the only group with a significant ROR (4.3; 95% CI [2.4–7.7], $P < 10^{-3}$). Regarding betalactams, both penicillins and cephalosporins were associated to drug hypersensitivity (ROR = 5.1; 95% CI [2.3–11.3], $P < 10^{-3}$) and (ROR = 3.1; 95% CI [1.3–7.4], $P = 0.01$), respectively.
Conclusion: Results on antibacterial agents' classes are in accordance with previous findings, indicating that betalactams are the most frequently associated with drug hypersensitivity. However, given the widespread use of these drugs, awareness should be raised among patients and prescribers about these risks.

1062
Adverse drug reactions during childhood: evaluation of 47 cases

Ozhan, AK; Altıntas, DU; Karagoz, DD; Karakoc, GB; Yilmaz, M
Pediatric Allergy & Immunology, Cukurova University, Adana, Turkey

Background: Adverse drug reactions can be seen in 5–15% of the patients treated with drugs. In this study we aimed to evaluate the profile of 47 children with drug allergy who admitted to Cukurova University, School of Medicine, Pediatric Allergy and Immunology outpatient clinic during last 2 years.
Method: A total of 47 children (13 girls and 34 boys) with the mean age and standard deviation of 6.95 ± 4.4 years were enrolled to the study. Detailed clinical history, physical examination findings, skin prick tests (SPT), specific Ig E levels and provocation tests were used for the diagnosis of drug allergy.
Results: Penicillin/amoxicillin group antibiotics were the most accused drugs with 32% followed by NSAIDs (18%), sefalosporins (14%), paracetamol (8%), metamizol (4%) and local anesthetics (3%). Reactions to more than one drug were determined in 23 cases (48%) Urticaria was the most common clinical finding with 57% followed by urticaria + angioedema (19%), angioedema without skin rash (10%), anaphylaxis (4%), Steven Johnsons Syndrome (4%) and urticarial vasculitis (4%). Provocation tests were performed in 11 patients who had multi drug use and in whom a unique suspicious drug was not detected with anamnesis and laboratory findings. Provocations were done with paracetamol ($n = 4$), NSAIDs ($n = 2$), amoxicillin ($n = 2$), clarithromycin ($n = 2$) and pencillin ($n = 1$). One subject with clarithromycin and another with NSAID showed urticarial rash during provocation.
Conclusion: Even though anamnesis, SPT and specific IgE tests hold important place in the diagnosis of drug allergy, provocation tests should always be kept in mind as the gold standart in selected patients.

1063
Unsubstantiated claims of 'snake products' as complementary and alternative medicine or just snake oil

Wong, HCG
Department of Medicine, Vancouver General Hospital, University of British Columbia, Vancouver, BC, Canada

Background: Complementary and alternative medicine (CAM) are readily available in different parts of the world. Many have claims not substantiated.
Method: Visits were made to markets, herbal shops and pharmacies in Hong Kong, China, Da Nang, Vietnam, Macau, China and Istanbul, Turkey. Some CAM with 'snake products' were selected and examined carefully.
Results: Four preparations with 'snake products', one from Hong Kong (1), one from Da Nang (2), one from Macau (3) and one from Istanbul (4) were noted to have claims of indications or effectiveness.

1 TRI-SNAKEGALL CHUAN PEI YE
Indications: Cough from common colds, pulmonary weakness and sputum excessive phlegm.
Ingredients: Tri-snakegall (three snake gall bile), Chuan Pei Ye (Fritillaria liquid), Ma Huang (Ephedra) and others.

2 SNAKE WINE
Usage: rheumatism, lumbago, sweat of limbs.
Ingredients: an entire snake in wine.
'Real speciality of Vietnam'

3 NOTHING TOOTH CHAN THREE SNAKES TRADEMARK OIL
Indications: paresis of extremities, dizziness and headache, muscle spasm, insect bites, burn, bruise, rheumatism.
Ingredients; wintergreen oil, peppermint oil, turpentine oil and others but no snake product identified.

4 SNAKE OIL
Claim: Treatment for hair loss.
Ingredients: Not listed.

Conclusion: CAM with label of 'snake' may not contain 'snake products'. Claims of indications or effectiveness of these CAM are not substantiated. Patients should be aware of the potential side effect and toxicity.

1064
Prevalence of quinolones allergy in an allergy unit

Jara Gutierrez, PF; Uriarte, SA; Fernandez-Nieto, M; Sastre, J
Alergologia, Fundacion Jimenez Diaz, Madrid, Spain

Background: Quinolones are relatively safe drugs with antibiotic therapeutic indications. The prevalence of quinolone allergy has increased in recent years.
Method: We retrospectively reviewed our database from August, 2011 to July, 2012, obtaining 626 patients with suspected allergy to drugs, though not including beta-lactams or antiinflammatory drugs. We selected patients with suspected quinolone allergy, performing standardised skin testing (ST) in these cases. Following informed consent, if the ST was negative, we performed oral or intravenous challenge test.
Results: We found 24 patients (24/626, 3.8%) with possible quinolone allergy. The diagnosis was confirmed in 8 (8/24, 33%) of them; five had positive ST, three negative ST, in which the challenge test was not performed by having a substantially suggestive anamnesis, one of this patient was diagnosed fixed drug eruption. In the intravenous challenge test (5/24, 20%), we noted a local irritative reaction in four patients (4/5, 80%). Challenge test was necessary for a correct diagnosis in 16 patients (16/24, 67%), which were negative. One patient (1/8, 12%) with quinolone allergy, had a history of allergy to other drugs. The most frequent quinolone studied was ciprofloxacin (75%).
Conclusion: In our allergy unit, quinolone allergy was shown to have a prevalence of 1.3% in patients studied by drug allergy and 33% in patients suspected of having quinolone allergy, increasing prevalence like in other centers. The diagnosis was supported by obtaining a positive ST, and others using medical records and challenge tests. The usefulness of ST was limited. The intravenous infusion drug may generate a local reaction, but not discard a drug allergy.

Poster Session 39

Clinical issues in drug allergy

1066
Passive transfer test in a patient with specific hypersensitivity to clavulanic acid

Cerda, JC[1]; Martorell, C[2]; Felix, R[1]; De las Marinas, MD[1]; Martorell, A[1]; Perez, I[3]; Iglesias, J[3]; Pineda, F[3]; Stahl Skov, P[4,5]
[1]University General Hospital, Valencia, Spain; [2]University Clinic Hospital, Valencia, Spain; [3]Department of Innovation and Development, Diater Laboratories SA, Madrid, Spain; [4]Department of Dermatology, University Hospital of Odense, Denmark; [5]Charite, Berlin, Germany

Background: We report new *in vitro* diagnostic techniques used to confirm the diagnosis of specific hypersensitivity to clavulanic acid (CA).
Method: A 31-year-old man presented skin rash and generalised pruritus a few minutes after taking the first dose of amoxicillin-clavulanic acid (AX/CA). Previously, this antibiotic had been well tolerated. Six months later, the patient tolerated well AX for 1 week. He was again treated with AX/CA, presenting with intensely pruritic generalised urticaria, followed by facial and lingual edema and dyspnoea 1 h after taking the first tablet. This resolved in 48 h after treatment with corticosteroids and antihistamines. Since then, beta-lactam antibiotics have not been prescribed. Skin prick test (SPT) and intradermal tests (IDT) were performed with penicillin G (PG) (10000 IU/ml), AX (20 mg/ml), AX/CA (20 mg/ml), benzylpenicilloyl octa-l-lysine (0.04 mg/ml) (PPL), sodium benzylpenicilloate (5 mg/ml) (DM), and potassium clavulanate (5–20 mg/ml) (DAP-clavulanic kit). Specific IgE levels to PG, penicillin V (phenoxymethylpenicillin potassium) (PV), AX and cefaclor (CF), UniCAP (Phadia) were determined. Histamine-release test (HRT) was made for CA, AX, AX/CA and PG (ReFlaB). A passive transfer test of the patient's serum to stripped basophils of a healthy subject was made and sensitised cells were challenged to the different drugs. Finally, a controlled oral challenge test (OCT) with PV and AX was made.
Results: SPT were positive for AX/CA at a concentration of 20 mg/ml and IDT was positive for CA at a concentration of 0.5 mg/ml. SPT and IDT were negative for PG, PPL, DM and AX. Controls were made with saline solution (−) and histamine (10 × 8 mm). Specific IgE to PG, PV, AX and CF was <0.35 kU/l. HRT was positive (class I) for CA and AX/CA (ReFlaB) and negative for AX and PG. When donor basophils were sensitised to the patient serum potassium clavulanate and AX/AC induced histamine release of 20% (300 μg/ml). No histamine release was observed when basophils were sensitised to non-allergic serum or when basophils were sensitised to patient serum pre-incubated with Omalizumab. OCT with PV and AX were negative.
Conclusion: HRT (ReFlaB, Denmark) and passive transfer tests, in combination with the DAP-clavulanic skin test kit, were useful and effective for the diagnosis of immediate hypersensitivity reactions to clavulanic acid. Passive transfer test reveals the presence of a specific-IgE to CA in the pacient serum.

1068
Local anaesthetics allergy: a case report

Lizarza, S[1]; Echeverria, JA[1]; Joral, A[1]; Lasa, EM[1]; Aranzabal, MA[2]; Echenagusia, MÁ[3]; Rey, M[4]
[1]Donostia Hospital, Allergy Unit, San Sebastian, Spain; [2]Allergy Unit, OSI Goierri-Alto Urola, Zumárraga, Spain; [3]Allergy Unit, OSI Bajo Deba, Mendaro, Spain; [4]Immunology Unit, Donostia Hospital, San Sebastian, Spain

Background: Local anaesthetics are very common drugs, usually very well tolerated. A variety of adverse effects associated with procedures involving local anaesthesia have been described but IgE- mediated reactions are extremely rare.
Method: We report the case of a 54-year-old woman who experienced 40 year ago facial angioedema after local anaesthesia at the dentist's office. Since then she claimed to be allergic to Procaine and Lidocaine; these allergies were confirmed in 2009 but allergy to Mepivacaine, Bupivacaine and Articaine was ruled out. Three years later, she was referred again because she suffered, immediately after she was given Mepivacaine, severe cutaneous itching, facial swelling, dizziness and throat discomfort.
Results: Prick and intradermal tests were performed with Procaine, Lidocaine, Mepivacaine, Bupivacaine, Articaine; a basophile activation test (BAT) was performed with Mepivacaine, Bupivacaine and Articaine. She was afterwards challenged with Bupivacaine and Articaine, with good tolerance of both drugs. The results are shown in the table:

Drug	Prick test	Intradermal test	Challege test	Bat
Procaine	+	N.D	N.D	N.D
Lidocaine	+	+++	N.D	N.D
Bupivacaine	Negative	Negative	Negative	Negative
Articaine	Negative	Negative	Negative	Negative
Mepivacaine	+	N.D	N.D	Positive

Conclusion: Although type I hypersensitivity reactions to amide-type local anaesthesics are rare, little is known about cross-reactivity among these drugs. We present a case report with confirmed allergy, by *in vivo* and *in vitro* tests, to Mepivacaine and cross reactivity *in vivo* to Procaine and Lidocaine. Also a extremely rare case of double allergy between esther and amide anaesthesics is shown.

1069
Captopril-induced DRESS: first reported case confirmed by patch test

Chaabane, A[1]; Ben Fredj, N[1]; Bel Hadj Ali, H[2]; Chadli, Z[1]; Boughattas, NA[1]; Aouam, K[1]
[1]Pharmacology, Medicine University, Monastir, Tunisia; [2]Dermatology, Monastir Hospital, Monastir, Tunisia

Background: Drug rash with eosinophilia and systemic symptoms (DRESS syndrome) is a severe and rare adverse drug reaction characterised by an association of a skin eruption, eosinophilia and a multivisceral involvement. Only one case of angiotensin-converting enzyme inhibitors-induced DRESS (ramipril) was reported in

the literature. We report a case of captopril-induced DRESS confirmed by skin tests.

Case report: A 59-year-old woman received a multidrug therapy including captopril, acebutolol and furosemide for hypertension. Three weeks after starting treatment, she developed a maculopapular itchy and edematous skin reaction, facial edema, fever and a cervical lymphadenopathy. The laboratory findings showed 2300/mm^3 of eosinophils associated to atypical lymphocytes. Alanine aminotransferase (ALT) was 67 IU/l (Normal range: 10–40 IU/l), aspartate aminotransferase (AST) was 57 IU/l (Normal range: 20–32 IU/l). The symptoms were thought to result from a hypersensitive reaction and the patient has withdrawn all the antihypertensive agents. The skin biopsy findings were in accordance with a hypersensitive skin reaction. The clinical and biological symptoms resolved completely 2 and 4 weeks after drug withdrawal, respectively. Six weeks later, patch tests were performed. Patch test to captopril (1% in petroleum) was strongly positive whereas patch test to acebutolol and furosemide remained negative. The captopril patch test was performed to a healthy control and revealed a negative result. A second series of patch tests to ramipril, delapril and perindopril was performed to evaluate cross reactivity with other angiotensin-converting enzyme inhibitors and showed negative results for all these drugs.

Conclusion: We add to the medical literature a first case of captopril-induced DRESS and pint out the usefulness of patch tests in diagnosing this reaction and evaluating the cross reactivity to other angiotensin-converting enzyme inhibitors.

1070
Ethambutol-induced DRESS: a case confirmed by patch test

Chaabane, A[1]; Chadli, Z[1]; Krifa, N[2]; Ben Fredj, N[1]; Boughattas, NA[1]; Aouam, K[1]
[1]Pharmacology, Medicine University, Monastir, Tunisia; [2]Oto-Rhino-Laryngology, Monastir Hospital, Monastir, Tunisia

Background: Drug rash with eosinophilia and systemic symptoms (DRESS syndrome) is a severe and rare adverse drug reaction characterised by an association of a skin eruption, eosinophilia and a multivisceral involvement. Antituberculosis agents have been rarely implicated in inducing DRESS. The most implicated drug were rifampicin and isoniazid. Only one case of ethambutol-induced DRESS was reported in the literature. We report the second case of ethambutol-induced DRESS confirmed by skin tests.

Case report: A 41-year-old woman has started an antituberculosis treatment based on a fixed combination of isoniazid, rifampicin, pyrazinamid and ethambutol (three tablets a day). Eight days later, she developed a maculopapular and itchy skin eruption, marked facial edema, oral mucosal erosion and fever. The laboratory findings showed 1600/mm^3 of eosinophils. Alanine aminotransferase (ALT) was 60 IU/l (Normal range: 10–40 IU/l), aspartate aminotransferase (AST) was 37 IU/l (Normal range: 20–32 IU/l). Lactate deshydrogenase (LDH) plasmatic level was at 421 IU/l (Normal range: 190–390 IU/l). The symptoms were thought to result from a hypersensitive reaction and the antituberculosis treatment was withdrawn. The clinical and biological symptoms resolved completely 2 and 3 weeks later, respectively. Because of the need of the antituberculosis treatment, the four drugs have been started again one at a time. The rechallenge of pyrazinamid, isoniazid and rifampicin was without any incident. However, 2 days after receiving ethambutol, the patient complained of a generalised pruritus with a marked increase of eosinophilis (1900/mm^3). Six weeks later, patch test to ethambutol (20% in petroleum) was performed and was strongly positive at 48 h reading. The ethambutol patch test was performed to a healthy control and revealed a negative result.

Conclusion: We add to the medical literature a second case of ethambutol-induced DRESS and point out that patch test appears to be useful and safe in diagnosing this reaction.

1071
Multiple hypersensitivity to antihistamines in a patient with chronic urticaria

Seifert, F[1]; Zirbs, M[1]; Eyerich, K[1,2]; Radopolou, E[3]; Ring, J[3]; Darsow, U[1,2]
[1]Dermatology and Allergy, Technische Universität München, Muenchen, Germany; [2]ZAUM-Center of Allergy and Environment, Technische Universität and Helmholtz Zentrum München, Muenchen, Germany; [3]Dermatology and Allergy, Technische Universitaet Muenchen, Muenchen, Germany

Background: Antihistamines (H_1-receptor-antagonists) are commonly used drugs in allergic diseases and pruritus. In spite of these drugs being generally well tolerated, some cases of hypersensitivity reactions (e.g. maculopapulous exanthema, fixed drug eruption, urticaria, photosensitivity and anaphylaxis) can be found in the literature. The allergic reaction is specifically connected to one single chemical group of antihistamines in most cases.

Case presentation: We present the case of a 21-year old female patient with a history of 1 year of chronically recalcitrant urticaria. The last 6 months before admission, she suffered from mild urticarial rashes every 2–3 days. She reported a severe exacerbation of the urticaria after H_1-antihistamine (cetirizine) intake, after general anaesthesia (with administration of dimetinden and cimetidine) and after intake of NSAID.

Repeated skin prick testing to the antihistamines cetirizine, fexofenadine and rupatadine, perioperatively used drugs and analgesics were negative. Oral, placebo-controlled provocation tests showed hypersensitivity to cetirizine, fexofenadine and rupatadine with sudden, severe urticarial reactions compared to the normally mild spontaneous urticarial rashes (in the majority of cases intravenous prednisolone had to be administered). Thus, one antihistamine representing the piperazines (cetirizine) and two representing the piperidines (fexofenadine and rupatadine) were not tolerated. Additionally, the patient's history suggests hypersensitivity to dimetinden (alkylamines) and cimetidine (H_2-antihistamine, guanidine-group). Further diagnostic tests with other chemical classes (azelastine, phenothiazines) are planned. This case demonstrates the possibility of hypersensitivity to multiple groups of H_1-antihistamines. Concurrent hypersensitivity to H_2-antihistamines has to be ruled out. In chronically recalcitrant urticaria, challange tests can lead to the diagnosis.

1072
Ondansetron hypersensitivity: a case report

Garcia Nunez, I[1]; Algaba Marmol, MA[2]; Escalona Peña, M[3]; Reina Ariza, E[1]
[1]Allergy Department, Hospital Quiron, Malaga, Spain; [2]Distrito Sanitario Osuna, Centro de Salud 'El Almorrón', Ecija, Spain; [3]Hospital Quiron, Malaga, Spain

Background: Serotonin antagonist hypersensitivity is very rare, being only described a few cases in the literatura. Our aim is to describe a patient with a drug hypersensitivity suspicion before the quimiotherapic treatment and after the premedication received and the protocol we performed to diagnose this patient.

Methods: A 29 years-old female with a breast ductal carcinoma came to our Allergy Department referring two anaphylactic episodes after intravenous premedication treatment (dexametasone 40 mg and ondansetron 8 mg) before quimiotherapic

treatment, needing medical treatment to control her symptoms.

Results: After explaining and signing the informated consent, we performed a Drug Provocation Test (DPT) single blind placebo controlled (all the provocations were like this one) with metilprednisolone 40 mg, confirming a good tolerance to this drug.

We performed skin prick test and intradermorreaction with ondansetron 0.02 mg/ml, presenting a positive intradermal result. To confirm these results, a DPT was performed, referring the patient after a 0.5 mg ondansetron intake odinophagia and generalised pruritus, with mild edema in pharynx and 98% O_2 saturation. We administered intravenous metilprednisolone 125 mg and intravenous dexclorpheniramine, being without symptoms after 1 h. The patient didn't needed to be observed as an inpatient.

A DPT with Dexametasone 40 mg and garnisetron were performed, confirming a good tolerance to these drugs. Other serotonin antagonists as tropisetron and palonosetron weren't tested, because cross-reactivity between ondansetron and tropisetron.

Conclusions:

1 We present an ondansetron hypersensitivity case report in an oncologic patient, with good tolerance to garnisetron.
2 A clinical report focusing in the implicated drug and the symptoms referred is very important to diagnose our patients.
3 All drugs implicated in a allergic reaction have to be tested in order to know their tolerance, except a good tolerance between the episode and the drug study.

1073
Selective IgE mediated reactions in a patient to clavulanic acid demostrated by histamine release

Cabañes Higuero, N[1]; Perez Perez, I[2]; Stahl Skov, P[3]; Pineda de la Losa, F[2]; Marchan Martin, E[4]; Iglesias Sanchez, JC[2]; Senent Sanchez, CJ[4]
[1]Hospital Virgen del Valle, Allergy, Toledo, Spain; [2]Diater Laboratories, Madrid, Spain; [3]University of Odense, Odense, Denmark; [4]Hospital Virgen del Valle, Toledo, Spain

Background: A precise diagnosis in betalactamic allergy is very important in order to prevent unnecesary treatment limitations. We present a case of 28 years old woman who, 30 min after oral administration of amoxicillin/clavulanic, presented a generalised urticaria. She was treated with corticosteroids and antihistamines with complete resolution. She had positive skin tests only to amoxicillin/clavulanic and clavulanic acid with negative tests to PPL, MDM, Penicillin G, ampicillin and amoxicillin. The oral challenge test with amoxicillin was negative.

Method: Histamine release from patient's basophils was tested by incubating blood with clavulanic acid using the HR-test kit (RefLab, Copenhagen Denmark). Further, histamine release after passive sensitisation with serum from the patient on stripped donor basophils was performed with the following drugs/compounds: DAP-Clavulanic (Potassium clavulanate), DAP-Amoxicillin (Sodium amoxicillin), Amoxicillin/Clavulanic, Penibiot (benzyl penicillin) & Clamoxyl. All compounds were tested in six concentrations in duplicate from 1000 to 1.9 μg/ml, dilution factor 3.5. Histamine release was detected by HR-Test from ReFLab. Control experiments included basophils sensitised with non-allergic serum and pre-incubation of sera with Xolair 1:1000.

Results: Histamine release from patient's basophils was positive to clavulanic acid with a maximal release of 98 ng histamine/ml but no release to amoxicillin. When donor basophils were sensitised to the patient serum Potassium clavulanate and amoxicillin/clavulanin induced histamine release of 15% at a concentration of 300 μg/ml. No histamine release was observed when basophils were sensitised to non-allergic serum or when basophils were sensitised to patient serum pre-incubated with Xolair. No histamine release was observed when sensitised basophils were challenged with sodium amoxicillin, penibiot or clamoxyl.

Conclusion: The patient's suspected allergic reactions to Clavulanic acid was confirmed *in vitro* by histamine release from patient's basophils. The reactions are most likely IgE mediated since 1) passive sensitisation with patient sera and subsequent challenge with clavulanate and amoxicillin/clavulanin induced histamine release whereas no release was observed to amoxicillin alone and 2) the histamine release was completely abolished when sera were pre-incubated with Xolair which prevents binding of IgE to the high affinity FcεRI receptor.

1074
Flare-up to docetaxel

Mateo-Borrega, MB; Vega-Castro, A; Alonso-Llamazares, A; Beitia-Mazuecos, JM
Allergy Section, Guadalajara University Hospital, Guadalajara, Spain

Background: The flare-up phenomenon has been more frequently reported as the development of eczematous skin lesions at previously affected skin sites on a former contact dermatitis episode after a systemic exposition to the culprit agent or after diagnostic procedures such as patch testing. It has been less frequently described the appearance of lesions at the sites of previously performed patch and intradermal tests with drugs. Docetaxel has been related with recall radiation dermatitis, but to our knowledge there are no previous reports of flare-up reactions induced by this drug.

Method: A 47 year old woman diagnosed with breast cancer was referred to our Allergy Department because she developed a pruritic maculopapular rash on her breasts, abdomen, arms and thighs 5 days after the 5th chemotherapy cycle with docetaxel and cyclophosphamide. She had received in all cycles premedication with dexamethasone and dexclorpheniramine. The patient had not been treated with radiotherapy. Skin prick and intradermal tests were performed with docetaxel, paclitaxel and ciclophosphamide after stopping treatment with antihistamines and corticosteroids used to control the symptoms previously described.

Results: Skin prick and intradermal tests were negative at immediate and delayed reading. Treatment with dexamethasone twice daily since 3 days before the following cycle was added. Three days after the 6th chemotherapy cycle the patient presented with erythematous plaques where intradermal docetaxel tests were performed followed after 2 days by the appearance of a maculopapular rash similar to the first one. The rash improved with antihistamines and corticosteroids in a few days. Nevertheless, the lesions at intradermal test sites evolved to erythematous-violaceous plaques and afterwards to hiperpigmented macules that persisted 2 months after their onset.

Conclusion: The lesions presented by our patient correspond to a flare-up phenomenon at intradermal testing sites with docetaxel.

1075
Allergy reaction to subcutaneous heparin. A case report

Burgos Montero, AM; Candon Morillo, R; Gonzalez Sanchez, LA; Moreno Mata, E; Ruiz Leon, B
Allergy Department, Hospial La Mancha Centro, Alcazar de San Juan, Spain

Background: We report a case of a 30 years old woman, with history of atopy (allergic rhinoconjunctivitis to pollens) who received subcutaneous enoxaparin as prophylactic treatment during bed rest for pregnancy risk. After 5 days of administra-

tion, itching and erythematous lesions appeared at the injection site. She had not other systemic symptoms. The treatment was interrupted and the lesions disappear within a month leaving residual hyperpigmentation. Previously, she had never been administrated heparins. She does not have reactions with other drugs.

Method: Patch test with lecture within 48, 96 h and a week before, skin prick and intradermal tests with enoxaparin, fraxiparine, heparin sodium and fondaparinux were performed. We made inmediate readings and 48 h before to analyze delayed positive tests. Subcutaneous challenge with enoxaparin, fraxiparine, heparin sodium and fondaparinux were made.

Results: Patch test, prick and intradermal tests were negative. Subcutaneous challenges with heparin sodium and fondaparinux were negative. Nevertheless, challenges with enoxaparin, fraxiparine were positive, so the patient had the same lesions as last time but they were immediate. She did not suffer a systematic reaction whereas the challenge.

Conclusion: Inmediate hypersensitivity to low molecular weight heparin is rare and the appearance of an eczematous reaction in subcutaneous puncture is attributed to a mechanism of delayed hypersensitivity. Diagnosis by skin prick test and patch test, not always, is definitive and heparin is a drug widely used as an anticoagulant and is indicated in the prevention and treatment of thromboembolic disorders. Although, we know that there is a high cross reactivity between low molecular weight, we wanted to know the exact profile of cross allergies. So, again, in this case, it is confirmed that the best alternative for the patient is the fondaparinux (as it is said in some articles, where the cross reactivity between fondaparinux and low molecular weight heparins is 10%) However, we also provide another alternative as sodium heparin.

1077
Etoricoxib-induced multilocular fixed drug eruption mimicking recurrent herpes simplex virus infection

Prucha, H[1,2]; Müller, T[2]; Zirbs, M[2]; Leon-Suarez, I[2]; Engst, R[2]; Ring, J[1,2]; Darsow, U[1,2,3]
[1]Christine Kühne Center for Allergy Research and Education (CK-CARE), Technische Universitaet Muenchen, Munich, Germany; [2]Department of Dermatology and Allergology, Technische Universität München, Munich, Germany; [3]ZAUM-Center for Allergy and Environment, Technische Universität and Helmholtz Center München, Munich, Germany

Background: Multilocular FDE usually appears as annular well circumscribed cutaneous and/or mucosal patches after a systemic exposure. Reexposure to this drug results in recurrence in exactly the same localisation. To date the newer cox-2-inhibitors like etoricoxib have rarely been reported to cause FDE.

We report the case of a 59-year old caucasian man with a prediagnosed questionable herpes simplex virus (HSV) infection on the lips and in the genital area. Lesions occured repeatedly, a hyperpigmented patch on the patients leg was known for years with recurrent inflammation. With the last acute relapse, a HSV infection and cutaneous borreliosis could be excluded, but in-depth allergy history revealed the association of the recurrent erythemas and blisters in loco parallel with the oral intake of etoricoxib 3 days preceeding the skin reaction.

Methods and results: Skin prick testing with etoricoxib was negative. Following oral challenge with etoricoxib, the patient developed typical multilocular fixed drug eruption lesions on his lower lip, on his right hand and the hyperpigmented patch on his right leg within 12 h. Histopathological assessment of these lesions was consistent with the diagnosis of a fixed drug eruption and later patch-testing in loco with etoricoxib was positive.

Conclusion: New cox-2-inhibitors like etoricoxib are increasingly used in the therapy of pain and rheumatoid diseases since there are only few allergic reactions to this group of medication, they present normally a good alternative in patients with contraindications for classic NSAIDs. HSV-infection and fixed drug eruption show some clinical similarities with regard to recurrence, blistering and localisation thus highlighting the importance of differential diagnosis.

1078
Cross-reactivity between carbapenems: two case reports

Noguerado, B; Pinto, C; Pineda, R; Martinez, P; Álvarez-Perea, A; De Barrio, M
Gregorio Marañón University General Hospital, Madrid, Spain

Background: Carbapenems are broad-spectrum betalactam antibiotics. They are used as a second line treatment and are restricted to hospital severe infectious diseases. Imipenem and meropenem have a similar structure with penicillins, with a beta-lactam ring attached to a modified thiazolidine ring and two different side chains. The side chain of imipenem is joined to beta-lactam ring in trans direction; meropenem has similar chemical structure with a methyl group in C1 and with a dimetilcarbamoilpyrrolidinyl group in C2. Strong data are lacking on the cross-reactivity between individual carbapenems.

Method: Case 1: A 65-year-old man, diagnosed in our service of non-immediate hypersensitivity to imipenem. In February 2012, the patient was hospitalised for acute pancreatitis in Intensive Care Unit. Because of lack response to other antibiotics and bad development, it was agreed to administer meropenem, with good tolerance.

Case 2: A 61-year-old woman, hospitalised because of high fever and uncontrollable vomiting. In September 2012, 72 h after meropenem administration, she developed a general erithematous rash, accompanied by oral, vaginal and rectal mucous affection, which was successfully treated with corticoids and antihistamines. It was followed by desquamation.

Allergic study: Skin tests (ID) were made with PPL, MDM, penicillin G, meropenem (2 mg/ml), imipenem-cilastatin (2 mg/ml), cilastatin (5% saline solution) and a single blind challenge test with imipenem-cilastatin and meropenem.

Results: Case 1: ID was positive (24 h) to imipenem-cilastatin and negative to PPL, MDM, PNG, cilastatin and meropenem. Challenge test with meropenem was negative.

Case 2: ID was positive (24 h) to meropenem and negative to PPL, MDM, PNG and imipenem-cilastatin. Challenge test with imipenem-cilastatin was negative.

Conclusion:

1 We present two cases of selective non-immediate hypersensitivity to different carbapenems.
2 Lack of cross-reactivity between imipenem and meropenem suggests that the involvement of the side chains in these hypersensitivity reactions may play an important role.
3 If carbapenem therapy is unavoidable, it may be possible to cautiously administer this drug in patients with non-immediate allergy to one of them, but it is necessary to confirm these data with extended studies.

1079
Hypersensitivity to a bowel preparation liquid

Barreira, P; Malheiro, D; Cadinha, S; Moreira da Silva, JP
Centro Hospitalar de Vila Nova de Gaia/Espinho, EPE, Drug Allergy Unit, Allergy and Clinical Immunology Department, Vila Nova de Gaia, Portugal

Background: Macrogols or polyethylene glycols (PEGs) are hydrophilic substances with different molecular weights, which are

extensively used as excipients in the food, cosmetic and drug industry. PEGs are believed to be chemically inert, nonetheless there are some reports of delayed and immediate hypersensitivity (HS) reactions to PEG-containing substances. PEG 3350 has a molecular mass of 3350 g/mol and it is widely used as an osmotic laxative, due to its lack of absorption in the GI tract. There are few reports in the literature of HS reactions to PEG 3350.

Method: We report the case of a patient with immediate HS to Klean Prep®.

Results: A 26-year-old Caucasian woman with allergic rhinitis, iron deficiency anemia and autoimmune thyroiditis was referred to our outpatient clinic for suspected drug allergy. She developed tachycardia, a generalised maculopapular erythematous pruritic rash and hand edema 10 min after the administration of 100 ml of Klean Prep® (macrogol 3350, anhydrous sodium sulfate, sodium bicarbonate, sodium chloride and potassium chloride) in preparation for a colonoscopy. She attended the emergency department and she was treated with parenteral corticosteroids and antihistamines and discharged with desloratadine. Symptoms and signs resolved in 24 h. Seven months later, skin prick and patch tests to Klean Prep® and PEG 4% were negative. The open oral challenge performed with Klean Prep® was positive, as the patient developed a maculopapular erythematous pruritic rash 10 min after the first administration (34.48 g = 2.95 g macrogol). The reaction subsided 1 h after treatment with parenteral corticosteroids and antihistamines. The patient was advised to avoid macrogol.

Conclusion: Despite the inert immunologic features of PEG, HS to these agents can occur and should be considered. The mechanism by which these reactions occur is not entirely clear. Since skin tests are not standardised, drug provocation test remains the gold standard for the diagnosis. To our knowledge this is the first case report with an oral provocation challenge to macrogol 3350.

1081
Drug allergy: pseudoephedrine

Martínez, PB; Perdomo, SV; Anibarro, PJC; Picon, SJ; Perez, IJS; Foix, PS
Servicio Alergología, Hospital Universitario de Burgos, Burgos, Spain

Background: Sympathomimetic drugs are greatly used among the population to treat common cold because they are very good nasal decongestant. Pseudoephedrine is one of the most used, generally associated with antihistamines to relieve nasal congestion. We report three cases with different skin manifestations, diagnosed drug allergy (Pseudoephedrine).

Method: Case 1: Man, 65 years old. He was admitted to the hospital with generalised purity skin erythema and eyelid edema. No other symptoms of anaphylaxis. No eosinophilia. He had made treatment with Cetirizine and Pseudoephedrine (associated) for 5 days. The skin lesions improved with corticosteroids and antihistamines treatment, with generalised skin peeling.

Case 2: Woman, 82 years old. She had had evanescent erythematous purity wheals on the skin. No angioedema. No other symptoms of anaphylaxis. She had made treatment with Pseudoephedrine and Ebastine (associated) days before. The skin lesions improved with corticosteroids and antihistamines treatment, without skin peeling.

Case 3: Woman, 66 years old. She presented micropapular generalised erythematous rash. No angioedema. No other symptoms of anaphylaxis. She had made treatment with Pseudoephedrine and Cetirizine (associated) days before. The skin lesions improved with corticosteroids without skin peeling.

Results: Case 1: The results of allergy study were: Skin tests with Cetirizine and Pseudoephedrine: negative. Patch test with Pseudoephedrine and Cetirizine: Negative. Cetirizine tolerance test: Negative. Pseudoephedrine tolerance test: Positive reappearing skin lesions. Patch tests (repeated): positive (++) with Pseudoephedrine.

Case 2: The results of allergy study were: Skin tests with Ebastine and Pseudoephedrine: Negative. Ebastine tolerance test: negative. Pseudoephedrine tolerance test: Positive presenting generalised hives in 4 h.

Case 3: The results of allergy study were: Patch test: positive (++) for Pseudoephedrine and Phenylephrine, (+) for Etilefrine and Ephedrine; negative for Cetirizine. Cetirizine tolerance test: Negative.

Conclusion: We present three cases with Pheudoephedrine proved allergy. They should avoid Pseudoephedrine and all drugs derived from the same family (Phenylamines) derivatives of Phenylpropanolamine (Ephedrine, Norefredina, Pseudoephedrine) and Phenylethanolamine derivatives (Phenylephrine).

1082
Paracetamol-induced fixed drug eruption at an unusual site

Gómez-Traseira, C[1]; Rojas Pérez-Ezquerra, PM[2]; Sánchez-Morillas, L[2]; González-Mendiola, R[2]; Rubio-Pérez, M[1]; Moral, A[2]; Laguna, JJ[2]
[1]Allergy, Hospital Central de la Cruz Roja, Madrid, Spain; [2]Hospital Central de la Cruz Roja, Madrid, Spain

Background: Paracetamol is a widely used analgesic/antipyretic drug for which several cases of fixed drug eruption (FDE) have been described.

FDE is a common non-immediate allergic reaction to a drug characterised by recurrence at the same site and a short delay between intake and onset of the reaction. Patch testing on lesional skin is considered to be helpful in the identification of the culprit drug in some cases.

Patient and methods: A 62-year-old woman was referred to our department because she had developed a maculopapular rash on the breasts and limbs 4 months previously after 7 days of treatment with paracetamol. Her history revealed five previous local adverse reactions to paracetamol, all of which were recurrences of similar lesions at the same location (papules on the hard palate).

Results: The results of skin prick test and intradermal tests with paracetamol with an immediate and late reading (24 h) were negative. Patch tests on her back with paracetamol and with a battery of non-steroidal antiinflammatory drugs (NSAIDs) were negative. Patch tests on residual lesional skin (hard palate) were impossible to carry out.

A single-blind oral challenge with paracetamol was necessary to confirm the diagnosis. Although the reaction for which the patient was referred to our department was a maculopapular rash, she developed a FDE after the challenge.

In order to investigate co-existing NSAID cross-intolerance, we performed an OCT with acetylsalicylic acid, and tolerance was good.

Conclusions: We report several delayed selective reactions induced by paracetamol in the same patient. Our findings could reflect two different clinical patterns of delayed allergic reactions, or, more probably, the initial phase of a unique clinical entity that was stopped by the corticosteroids prescribed during the challenge. However, we were unable to confirm these hypotheses. The uncommon anatomical site of the lesions (hard palate) is noteworthy.

1083
Multiple drug hypersensitivity: a case report

Aranzabal, MA[1]; Echenagusia, MA[2]; Joral, A[3]; Navarro, JA[3]; Lizarza, S[3]; Lasa, EM[3]
[1]Unit Allergy, OSI Goierri-Alto Urola, Zumarraga, Spain; [2]Unit Allergy, OSI Bajo Deba, Mendaro, Spain; [3]Department of Allergology, Hospital Universitario Donostia, San Sebastian, Spain

Background: The term multiple drug hypersensitivity describes immune-mediated reactions to structurally unrelated drugs. These reactions are facilitated by generalised immune stimulations such as those caused by viral infections as Epstein-Barr virus (EBV) or other herpes virus infections.

Method: A 16-year-old boy experienced a generalised rash during an infectious mononucleosis that was complicated with hepatitis and autoimmune hemolytic anemia. Before the onset of the eruption he had been treated with amoxicillin, paracetamol and ibuprofen and afterwards with metamizol and with cloxacillin first and with levofloxacin later because of a *Staphylococcus aureus* infection. As the eruption worsened, both antibiotics were withdrawn. A month later he was discharged with linezolid, omeprazole and ibuprofen with good tolerance.

Six months later, 4 days after starting treatment with clindamycin and 2 days after beginning therapy with metamizol, ibuprofen and omeprazole because of teeth extraction, another generalised pruriginous macular rash appeared.

Results: In the first episode skin prick tests (SPT), intradermal test (IT) and oral challenge test (OCT) were negative to levofloxacin. SPT and IT with amoxicillin, ampicillin, penicillin, cloxacillin and PPL and DM gave negative at 20 min lecture. At 24 h SPT and ID were positive to amoxicillin and ampicillin and negative to penicillin, PPL, DM, cloxacillin, cefuroxime, ceftazidime, and ceftriaxone. OCT with cefuroxime was well tolerated. In the second event SPT, IT and patch tests were performed with clindamycin, metamizol, ibuprofen and omeprazole and a delayed reaction was observed with metamizol and omeprazole in IT and patch test (at 24 h). OCT with ibuprofen was well tolerated. Patch tests were also positive to rabeprazole, lansoprazole, esomeprazole and pantoprazole.

Conclusions: We present a case of multiple drug hypersensitivity (aminopenicillins, metamizol and proton pump inhibitors) in a teenager who suffered an infectious mononucleosis. The PT and the IT have been useful tools in identifying the causal agents. EBV infection might have played a role in the development of drug hypersensitivity. We have also observed cross reactivity between proton pump inhibitors.

Poster Session 40

Epidemiology of food allergy

1086
Prevalence of immediate-type food allergy in early childhood in Seoul, Korea

Han, Y[1]; Kim, J[1]; Park, M[1]; Ahn, K[2]; Do, J-R[3]
[1]Environmental Health Center for Atopic Diseases, Samsung Medical Center, Seoul, Korea; [2]Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [3]Korea Food Research Institute, Seoul, Korea

Background: We performed this study to determine the prevalence and causative foods of immediate-type food allergy (FA) in early childhood in Korea.
Method: A questionnaire-based, cross-sectional study was performed between September and October 2011. Children aged 0–6 years were recruited from 301 public child care centers in Seoul. Parents were asked to complete a questionnaire on FA. Those children with FA were classified into 'perceived FA, ever', 'immediate type FA, ever', and 'immediate type FA, current' according to the algorithm.
Results: A total of 16 749 children were included in this study. The prevalence of 'perceived FA, ever', 'immediate type FA, ever', and 'immediate type FA, current' was 15.1%, 7.0%, and 3.7%, respectively. 'Immediate type FA, current' was reported by 182 (4.9%) out of 3738 children in 0–2-year olds, 262 (3.4%) out of 7648 in 3–4-year-olds and 177 (3.3%) out of 5363 in 5–6-year-olds. Hen's egg (126/621) was the most frequent causes as the individual food in children aged 0–6 years, followed by cow's milk (82/621), peanut (58/621) and soybean (17/621). In food groups, fruits (114/621), tree nuts (90/621) and crustaceans (85/621) were the major foods causing allergy. The three leading causes of food-induced anaphylaxis were hen's egg (22/47), peanut and/or tree nuts (19/47), and cow's milk (15/47).
Conclusion: The prevalence of immediate type FA in early childhood in Seoul is 3.7%, and was higher in younger age group. The most frequent causative foods are peanuts and/or tree nuts, hen's egg, fruits, crustaceans and cow's milk.

1087
Food allergy prevalence and its sensitisation from infancy to 7 years old in Japan

Sugizaki, C; Ebisawa, M
Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, Allergy, Sagamihara, Japan

Background: We have followed up birth cohort in Sagamihara to clarify profiles of allergic diseases during the first 7 years of life. In this report, we clarified itemized reported food allergen avoidance rate and its sensitisation rate in the first 7 years.
Method: Using the mass medical examination system at the age of 4 months old, we obtained basic information on subjects' profile from 1/1/02 to 12/31/02. We followed up the subjects, whose parents agreed to the purpose of this study, at the age of 8, 12 months, 3, 5 and 7 years using questionnaire by mail. Number of study subjects was 5247 during infancy, 4505 at 3 years, 3604 at 5 years, and 3247 at 7 years, respectively.
Results: Responses were obtained from 4214 subjects (80.3%) at the age of 8 months, and 4068 (77.5%) at 12 months, 2888 (64.1%) at 3 years, 2135 (59.2%) at 5 years and 2165 (66.7%) at 7 years.

The incidence of chronic eczema with suspicion of atopic dermatitis at 8 months, 1, 3 and 5 years was 20.1%, 14.4%, 14.8%, 15.5%, and 13.8%, respectively. The food avoidance rate due to food allergy at 8 months, 1, 3, 5 and 7 years was 19.5%, 13.1%, 4.9%, 3.7% and 3.1%. The diagnosis rate of bronchial asthma at 1, 3, 5 and 7 years was 3.2%, 8.7%, 14.1% and 14.9%. To our surprise, the diagnosis rate of Japanese cedar pollinosis at 3, 5 and 7 years was 3.0%, 10.7% and.21.1%. The percentages of patients with any kind of allergic diseases at 3, 5, and 7 years were 24, 1%, 33.3%, and 39.2%, respectively.

Itemized food allergen avoidance rates with tendency to decrease subsequently at 8 months, 1, 3, 5, and 7 years were seen in hen's egg, cow's milk, wheat, and soybean. The rates tended to increase at 8 months, 1, 3, 5, and 7 years were seen in shrimp and peanut. Among subjects received medical examination (maximum at 7 years by 18.2%), sensitisation rates to hen's egg (5.9%), cow's milk (2.6%), wheat (1.4%), and soybean (1.1%) reached to maximum at 3 years, followed by gradual decrease with aging. The rates to buckwheat, shrimp, peanuts, sesame tended to increase until 3 years and those to peanuts and shrimp stayed at the same levels.
Conclusion: Facts of atopic march were clarified in this cohort study as has been suspected previously. Although these data were based on reports from guardians, the prevalence of common food allergies and its change with aging was clarified in this study.

1088
Anisakis simplex hypersensitivity in central and southern Italy: clinical features

Centrone, M[1,2]; Colagiovanni, A[1]; Buonomo, A[1]; Rizzi, A[1]; Aruanno, A[1]; Pascolini, L[1]; Rizzi, G[1]; Di Rienzo, A[1]; Mezzacappa, S[1]; Nucera, E[1]; Schiavino, D[1]
[1]Policlinico A. Gemelli, Roma, Italy; [2]Universita Cattolica del Sacro Cuore, Roma, Italy

Background: The nematode Anisakis simplex (AS) is a worldwide distributed parasite that infects consumers of raw or undercooked parasitized fish. The ingestion of anisakis infected fish could cause an acute IgE-mediated generalised reaction (urticaria-angioedema-anaphylaxis).
Method: A retrospective study was conducted on 112 patients with suspected food allergy enrolled from January 2007 to January 2012. Inclusion criteria were positive skin prick test and/or positive specific IgE for Anisakis simplex. We evaluated the clinical characteristics of patients, the benefit of fish-free diet and of the treatment with mebendazole in symptomatic patients.
Results: Ninety-seven of 112 patients included in the study showed evident symptoms after fish intake. Seventy-three of them reported cutaneous symptoms (31 urticaria, 20 pruritus and 22 angioedema); 26 had gastrointestinal symptoms (six nausea and vomiting, nine diarrhea, 11 colic pains and meteorism); 42 of 112 patients followed a free-fish and seafood products diet; in those who had gastrointestinal symptoms, 70% had benefit from diet with resolution of symptoms. The diet was effective in the 65% of patients with acute SOA (urticarial-angioedema syndrome)

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

and in the 38% of the patients with chronic urticaria. Ten patients did not respond to diet and were treated with mebendazole, 40% of them have benefits. Thirty-two patients correlated the onset of symptoms to the ingestion of a particular fish species, in particular marinated anchovies were among the species most involved. Forty-nine patients also performed skin prick test for dermatophagoides and 15/49 (31%) showed a positive test.

Conclusion: Anisakis simplex has a pathogenetic role in hypersensitivity reactions and gastrointestinal disorders. It could play an equally important role in chronic urticaria although the pathogenetic mechanism is not fully known. In symptomatic patients a free-fish and seafood products diet show clinical improvement while the efficacy *in vivo* of an anthelmintic needs further study. The fish species, like anchovies, associated with sensitisation in Italy reflect the spread of the Anisakis simplex larvae in seafood of the Mediterranean Sea. The results confirm the evidence of cross-reactivity between Anisakis simplex and Dermatophagoides. Prevention methods by freezing fish products at −20° for at least 24 h and adequate cooking are able to kill the parasite but not to cancel entirely the allergenicity (some allergens found to be resistant to temperatures of 100 °).

1089
Patterns of sensitisation in the first decade of life

Patil, VK[1]; Grundy, J[1]; Glasbey, G[1]; Dean, T[2]; Arshad, SH[1,3]; Venter, C[1,2]
[1]David Hide Asthma & Allergy Research Centre, Newport, United Kingdom; [2]University of Portsmouth, Portsmouth, United Kingdom; [3]University of Southampton, Southampton, United Kingdom

Background: Prevalence of sensitisation change from early childhood to later childhood, with sensitisation to some allergens (milk and egg) being common in early childhood and other allergens (nuts, fish and aeroallergens) in later childhood. In a prospective longitudinal birth cohort, we looked at the patterns of sensitisations in the first 10 years of life.

Method: The FAIR (Food Allergy and Intolerance Research) birth cohort ($N = 969$) was established on the Isle of Wight (UK) between September 2001 and August 2002 to prospectively study the natural history of food allergy. Participants were followed up at 1, 2, 3 and 10 years of age. Skin prick tests (SPT) were performed using nine common aero and food allergens; HDM (house dust mite), cat, grass, milk, egg, wheat, peanut, cod and sesame. Sensitisation was defined as ≥3 mm diameter skin prick test wheal. Participants who underwent SPT at 1, 3 and 10 years ($n = 426$) were included for the analysis of sensitisation patterns over the first 10 years of life.

Results: SPT at all three time points was available for 426 participants; of these, 122 (28.6%) were sensitised on at least one occasion. The majority of the children were never sensitised (304/426; 71.4%). Small numbers were persistently sensitised (10/426; 2%) at 1, 3 and 10 years. 66/426 (15.5%) children were not sensitised at 1 and 3 years but developed sensitisation at 10 years. Of those who were not sensitised at 1 year, 34/426 (8%) developed sensitisation at 3 years and remained sensitised at 10 years. No child was only sensitised at 1 year. Early childhood sensitisation at 1 and 3 years only was seen in 2/426 (0.5%) children as they were not sensitised at 10 years. Three (0.7%) children showed transient loss of sensitisation at 3 years and seven (1.6%) showed transient sensitisation at 3 years. Out of 15 (3.5%) children who were sensitised at 1 year, 11 (73.3%) and 13 (86.6%) were sensitised to at least one new allergen at 3 and 10 years respectively.

Conclusion: The majority of children remain non-sensitised in the first decade of life. Some develop sensitisation only in later childhood. Those sensitised in the first year of life tend to remain sensitised and with higher chance of developing new sensitisations throughout childhood.

1090
Reassessing foods implicated in the celery-mugwort-spice syndrome

Hemmer, W; Fellinger, C; Sesztak-Greinecker, G; Wöhrl, S; Jarisch, R; Wantke, F
Floridsdorf Allergy Center, Vienna, Austria

Background: Mugwort pollen allergy may be associated with adverse reactions to foods such as celery, spices and others. The prevalence of such cross-reactions in mugwort pollen-sensitised patients is not well known and the alleged contribution of certain foods needs to be critically reassessed.

Method: One hundred patients with a positive skin prick test to mugwort pollen were skin tested with up to 40 different foods by prick-to-prick. 40/100 patients were preselected based on a previous allergic reaction to a mugwort-associated food. To avoid overlap with other secondary food allergies, no patients co- sensitised to birch pollen, profilin, or latex were included into the study.

Results: Cross-sensitisation was extremely common in selected as well as unselected patients to camomile tea (79%/68%) and sunflower honey (78%/52%), both containing pollen allergens from plants belonging to the same plant family as mugwort (Asteraceae). Among botanically unrelated foods, positive reactions were most often seen with celeriac (85%/40%), celery (67%/46%), dill (52%/25%), mango (59%/26%) and lychee (43%/16%). Sensitisation to pistachio (22%/6%), cashew nut (17%/2%) and sunflower seeds (16%/5%) was less common and largely confined to the food-allergic group. Reactivity with carrot, parsley, tomato, bell pepper, cucumber, onion, melon, banana, peach, soy and hazelnut was unusual and partly linked to concomitant Art v 3 sensitisation. Among spices, only those from the Apiaceae family (caraway, coriander, cumin, curry) played a role (56%/23%), whereas pepper, ginger, cardamom, cloves, cinnamon and Lamiaceae spices (basil, marjoram, etc.) were throughout negative. With regard to clinical reactions, celeriac, honey and mango were most important in terms of frequency and severity, followed by camomile, lychee and curry powder. LTP (Art v 3) sensitisation was generally rare (12%/3%) and unlikely is the major cause of mugwort pollen-related food allergy.

Conclusion: Cross-reactivity with honey, camomile, celeriac, mango, lychee and Apiaceae spices is widespread in mugwort pollen allergy affecting 16–68% of unselected patients. Apart from celery and spices, the scale of mugwort-associated foods clearly differs from that of birch pollen-associated foods and thus does not rationalize the delineation of a 'celery-carrot-birch-mugwort-spice syndrome' as proposed previously.

1091
The prevalence of food allergy in Kazakhstan

Nurpeissov, TT; Nurpeissov, TN; Nurpeissov, T; Abdushukurova, G; Turdiyeva, Z
SRI of Cardiology and Internal Diseases, Republican Allergological Center, Almaty, Kazakhstan

Background: The prevalence of allergic diseases in Europe and USA is great and growing, but the data about allergy epidemiology in Kazakhstan is almost absent. At the moment Kazakhstan residents have many of risk factors similar to Europe citizens. The food allergy is one of the biggest allergic problem occuring in children and adults, seriously decreasing their quality of life.

The aim of this study was to determine the food allergy prevalence by different methods among children and adults in different towns of Kazakhstan.

Method: Two-stage randomised clustered population study was conducted to determine the prevalence and morbidity structure of food allergy. At the first phase random buildings were chosen, then all inhabitants were asked to fill the questionnaire. For children their parents gave answers also. At the second stage specialised survey and examination including prick tests and/or specific IgE detection in persons with announced allergy was conducted. The most common for Kazakhstan food allergens were used.
Results: Five thousand five hundred and twelve persons, including 3759 adults and adolescents over 14 years and 1753 children, were involved in this study. The presence of food allergy has been marked in 21.3% of total amount of respondents (1560 persons). There were 562 adults and 998 children, that makes 15.0% and 56.9% of total respondents of same age group, respectively.

After the detailed examination the real presence of food allergy (strong correlation between episodes of allergic reactions and certain food products, good efficacy of elimination, positive *in vitro* and/or *in vivo* tests) was estimated only in 3.7% of respondents – 2.8% for adults and adolescents, and 5.6% for children, respectively.
Conclusion: Data obtained in the study suggests that:

1 The difference between suspected (by patients themselves) and real prevalence of food allergy in Kazakhstan is huge (21.3% vs 3.7%, $P < 0.01$).
2 The biggest difference revealed in children (up to 10 times).
3 Nevertheless food allergy more common for children than among adults (up to two times).
4 The prevalence of food allergy in Kazakhstan is high that needs further investigations and prevention activity.

1092
Children and adults with multiple plant-food allergy: comparison of sensitisation profiles

Pascal, M[1]; Bartra, J[2]; Jiménez-Feijoo, R[3]; Bosque, M[4]; Malet, A[5]; Folqué, MM[3]; Sánchez-López, J[2]; Valero, A[2]; Juan, M[1]; Vilella, R[1]; Plaza, AM[3]; Yagüe, J[1]
[1]Servei d'Immunologia, Hospital Clínic de Barcelona, CDB, Barcelona, Spain; [2]Unitat d'Allèrgia, Servei de Pneumologia, Hospital Clínic de Barcelona, ICT, Barcelona, Spain; [3]Immunoallèrgia, Hospital Sant Joan de Déu, Esplugues de Llobregat, Spain; [4]Pneumologia, Allèrgia i Immunologia Clínica Pediàtrica, Hospital Parc Taulí, Sabadell, Spain; [5]Allergo Centre, Barcelona, Spain

Background: Multiplexed component resolved diagnosis (CRD) is useful to evaluate the panorama of sensitisation of patients with multiple plant food allergy and to elucidate the nature of proteins triggering allergic reactions.
Aim: To analyze at a molecular level the panorama of sensitisation in children and adults allergic to multiple plant-foods and to compare their sensitisation profiles.
Methods: A retrospective, observational and descriptive study of the sensitisation profile of two cohorts of polysensitised patients (children and adults) from the same geographical area derived to our lab for multiplexed-CRD analysis on a microarray format during 1 year. All patients had been derived under the same clinical criteria: diagnosis of plant-food allergy to at least two non-taxonomically related plant-foods (with clinical history of allergic reaction, positive skin prick test and/or specific IgE to culprit foods).
Results: The microarray results of 106 children (median age [range] years-old: 11 [3–18]) and 55 adults (32 [20–61]) were analyzed. In children, the panorama of sensitisation to plant-food allergens showed a high prevalence of sensitisation to storage proteins of nuts, seeds and/or legumes (75%), especially walnut and peanut components, with concomitant sensitisation to panallergens, mainly LTP (Lipid Transfer Protein), but also without sensitisation to cross-reactivity molecules (18%). In contrast, in adults the panorama of plant-food sensitisation was restricted to LTP (85%), with occasional sensitisation to storage proteins (which were mainly walnut components), profilins and PR-10. Regarding LTP, most children and adults showed sensitisation to almost all LTPs of the microarray. In patients showing sensitisation to only one LTP, that was Jug r 3 or Pru p 3.
Conclusion: In our area, children and adults with multiple plant food allergy differ significantly in their panorama of sensitisation to storage proteins, whereas the high prevalence and broad LTP sensitisation profile seems to be similar. LTP sensitisation may start early in life and persist until adulthood, but prospective studies are still required. The high prevalence of LTP sensitisation is consistent with a Mediterranean area, but the high prevalence of storage proteins observed in children has to be taken into account.

1093
Degree of concordance of characteristics of food allergy in siblings

van Ginkel, DC[1]; Flokstra-de Blok, BMJ[2]; Koppelman, GH[1]; Dubois, AEJ[1]
[1]Department of Pediatric Pulmonology and Pediatric Allergy, GRIAC Research Institute, University Medical Center Groningen, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [2]Department of General Practice, GRIAC Research Institute, University Medical Center Groningen, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Background: The prevalence of food allergy has increased in recent decades and food allergy is currently the leading cause of anaphylaxis in children in the US. Research has shown that sensitisation to food allergens is strongly genetically determined. We tested the hypothesis that siblings are more similar with regards to the main phenotypic characteristics of food allergy compared with matched controls.
Method: In the University Medical Center Groningen, data was obtained from double-blind placebo-controlled food challenges (DBPCFCs) performed as part of routine care, including information on the history, co morbidities, immunological test results and challenge results. Siblings were identified by address and name and 56 variables were compared, including other DBPCFCs, number of suspected foods, positive test reactions to foods, eliciting dose, specific IgE, atopic score of the parents and severity scores of the home reaction by history and test reaction during the DBPCFC. Controls were selected matched for age, gender and parental atopic score. Data was analysed using the one-sided sign test with $\alpha = 0.05$.
Results: In total, 685 children were enrolled which included 21 sibling pairs. Of all 56 tested variables, 19 showed more concordance among siblings compared to matched controls of which two were significant; suspected food ($P = 0.033$) and threshold dose ($P = 0.038$).
Conclusion: The results show that the majority of the characteristics of food allergy did not show a significantly stronger concordance between siblings compared with matched controls. The variables which showed more concordance were eliciting dose and tested allergen. The concordance for tested allergen may be explained by heightened parental suspicion for a certain food being transferred from one sibling to the next. Concordance of the eliciting dose in siblings is of interest and may reflect a relatively large genetic influence on this variable. However, the overall conclusion these results suggest is that siblings are not more alike than matched controls in terms of food allergy characteristics. Clinicians may thus

reassure parents that siblings of allergic patients do not necessarily have the same degree or form of food allergy.

1094
The prevalence of food allergy in *Opisthorchis felineus* infection endemic region – the EuroPrevall study

Fedorova, O[1]; Yevdokimova, T[1]; Fedotova, M[1]; Janse, J[2]; Yazdanbakhsh, M[2]; Wong, G[3]; Kummeling, I[4]; Burney, P[4]; Fernandez-Rivas, M[5]; van Ree, R[6]; Ogorodova, L[1]; Kamaltynova, EM[1]
[1]Department of Pediatrics, Siberian State Medical University, Tomsk, Russia; [2]Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands; [3]Department of Paediatrics and School of Public Health, Chinese University of Hong Kong, Hong Kong, China; [4]Department of Respiratory Epidemiology and Public Health, National Heart and Lung Institute, Imperial College London, London, United Kingdom; [5]Hospital Clinico San Carlos, Madrid, Spain; [6]Department of Experimental Immunology and Department of Otorhinolaryngology, Academic Medical Center, Amsterdam, The Netherlands

Background: It has been reported that the prevalence of allergies varies due to numerous environmental factors including the prevalence of helminth infestation. Our recent investigation has shown that Opisthorchis felineus invasion modifies the course of allergic diseases. An epidemiological survey is needed to analyze the protective effect of the parasite on the development of food allergy.
Purpose: To investigate the relationship between Opisthorchis Felineus infection and food allergy in children from Tomsk Oblast (West Siberia, Russia).
Method: The cross-sectional study in random samples of schoolchildren aged 7–10 years (n = 13010) was performed as a part of EuroPrevall project (FP6- FOOD-CT-2005-514000). There were three samples: Tomsk city and rural areas with high and low opisthorchiasis level. The case-control sample was recruited for the second stage (n = 1288). Thus who reported adverse reactions to food in the screening stage in each sample were considered as cases, children without reported reactions were controls. The standardised screening questionnaires were used at the screening stage. The case-control stage included the completion of a clinical questionnaire, skin-prick test, serum specific IgE measurement (ImmunoCAP, Phadia, Sweden). The Opisthorchis Felineus parasites were determined in stool samples by the PCR analysis. Probable food allergy was defined as the combination of reaction within 2 h after food ingestion together with specific IgE $\geq$ 0.35 kU/l and/or positive skin-prick test to the same food (mean diameter of wheal $\geq$3 mm).
Results: The prevalence of self-reported symptoms to EuroPrevall priority foods is lowest in the rural sample with high prevalence of opisthorchiasis compared with the urban sample and rural sample with low prevalence of helminthiasis (9.2%, 11.5% and 10.5% respectively, $P < 0.05$). The prevalence of probable food allergy is 1.08% in children of Tomsk Oblast population and it is lower in comparison with other EuroPrevall centres in Europe. The prevalence of probable food allergy is significantly lower in rural regions with high level of opisthorchiasis (0.79%) in contrast with rural regions with low level of opisthorchiasis (1.36%; OR 0.58; CI 95% 0.36–0.91; P = 0.01) and urban population (1.18%; OR 0.66; CI 95% 0.41–1.04; P = 0.06).
Conclusion: The food allergy prevalence in children varies a rural to urban gradient and is ecologically associated with chronic opisthorchiasis prevalence.

1095
Prevalence of fruit allergy in general risk children in Japan

Nagao, M[1]; Tokuda, R[1]; Sugimoto, M[1]; Kondo, M[1]; Kainuma, K[1]; Amano, N[2]; Chuang, T[3]; Burns, DH[3]; Fujisawa, T[1]
[1]Institute for Clinical Research, Mie National Hospital, Tsu, Japan; [2]Siemens Healthcare Diagnostics K.K., Tokyo, Japan; [3]Siemens Healthcare Diagnostics Inc., Tarrytown, NY, United States

Background: Fruit allergy is a common food allergy in adults and is mostly 'pollen-related'. However, prevalence of fruit allergy in children and possible relationship with pollen allergy, especially in general population, is still to be studied.
Objectives: To describe prevalence of fruit sensitisation and allergy in general risk children in Japan and to explore the relationship between sensitisation/allergy to fruit and common inhalant allergens in Japan including birch and Japanese cedar pollen.
Method: A cohort of 251 children (M/F; 123/128) who joined 'The Lung Function Study', which recruited 'healthy' children to determine reference values for exhaled nitric oxide. They had neither acute nor chronic diseases other than allergy. Mean age (±SD) was 9.6 ± 2.8. The ISAAC questionnaire was used for the assessment of asthma, allergic rhinitis and atopic dermatitis. Questionnaire asking about food-induced symptoms was also administered. Specific IgE to house dust mite (HDM), Japanese cedar pollen (JCP), Japanese cypress, orchard, ragweed, birch, apple, kiwi, tomato and melon were quantified by IMMULITE® 2000 3gAllergy™ specific IgE assay.
Results: The cohort consisted of 33% healthy asymptomatic, 52% allergic rhinitis, 12% asthma with rhinitis and 3% asthma without rhinitis. Prevalence of sensitisation and self-reported allergy to any fruit was 30% and 4.4%, respectively. Ninety nine percent of subjects who sensitised to any fruit were sensitised to any pollen and 41% of pollen-sensitised subjects were sensitised to any fruit. Twelve percent of asymptomatic and 39% of allergic rhinitis subjects were sensitised to both pollen and fruit. ROC analysis revealed that cut-off values of IgE for apple, melon, tomato and kiwi allergies were 0.17, 0.15, 0.14 and 0.22 IU/ml, respectively, with sensitivity of 40–60% and specificity of 80–90%. Multiple linear regression analysis found significant associations of sensitisation to apple and birch, tomato and ragweed, melon and ragweed, and kiwi and orchard.
Conclusion: We found high rate of fruit sensitisation and its strong association with pollen allergy and allergic rhinitis in children. Further study will focus on allergen components.

1096
Nut allergy profiles in Valencia, a Mediterranean area

Nieto Cid, M[1]; Giner, A[2]; Calderón, R[3]; Calaforra, S[2]; Pérez-Gómez, MA[2]; Ramón, L[2]; Mazón, Á[3]; Pineda, F[4]; Nieto, A[3]; Hernández, D[2]; Alergia La Fe
[1]Allergy, Hospital La Fe, Valencia, Spain; [2]Hospital La Fe, Valencia, Spain; [3]Pediatric Pulmonology & Allergy Unit, Hospital La Fe, Valencia, Spain; [4]Laboratorios Diater, Madrid, Spain

Background: Allergy to nuts is a very heterogeneous problem, with significant different clinical phenotypes depending on the particular nut/s, the age, the country, etc.

The aim of the study was to evaluate the possible clinical and biological profiles in adults and children presenting clinical reactions against at least one nut. Additionally, we also tried to evaluate whether lipoproteins contained in nuts would have some allergologic relevance.
Method: Fifty four patients (33 adults/21 children) complaining from allergic symptoms with nuts were evaluated by a detailed clinical history, skin prick tests (SPT) and serum sIgE against almond, peanut, walnut, cashew, hazelnut, chestnut, pistachio, pine-nut, sunflower seed and maize, as well as lipid transfer protein, profilin and hydrosoluble and liposoluble protein fractions from almond, peanut, hazelnut, and walnut. The Ethical Committee approved the study, and an informed consent was obtained from the patients and/or parents.
Results: The nuts most frequently responsible for clinical symptoms were walnut (33%), peanut (33%) and almond (20%).

There were no differences between adults and children.

Results of SPT and *in vitro* analysis showed differences between adults and children related to sensitisation against panallergens.

1 Adults: LTP 25/33 (76%) PROFILIN 3/33 (9%)
2 Children: LTP 2/21 (9.5%) PROFILIN 8/21 (38%)

Additionally, there were also differences related with sensitisation to lipo-soluble and hydro-soluble fractions depending of the nut.

1 Both hydro + lipo: Almond 37.5%, Walnut 63%, Hazelnut 54%, Peanut 20%
2 Only hydro: Almond 12.5%, Walnut 28%, Hazelnut 42%, Peanut 70%
3 Only lipo: Almond 50%, Walnut 9%, Hazelnut 4%, Peanut 10%

There was an inverse correlation between age and allergy to almond.

Conclusion:

1 The pattern of allergy to nuts is different in Spain compared to other countries.
2 More comprehensive investigation regarding allergy to nuts is needed since the great heterogeneity detected would have relevant clinical, therapeutic and prognostic implications
3 Negativity of SPT and sIGE with commercial extracts in symptomatic patients would be explained by a sensitisation against lipoproteins, which are commonly removed during the delipidation process applied in the manufacturing of diagnostic extracts.

1097
A closer look at allergy towards nonspecific-lipid transfer proteins in a birch endemic region

Van Gasse, A; Sabato, V; Hagendorens, M; Bridts, C; De Clerck, L; Ebo, D
Immunology-Allergology-Rheumatology, University of Antwerp and Antwerp University Hospital, Antwerpen, Belgium

Background: Non-specific lipid transfer proteins (ns-LTP) can cause severe plant food allergy. Sensitisation towards ns-LTP is mostly described in Southern Europe, where it appears to be dominated by gastrointestinal exposure to Pru p 3, the ns-LTP from peach (*Prunus persica*). In contrast, less is known about sensitisation towards ns-LTP in Northern Europe. This study aims to evaluate the prevalence, origin and clinical relevance of ns-LTP sensitisation in patients attending the Antwerp outpatients clinic for allergology

Method: Four hundred and five patients (pre-, schoolchildren and adults, 233 male) with inhalant, food and/or latex allergy were studied. Investigations implied a questionnaire, skin tests and quantification of IgE towards two pollen ns-LTPs (Art v 3 from mugwort (*Artemisia vulgaris*) and Par j 2 from pellitory of the wall (*Parietaria judaica*)) and two food ns-LTPs (Pru p 3 and Cor a 8 from hazelnut (*Corylus avellana*)) by microarray.

Results: Sensitisation towards a ns-LTP was found in 33/405 (8%) of the patients and was most frequently depicted by IgE towards Pru p 3. However, respectively 7 and 4 of the 33 patients sensitised towards ns-LTP presented with only sIgE towards Art v 3 and Cor a 8. Of the ns-LTP positive patients, 42, 9% showed a systemic (severe) allergic reaction

Conclusion: In our regions sensitisation towards ns-LTP can result from several sources and routes of exposure and there is no single biomarker enabling to identify all patients sensitised towards ns- LTP. Larger collaborative studies are needed to further explore our findings and clinical relevance of LTP.

1098
Sensitisation profile in a group of Spanish patients allergic to lipid transfer protein

Barbarroja-Escudero, J; Antolin-Amerigo, D; Sanchez-Gonzalez, MJ; Alvarez-Mon, M; Rodriguez-Rodriguez, M
Allergy Department, Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Spain

Background: Lipid transfer protein (LTP) induces systemic allergic reactions in sensitised patients, usually anaphylaxis. Vegetables are the culprit food in most cases, and subsequently, fresh fruits and nuts.

Method: We recruited 46 patients (39 women) sensitised to LTP over a 6-month study period. This IgE-mediated allergy was demonstrated by anamnesis, skin-prick tests, prick-by-prick testing and/or ImmunoCAP assay.

Results: Of 46 patients, 26 suffered from anaphylaxis (56.52%), six systemic urticaria/angioedema (13.04%), five oral allergy syndrome (OAS) (10.86%), three contact urticaria (CU) (6.52%), two anaphylaxis and OAS (4.34%), two anaphylaxis and CU (4.34%), two urticaria and OAS (4.34%), one rhinitis (2.17%), and one protein contact dermatitis (2.17%), due to LTP allergy. The mean age was 32 years old. Thirteen patients showed pollen allergy, of which five were allergic to profilin. The culprit foods were, in order of frequency: rosacea fruits (39 patients, 84.78%), nuts (23p, 50%), tropical fruits (5p, 10.86%), legumes (6p [3p almorta], 13.04%), cereals (3p, 6.52%), grape (3p, 6.52%), lettuce (2p, 4.34%), asparagus (1p, 2.17%), and pomegranate (1p, 2.17%). The mean specific IgE to LTP was 8.71 kU/l.

Conclusion: In our series of 46 patients allergic to LTP, the most frequent clinical picture registered was anaphylaxis, followed by systemic urticaria/angioedema.

The most recurrent culprit foods were rosacea fruits, followed by nuts.

Elevated specific IgE levels against LTP were not always correlated with the most severe reactions.

1099
Frequency of lipid transfer protein (Pru p 3) and profilin (Pru p 4) sensitisation in 1052 patients referenced to an immunoallergology department in Lisbon

Silva, PM; Pestana, L; Costa, AC; Barbosa, MP
Imunoallergology, Hospital de Santa Maria, Lisboa, Portugal

Background: Lipid transfer proteins (LTP) and profilins are the most important panallergens in the clinical management of patients with allergy to pollen and plant food (mainly tree nuts and fresh fruits) in the Mediterranean countries. Knowledge of the prevalence of sensitisation to these allergens in the population would be extremely useful. In this study we analyze the frequency of sensitisation to LTP and profilin in our allergy clinic in Lisbon.

Method: We performed skin prick tests to Pru p 3 and Pru p 4 components (peach's LTP and profilin respectively) in all patients referred to our hospital's allergy department in Lisbon during a period of 6 months. Afterwards, anamnesic features of all sensitised patients were assessed.

Results: We tested 1052 patients (64% female, mean age 34 years) and found 47 (4.5%) Pru p 3 and 42 (4%) Pru p 4 sensitisations. Co-sensitisation was present in seven patients. Polinosis was present in 70% of the LTP-sensitised, 80% of profilin-sensitised and 86% of the co-sensitised groups, whereas plant food allergy was present in 48%, 23% and 71.4% respectively. Cutaneous, respiratory and gastrointestinal manifestations of food allergy were significantly more frequent in the LTP and co-sensitised groups ($P < 0.001$).

Conclusion: We found lower rates of sensitisation than in other studies performed in Mediterranean populations. Although 52% of patients were asymptomatic, LTP sensitisation, regardless of profilin co-sensitisation, was more often associated with

plant-food allergy and presented more severe manifestations.

1100
Hepatitis A infection associated with protection against food allergy in children

Wong, GW[1]; Li, J[2]; Leung, TF[1]; Yung, E[1]; Zhong, NS[2]
[1]Paediatrics, Chinese University of Hong Kong, NT, Hong Kong; [2]Guangzhou Institute of Respiratory Disease, Guangzhou, China

Background: Food allergy is a common condition affecting children and adults in developed countries. Very little is known regarding the possible protective factors against the development of food allergy.
Method: Random samples of primary schoolchildren were recruited from urban and rural regions of China. Cases and controls were then recruited for detailed assessment to evaluate for possible food allergies. Objective evidence of sensitisation including SPT and serum specific IgE were performed. Serum samples were antibodies against common fecal-oral infections including hepatitis A, salmonella, and toxoplasmosis.
Result: A total of 16 875 Chinese school children aged 6- to 11-years from Hong Kong, and two regions in mainland China (Guangzhou city and rural Shaoguan) were screened with an overall response rate of 92%. Among them, 1121 subjects were recruited for the detailed case-control phase of the study according to the standardised EuroPrevall protocol. The prevalence of parents' reports of more than four episodes of adverse food reactions varied widely and they were 3.9% in Hong Kong, 2.3% in Guangzhou (urban), 1% in Shaoguan (rural). Defining probable food allergy as having symptoms with a certain food within 2 h of ingestion and positive SPT/serum specific IgE to that food, the prevalence of probable food allergy was 2.0% in Hong Kong, and only 0.5% in mainland China. Serological evidence of past hepatitis A infection was very common ranging from 81% in Guangzhou to 10% in Hong Kong. After adjustment for place, gender and age, presence of hepatitis A antibody was associated with protection against probable food allergy (OR 0.21; 95% CI: 0.10–0.41) with $P < 0.01$. Presence of antibodies against toxoplasmosis and salmonella was not associated with protection against food allergies.
Conclusion: This is the first comparative study of epidemiology of food allergy in urban and rural China. This study demonstrated that the prevalence of food allergies is very low in rural populations. Evidence of past hepatitis A infection was associated with lower prevalence of food allergies. Further studies are needed to clarify the possible causal role of infections and food allergies in children.

Supported by European Commission EUROPREVALL project (FOOD-CT-2005-514000) and the Hong Kong Research Grant Council GRF477110.

1101
Food allergy and its risk factors in pre-school children in Guangdong Province: an epidemiological survey

Luo, J; Sun, B; Huang, H; Zheng, P; Huang, J
State Key Laboratory of Respiratory Disease, Guangzhou, China

Background: To investigate the food allergy and its risk factors in pre-school children in Guangdong Province, China through a self-designed questionnaire. The results of the present investigation are believed to deepen the understanding of food allergy in children and serve as strong evidence for prevention and treatment of allergic diseases in children.
Method: The questionnaire was designed according to the epidemiological characteristics of local allergic diseases in Guangdong Province. Parents of kindergarten children in different areas in Guangdong Province, China were selected to fill in the questionnaire. Relevant investigators followed the parents by phone call to confirm the effectiveness of the questionnaires collected. The results were analyzed by descriptive statistics, Chi-square test, logistic regression model and spearman correlation analysis.
Results:

1. Of 2761 questionnaires that had been handed out, 2540 were valid, giving a valid answer rate of 92%. Of the valid, 1331 cases were male and 1209 cases female, with an average age of 4.6 ± 1.1 years. The common food items leading to adverse food reactions were seafood, high-protein food, dried fruit.
2. Eggs, milk and milk products were the most common food leading to adverse food reactions in children aged 0–3 years. This ratio was reduced dramatically after the age of four.
3. Applied logistic regression analysis of children allergic factors in children food allergies, we can found that the first degree relatives suffered from food allergy and allergy rhinitis, the risk of children with food allergies increased.
4. Analysis of the relevance of various types of allergy revealed by spearman correlation analysis, food allergy and drug allergy, atopic dermatitis, bronchial asthma, eye allergy, allergic rhinitis, the correlation coefficient were statistically differences.

Conclusion: The incidence of food allergy in children in Guangdong Province is higher than we expected, close to those in the United States and Europe, though the allergens are not completely consistent. Although allergy to high-protein molecules is common in infants whose gastrointestinal function is immature, allergy to milk, dairy products and eggs will be reduced with increasingly improved digestive function and the immune system in most young children. The best way to determine food allergens for children who were allergies or have a family history of allergy is go to the hospital for a test.

Poster Session 41

Food allergy: anaphylaxis

1103
The prevention of anaphylaxis of leukotriene receptor antagonists on rush specific oral immunotherapy in children with food allergy

Soejima, K[1]; Taniuchi, S[1]; Takahashi, M[1,2]; Hatano, Y[1]; Nakano, K[1]; Kaneko, K[1]
[1]Pediatrics, Kansai Medical University, Osaka, Japan; [2]Pediatrics, Izumiotsu Municipal Hospital, Osaka, Japan

Background: The aim of the study was to elucidate whether leukotriene receptor antagonists (LTRAs) can prevent severe allergic reactions, which occur during rush specific oral immunotherapy (rSOIT) in children with food allergies.
Method: Ten children with food allergies (four with hen's egg allergy, two with wheat allergy, and four with cow's milk allergy; aged between 7 and 12 years; median, 8.5 years) who were receiving LTRAs during rSOIT were retrospectively selected from among 63 children undergoing rSOIT. Patients receiving LTRAs from the start of rSOIT were excluded from the study. In the rush phase of rSOIT, the initial dose after open food challenge (OFC) was set at approximately one-tenth of the threshold dose in OFC. After the administration of the initial dose, the subsequent doses were increased by approximately 1.2 times of the previous dose and were administered every 2 h, four times a day. The target doses of hen's egg, wheat (udon noodle), and cow's milk in the rush phase were 50, 200 g, and 200 ml, respectively. The ingestion of the target dose was continued at home every day for at least a year in the maintained phase.
Results: Three children with hen's egg allergy and one with wheat allergy experienced intractable abdominal pain during the rush phase; therefore, the loading dose was not increased in these children. However, the administration of LTRAs prevented their symptoms, resulting in the completion of the rush phase. The administration of LTRAs was also effective in the prevention of anaphylaxis during the maintained phase in three children with cow's milk allergy and one with wheat allergy.
Conclusion: The findings from this retrospective study suggest that the administration of LTRAs is useful for the prevention of adverse allergic reactions such as anaphylaxis and abdominal pain during rSOIT.

1104
Reasons for unsuccessful adrenaline auto-injection and the benefits of training

Gyllfors, P[1]; Strand, V[1]; Arvidsson, P[2]; Strodl Andersen, J[3]
[1]Asthma and Allergy Clinic, St Göran's Hospital, Stockholm, Sweden; [2]ALK Nordic, Kungsbacka, Sweden; [3]ALK, Global Clinical Development, Horsholm, Denmark

Background: Adrenaline auto-injectors (AAIs) are indicated for the emergency treatment of severe acute allergic reactions (anaphylaxis). But many reports exist of failure to use a prescribed AAI and of unsuccessful or accidental administration. For this reason, training is essential.
Method: A randomised, open-label, 2-way cross-over, single-centre, non-inferiority study was conducted. Subjects: ≥16 years, body weight ≥50 kg, AAI prescription ≥2 years. Two AAIs were evaluated: Jext® (ALK, Denmark) and EpiPen® (Meda, Sweden). Primary endpoint: proportion of subjects with a successful self-injection. Based on literature, a failure rate of 40% was anticipated in the power calculation. The trial comprised two sessions 1–4 h apart. The AAIs contained adrenaline in line with Swedish recommendations for AAI training. Six fulfilled criteria were required for successful self-injection: correct identification of the safety cap, correct removal of the safety cap, identification of the needle, use of sufficient force, appr. 90° angle, and ≥10 s *in situ*. These criteria were evaluated by a trained nurse present at the sessions. In addition, the sessions were filmed to enable confirmation of these evaluations.
Results: Ninety-one subjects were randomised. Eighty-nine completed the study. Forty-eight percent were male. Ninety percent were Caucasian. Mean age: 34 years (median, 28; min–max, 17–75). The primary results are reported elsewhere. Fifteen subjects had a different outcome for the two sessions: 11 failed in session 1 and succeeded in session 2, whereas four succeeded in session 1 and failed in session 2. The overall failure rates and the three primary reasons for failure are found in T1.
Conclusion: The overall rate of failure was lower than expected, perhaps reflecting the controlled setting. However, one in five still failed to achieve a successful self-injection. The rate of failure was numerically lower for session 2 than for session 1 suggesting that session 1 provided a training effect. The primary reasons for failure suggest that important factors for optimising adrenaline administration could be regular training in handling the AAIs, focusing on adequate time, angle and force needed for achieving a successful injection.

1105
Factors influencing anaphylactic responses in an oral ovalbumin food allergy model under gastric acid suppression

Diesner, SC[1,2]; Schultz, C[1]; Pfitzner, B[3]; Assmann, VE[1]; Krishnamurthy, D[1]; Kreiner, K[1]; Weichselbaumer, M[1,4]; Starkl, P[1]; Eiwegger, T[2]; Szepfalusi, Z[2]; Fehrenbach, H[5]; Jensen-Jarolim, E[1,4]; Hartmann, A[3]; Pali-Schöll, I[1,4]; Untersmayr, E[1]
[1]Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria; [2]Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria; [3]Department of Microbe-Plant Interactions, Helmholtz Center Munich, Neuherberg, Germany; [4]Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna, Vienna, Austria; [5]Research Center Borstel, Leibniz Centre for Medicine and Biosciences, Priority Area Asthma & Allergy, Borstel, Germany

	Jext (N = 90) n (%)	Epipen (N = 89) n (%)	Session 1 (N = 90) n (%)	Session 2 (N = 89) n (%)
<10 s *in situ*	10 (11)	12 (13)	13 (14)	9 (10)
<90° angle	7 (8)	10 (11)	10 (11)	7 (8)
Insufficient force	2 (2)	6 (7)	2 (2)	6 (7)
Overall failure rate	18 (20)	21 (24)	23 (26)	16 (18)

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

Background: Recently, we have established a murine food allergy model, which is based on allergen feedings under gastric acid-suppression. This immunisation protocol has repeatedly been demonstrated to induce IgE-mediated food allergy, including anaphylactic symptoms, mucosal influx of allergy effector cells and elevated Th2 cytokines. As in human patients, not all animals being treated with anti-ulcer drugs developed elevated allergen-specific IgE upon oral sensitisation. Thus, in this study we aimed to characterise differences of animals with high IgE titres and compare them with IgE non-responders.

Methods and results: Out of the BALB/c animals being subjected to our oral ovalbumin (OVA) immunisations under acid suppression, 10 mice revealed high OVA-specific IgE and IgG1 titers (IgE+/IgG1+), five were only IgG1 positive (IgE−/IgG1+) and 10 animals did not show elevated antibody titers (IgE−/IgG1−). After oral and intravenous OVA provocation only the IgE+/IgG1+ animals showed a significant reduction of body temperature, high levels of mouse mast cell protease-1 (mMCP-1), elevated total IgA and OVA-specific IgA levels in intestinal lavage fluid, the latter being also increased in IgE−/IgG1+ mice. These findings were accompanied by an increase of blood hematocrit, haemoglobin, red blood cell count and monocytes after OVA provocation, only in the IgE responders. Gastric pH measurements 15 min after intravenous anti-ulcer medication revealed no differences on drug responses between the 25 animals. In FACS analysis of spleen cells, no substantial differences in T-regulatory cell distribution could be observed. Cytokine analysis of stimulated spleen cell supernatants revealed significantly elevated levels of the Th2 markers IL5 and IL13, but also of IL2. Additionally, we observed higher levels of IL22 in the IgE responding group as well as in the non-responders. Histological analysis did not reveal any visible differences in the gastrointestinal morphology between the three groups. Interestingly, comparison of microbiota revealed differences regarding the composition of bacterial communities between the groups.

Conclusions: These data clearly indicate that anaphylaxis in our model was dependent on OVA-specific IgE rather than IgG1 and was accompanied by changes in blood cell count and cytokine levels. Interestingly, slight differences in microbiota composition were observed between the three different groups.

Supported by FWF grants P21884 and P21577.

1106
Beta-tryptase for the diagnosis of anaphylaxis

Guilarte, M; Sala, A; Labrador-Horrillo, M; Esteso, O; Luengo, O; Cardona, V
Hospital Universitari Vall d'Hebron, Barcelona, Spain

Background: The diagnosis of anaphylaxis is primarily based in clinical symptoms since no reliable marker is currently available. Currently, total serum tryptase is the only mast cell mediator widely available for the diagnosis of anaphylaxis. Nevertheless, in most cases it is not sufficient by itself to confirm the diagnosis of anaphylaxis. The isoform beta-tryptase is massively released during anaphylactic mast cell activation. Our aim was to study the role of beta-tryptase for the improvement of the diagnosis of anaphylaxis.

Methods: Fifty patients with an acute anaphylaxis attending the emergency department during a period of 6 months were evaluated. Only patients fulfilling the NIAID/FAAN criteria were selected. A blood sample was obtained at different timepoints: T1: 1–2 h after the beginning of the clinical symptoms, T2: 4–6 h and T3: 6–12 h after the beginning of the clinical symptoms. Additionally, a baseline sample (TB) was also obtained. Beta-tryptase was measured by ELISA in serum samples, and correlated with total tryptase levels (EIA UniCAP).

Results: There was an increase in tryptase and beta-tryptase values during anaphylaxis as shown by a significant main effect for timepoint respectively ($F_{2,56}$: 18.59; $P < 0.0001$ and $F_{3,56}$: 32.25; $P < 0.0001$). Beta-triptase peak levels were correlated with total tryptase peak levels ($R = 0.53$; $P < 0.0001$). No correlation was observed between beta-tryptase and severity ($R = 0.107$; $P = 0.46$). In 40% of the patients, total tryptase at T1 was <11.4 μg/l. In those patients, total tryptase and beta-tryptase levels were different in T1 comparing with TB ($P = 0.02$ and $P = 0.04$ respectively). However, ratio between T1 and TB total tryptase was <2 in 30% of the patients (15/50). Of those, beta-tryptase ratio was ≥2 in only one patient. In contrast, when total tryptase ratio was ≥2, only 14% of patients had a beta-tryptase ratio < 2.

Conclusion: Beta-tryptase determination does not seem to significantly improve the diagnostic performance of total serum tryptase.

1107
Food-induced anaphylaxis in Albacete, Spain

Soto Vargas, G; Flores González, MP; Palomeque Rodriguez, MT; Lara De La Rosa, MP; Martínez Borque, N; Asensio Sanchez, MT; Gónzalez Martín, MI; Torrecillas Toro, M
Allergology, Complejo Hospitalario Universitario Albacete, Albacete, Spain

Background: To determine the characteristics of food-induced anaphylaxis (FIA) sent to our Allergy department in the Complejo Hospitalario Universitario de Albacete.

Method: Data were taken from the patients with FIA sent to our department during 2012.

Results: One hundred and forty-four patients were included, between 10 and 73 years of age, with an average age of 30.7 years at the time of the diagnosis. The organs affected from largest to smallest frequency, were: skin, respiratory tract, digestive, cardiovascular and neurological.

About the degrees of anaphylaxis we found six patients with anaphylaxis degree I, 12 patients of degree II, 14 degree III, 111 patients degree IV and one patient of degree V.

With respect to the animal proteins we found 10 allergic patients to egg, 12 to crustaceans, nine to anisakis and three to blue fish.

Table 1 Allergic to vegetal proteins

Vegetal proteins	LTPs*+ Profilin−	LTPs− Profilin+	LTPs+ Profilin+	LTPs− Profilin−
Nuts (81)	58	6	10	7
Rosaceous (48)	34	5	7	2
Peach (35)	20	4	10	1
Banana (27)	16	4	5	2
Melon (24)	11	6	6	1
Kiwi (22)	11	3	5	3
Grape (19)	13	1	3	2
Spice (18)	15	0	2	1
Watermelon (16)	7	7	2	0

*Lipid transfer proteins.

Ninety-one percent of the 144 patients with FIA were sensitised to pollens: 86% had rhinoconjunctivitis and 51% allergic bronchial asthma.

Conclusion:

1 The majority of FIA made debut in early ages.
2 Most of the anaphylactic reactions were degree IV. The most affected organ was the skin and the most frequently implicated foods were nuts and rosaceous, with the peach being the principal between vegetables, and eggs and crustaceans, between those of animal origin.
3 Sixty-three percent of the patients who presented FIA had rhinoconjunctivitis and/or asthma by pollens (olive tree, poaceae and chenopodiaceae by this order).
4 The majority of the allergic patients to vegetal proteins were sensitised to LTPs.

1108
Frequency and risk factors for food induced anaphylaxis in Turkish children

Cavkaytar, O; Arik Yilmaz, E; Buyuktiryaki, B; Soyer, OU; Tuncer, A; Sackesen, C
Department of Pediatric Allergy, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Background: Hospital admissions due to food induced anaphylaxis (FIA) has doubled in the last decade. The aim of this study is to find out the frequency of FIA and related risk factors among Turkish children.

Method: The diagnosis of food allergy was determined in children with a specific clear-cut history of food-related symptoms that developed in early phase after the ingestion of a certain food together with the demonstration of IgE mediated sensitisation by positive skin prick tests or serum sIgE. The definition of anaphylaxis and its severity were determined according to the EAACI position paper. Kruskal-Wallis, chi-square and binary logistic regression tests were used in statistical analysis.

Results: FIA was determined in 176 (33%) of 537 children with food allergy. One hundred and thirty-five of them (76%) experienced anaphylaxis in the history, 77 of them (%43.5) experienced anaphylaxis during food challenge test at our clinic. Allergic rhinoconjunctivitis, asthma, having multiple food allergies, aeroallergen sensitisation, familial history for atopic diseases were more frequent in children who experienced FIA compared to the cases without anaphylaxis ($P < 0.0001$, $P < 0.0001$, $P = 0.002$, $P = 0.015$, $P = 0.006$, respectively). Total IgE, the age of onset of symptoms related to food allergy and the age of initial hospital admission for allergic symptoms were higher in patients with FIA ($P < 0.0001$, $P < 0.0001$, $P = 0.012$, respectively). The foods inducing anaphylaxis were cow's milk ($n = 90$, 51%), hen's egg ($n = 40$, 23%), hazelnut ($n = 18$, 10%), lentil ($n = 10$, 6%), fish ($n = 4$, 2%) wallnut ($n = 4$, 2%), peanut ($n = 3$, 2%) and beef ($n = 3$, 2%). The severity of anaphylaxis was mild in 36% ($n = 64$), moderate in 57% ($n = 101$) and severe in 6% ($n = 10$) of the study group. Asthma (OR: 3.0, 95%CI: 1.8–5.0, $P < 0.0001$) and peanut allergy (OR: 2.6, 95% CI: 1.3–5.0, $P = 0.005$) were defined as risk factors for FIA in multivariate regression analysis. The median age for both onset of symptoms and for the initial hospital admission for allergic symptoms was higher in patients with moderate to severe anaphylaxis compared to patients with mild anaphylaxis [0.5 (0.3–0.6) vs 0.4 (0.3–0.5) years] and [2.5 (1–6) vs 1.2 (0.7–2.9) years], ($P = 0.033$ and $P = 0.005$, respectively).

Conclusion: Our results show that one-third of the children with IgE mediated food allergy may experience anaphylaxis and co-existing asthma is a significant risk factor for the development of FIA in Turkish children with food allergy.

1109
Reduced shrimp allergenic potency by *in vitro* digestion

Gámez, C[1,2]; Mazzeo, C[1,2]; del Pozo, V[1,2]; Sanz, V[1,2]; Sastre, J[2,3]
[1]Immuno-allergy, IIS-Fundación Jiménez Díaz, Madrid, Spain; [2]CIBER de Enfermedades Respiratorias, Palma de Mallorca, Spain; [3]Allergy, IIS-Fundación Jiménez Díaz, Madrid, Spain

Background: Shrimp allergy is one of the most important causes of food allergy being the mainstay of treatment a strict avoidance. To find potential hypoallergenic extracts to be use for immunotherapy is still an unmet need. The aim of this work was to assess the effect of simulated gastrointestinal digestion on the allergenicity of *Solenocera melantho* proteins.

Method: The digestive stability of allergenic proteins from raw and boiled *S. melantho* extract in simulated gastric fluid (SGF) was investigated by SDS-PAGE and immunoblotting with sera from shrimp allergic patients. Allergenicity of purified *S. melantho* tropomyosin in SGF was also studied. The allergic reactivity of untreated and digested boiled shrimp extracts (UBSE and DBSE, respectively) was studied by different tests: Skin Prick Test (SPT), basophil activation test (BAT) and T-cell proliferation assays.

Results: Within the SGF system, raw and boiled *S. melantho* proteins were rapidly degraded in a short period of time (5 min), while tropomyosin was more resistant, and only after 60 min of treatment, tropomyosin was completed digested. Immunoblottings using a sera pool from shrimp allergic patients showed that the pepsin digestion reduces the IgE binding to DBSE and to tropomyosin, indicating a reduction of allergenicity.

When SPTs were performed with treated and untreated shrimp extracts, we observed an important reduction in mean wheal diameters (61.4 ± 41.6 vs 19.7 ± 37.5 mm, $N = 5$).

BAT using both extracts showed that UBSE induced a two-fold activation in comparison with DBSE (85.4% vs 43.04%, $N = 3$).

Finally, we demonstrated that UBSE and DBSE induced comparable proliferative response in purified T cells from shrimp allergic patients.

Conclusion: *In vitro* digestion with SGF fluid is effective in reducing IgE binding of *S. melantho* proteins, even to its major allergen tropomyosin. SPTs and BATs prove the reduced allergic activity of DBSE. Moreover, proliferation assays show that DBSE could preserve T cell epitopes. This data open the opportunity to use digested shrimp extract to obtain a safe vaccine for seafood allergy.

1110
Anaphylaxis audit in a busy metropolitan emergency department: a review of real life management compared to best practice

Murad, A; Katelaris, CH
Immunology, Campbelltown Hospital, Sydney, NSW, Australia

Background: A number of studies have highlighted deficiencies in anaphylaxis management in emergency departments when procedures are compared to recommended best practice. We undertook a survey of our hospital's performance in the management of anaphylaxis with the aim of identifying any deficiencies.

Methods: Paediatric and adult admissions to the Emergency Department of a busy hospital in Southwest Sydney were tracked over a 5 month period. Anaphylaxis presentation was graded based on presenting symptoms and signs using the Brown grading system. Use of all medication during resuscitation and management was documented. Observation period before discharge was noted. These measures were compared to best practice as outlined in management guidelines.

Results: A total of 38 patients presented with anaphylaxis. Of these three had severe anaphylaxis, 13 had moderately severe anaphylaxis and 12 had mild anaphylaxis. A number of deficiencies in 'real life practice' emerged. Anaphylaxis was not always recognised and often not graded. This led to inappropriate management most commonly in the moderate severity group, with adrenaline being withheld in 12 of those cases. Identification and management of severe cases were more prompt and appropriate with adrenaline being used on all three occasions. Another frequent problem identified was the use of sedating antihistamines, usually given in the parenteral form, with 14 of the 38 patients receiving promethazine, in spite of recommendations against their use. Observation time was inadequate, especially in those with mild or moderately severe anaphylaxis with 18 patients being observed for <4 h. Finally, despite recommendation to ED staff to refer all patients presenting with anaphylaxis for follow up consultation in the Immunology/Allergy department, this was not universally followed through with only 11 of the 38 being referred.

Conclusion: A number of deficiencies in the management of anaphylaxis presentations have been identified. These deficiencies are not unique to this emergency department and call for targeted educational activities within the departments themselves.

1111
Diagnosis and treatment of food anaphylaxis in Albacete, Spain

Soto Vargas, G; Flores González, MP; Palomeque Rodriguez, MT; Torrecillas Toro, M; Lara De La Rosa, MP; González Martín, MI; Prieto Montaño, P; Martínez Borque, N
Allergology, Complejo Hospitalario Universitario Albacete, Albacete, Spain

Background: To determine the diagnosis characteristics of food-induced anaphylaxis (FIA) sent to our Allergy department in the Complejo Hospitalario Universitario de Albacete, the way they were treated and the helping factors.

Method: Data were taken from the patients with FIA sent to our department during 2012.

Results: One hundred and forty-four patients were included. Forty-four patients had anaphylaxis by a single food and 100 by several foods.

Of the total cases, 35 patients had one episode of anaphylaxis, 21 patients two episodes, 17 of them three episodes and 71 patients four or more episodes.

The time passed from the episode to the diagnosis was from 24 h to 360 months, being the average of 32.5 months.

<2	2–6	6–12	12–24	Major of 24
31	30	11	21	51

Time until the diagnosis (months).

Of the total, 27 had being prescribed adrenaline at the time of the first consultation in Allergology (117 not) and 10 had received it at the emergency room (134 not).

In relation to the helping factors we found that execirse was involved in 17 patients, NSAIDs in six, alcohol in three cases, and fasting and ACE-inhibitors with one case each one.

Conclusion:

1 Sixty-nine percent of the patients with FIA were allergic to several foods.
2 It is of interest the great delay in the derivation of the FIA to our department; 78% of the patients were diagnosed more than 2 months passed the first episode, being of more than 2 years in the 35% of the cases.
3 Most of the patients did not have prescribed adrenaline previously, when arriving at the department of Allergology, and to a minority (6%) it had been administered only in the emergency room.
4 19% had helping factors associated, being exercise and NSAIDs intake, the most frequently involved.

1112
Comparative evaluation of handling characteristics of two adrenaline auto-injectors

Strand, V[1]; Gyllfors, P[1]; Arvidsson, P[2]; Strodl Andersen, J[3]; Taffo, P[2]
[1]Asthma and Allergy Clinic, St Göran's Hospital, Stockholm, Sweden; [2]ALK Nordic, Kungsbacka, Sweden; [3]ALK, Global Clinical Development, Horsholm, Denmark

Background: Adrenaline auto-injectors (AAIs) are indicated for the emergency treatment of severe acute allergic reactions (anaphylaxis). But many reports exist of failure to use a prescribed AAI and of unsuccessful or accidental administration. A new AAI, Jext® (ALK, Denmark) has been developed with the intention of providing simpler administration and instructions, longer shelf life and shielding of the needle after use.

Method: A randomised, open-label, 2-way cross-over, single-centre, non-inferiority study was conducted. Subjects: patients with an AAI prescription ≥2 years but asymptomatic at study visit, age ≥16 years, body weight ≥50 kg. Primary objective: to demonstrate non-inferiority of Jext compared with EpiPen® (Meda, Sweden) for the proportion of subjects with a successful self-injection. Secondary objectives: to compare the handling characteristics regarding subject preference, and time and hesitance to perform the injection. The trial comprised two sessions 1–4 h apart. AAIs with 150 μg adrenaline were used, giving 300 μg in total, i.e. not exceeding the standard dose for adults. Based on literature, the premise for the power calculation was an anticipated success rate of 60%.

Results: Ninety-one subjects were randomised. Eighty-nine completed the study. Forty-eight percent were male. Ninety percent were Caucasian. Sixty-eight (76%) subjects had a successful injection with EpiPen, and 71 (80%) were successful with Jext. Fifteen subjects had a different outcome for the two sessions; nine were successful with Jext and six were successful with Epipen. The non-inferiority test was inconclusive (odds ratio 3.0 in favour of Jext; 90% confidence limits, 0.3–77). Seventy-five percent preferred Jext, 15% preferred Epipen, and 10% had no preference. The time to perform the injection and the degree of hesitance was similar. In the artificial study setting of adrenaline administration without clinical manifestations of anaphylaxis, numerically higher frequencies of dizziness and headache were observed for Jext (six and three events, vs 1 and 0 events for Epipen). A numerically higher frequency of injection site pain was observed for Epipen (21 vs 8 events for Jext).

Conclusion: Eighty percent of the subjects had a successful injection with Jext vs 76% with Epipen, but the trial was inconclusive with respect to non-inferiority. The overall rate of successful injections was higher than expected, leading to lower power for confirmation of non-inferiority. No safety concerns were observed. Seventy-five percent of the patients preferred Jext over Epipen.

1113
Anaphylaxis episodes due to goat's and sheep's milk as hidden food allergen

Acosta Rivera, M[1]; Farias Aquino, GE[1]; Rosado Ingelmo, A[1]; Pastor-Vargas, C[2]; Sanz Serrano, V[2]; Alonso Diaz de Durana, MD[1]
[1]Allergy, Hospital Universitario Fundación Alcorcón, Alcorcón, Spain; [2]Immunology, Fundación Jiménez Diaz, Madrid, Spain

Introduction: Allergy to goat's and sheep's milk (GSM) without cow's milk (CM) allergy is rare. Caseins have been reported

the most implicated allergens. We report two cases of repeated anaphylaxis episodes after ingestion of GSM as a hidden food allergen without any clinical CM allergy.

Methods: *Case* 1. A 6 years old female experienced two episodes of anaphylaxis 30 min after eating pizza made from different cheeses in the same restaurant in different days. She tolerated cheeses in other occasions.

Case 2. A 23 years old male, experienced during the last 12 years, recurrent episodes of anaphylaxis minutes after eating processed food (pasta, pizza). The last episode was 10 min after eating food that contained sheep milk. Both patients have always tolerated CM or their products. Skin prick tests (SPT) were carried out using commercial extracts CM, casein, alpha-lactalbumin and beta-lactoglobulin. Skin prick-prick tests with cow's, goat's and sheep's cheese were also performed. Total serum IgE and specific IgE to cow's milk and its fractions, goat's milk (GM) and sheep's milk (SM) were carried out. SDS-PAGE of CM, GM, SM and Immunoblotting was performed.

Results: Skin prick tests SPT with CM proteins were negative in both patients. Prick-by-prick tests were positive to goat's and sheep's cheese and negative to cow's cheese. Total serum IgE was 221 KU/l (patient 1) and 468 KU/l (patient 2). Specific IgE antibodies by CAP were negative (<0.35 KU/l) to all CM proteins in both patients. Results for Specific IgE to GM and SM were as follows: patient 1 (31.6 and 23.9 kU/l) and patient 2 (17.5 and 13.2 kU/l). Goat's and sheep's immunoblotting showed IgE-binding bands which might correspond to caseins.

Conclusions: We report two patients with repeated allergic reactions and a strong IgE-reactivity to GM and SM proteins but not to CM proteins. A strict avoidance of GSM, a correct labelling and identification of the dairy products should be necessary in GSM allergy because small amounts can elicit severe reactions. GSM should be considered as a hidden food allergen in cases of anaphylaxis due to processed foods.

1115
Allergy to rabbit meat ingestion after sensitisation by inhalation

Martorell, C[1]; Morales, C[1]; Bartolomé, B[2]; Ortega, S[1]; Raducan, I[1]; Peláez, A[1]
[1]Hospital Clínico Universitario, Allergy Service, Valencia, Spain; [2]Research & Development, Bial-Aristegui, Valencia, Spain

Background: A 20-year-old woman who since childhood has suffered from allergic rhinoconjunctivitis resulting from exposure to rabbits; she also displays symptoms by exposure to dogs, cats and horses. In April, 2012, immediately after eating 'paella' (rice, saffron, chicken, rabbit meat, tomatoes, green beans, artichoke and butter beans (*Phaseolus lunatus*)) she developed urticaria, dyspnoea, stridor, disphonia, abdominal pain and dizziness. Thereafter she has not eaten rabbit meat and has tolerated the rest of the 'paella' ingredients.

Method: Skin prick tests with aeroallergens extracts: mites, fungi, pollens and epithelium from dogs, cats, rabbits. Skin prick tests with rabbit meat extract, cow milk; and with lactalbumin, lactoglobulin and caseinSerum specific IgE (EAST) determination to epithelium, urine and meat from rabbit; epithelium from dog and cat; chicken, beaf and pork, as well as BSA. SDS-PAGE immunoblotting technique was performed to detect the IgE binding proteins. Immunoblotting-inhibition was carried out with rabbit meat extract in solid phase.

Results: Skin tests were positive to epithelium from rabbit, dog, cat and horse as well as to rabbit meat. Specific IgE (kU/l) to rabbit epithelium, urine and meat: 38, 13.6 and 6 respectively. SDS-PAGE immunoblotting technique detected a 67-kDa (probably albumin) IgE binding band in rabbit extracts from meat, epithelium and urine. In meat rabbit extract and epithelium was also detected a 40-kDa binding band; and in meat and urine a 28-kDa one. Low intense IgE binding bands were also detected in extracts from dog, cat, horse and cow epithelium and in cow urine. The immunoblotting-inhibition showed that extracts from rabbit epithelium and rabbit urine produced a total IgE binding inhibition in rabbit meat extract.

Conclusion: We present a case of allergy to rabbit meat resulting from previous sensitisation to proteins from rabbit epithelium and urine by inhalation.

The patient is sensitised to proteins from other mammals but it seems that the primary sensitisation agent was rabbits.

1116
Anaphylaxis to raw egg by contact to burned skin in an otherwise tolerant child

Roumpedaki, E[1]; Douladiris, N[1]; Pananaki, A[1]; Maggina, P[2]; Stamogiannou, L[2]; Papadopoulos, NG[1]
[1]Allergy Department, 2nd Pediatric Clinic, National and Kapodistrian University, Goudi, Athens, Greece; [2]1st Department of Pediatrics, 'P & A Kyriakou' Children's Hospital, Goudi, Athens, Greece

Introduction: Anaphylaxis is a serious, allergic reaction of immediate onset caused by various triggers. Anaphylaxis triggered after contact with a food allergen is known as well, yet this implies that the implicated allergen is not tolerated by any other route. We describe a child suffering anaphylaxis after contact with a food allergen, being tolerated in his diet, under special circumstances i.e. a skin burn.

Case presentation: A boy 20 months old has been admitted to the emergency room of our hospital with an altered level of consciousness and a second degree burn at the neck and chest area of about 4% of body surface, after accidentally pouring hot coffee on him. His parents referred having covered the burned area with three raw egg whites, as a folk remedy and about 10 min later, the boy lost consciousness and vomited repeatedly. At his arrival at the hospital about an hour later, he suddenly presented flushing, lips oedema and cyanosis with a persistent low blood pressure (70/45) and wheezing. Adrenaline i.m. has been administered, as well as saline i.v., methylprednisolone i.v. and cetirizine. Serum tryptase was measured about an hour and a half from the onset of reaction and resulted 29.8 μg/l (normal value <11.4 μg/l).

No prior history of atopy has been registered at allergic evaluation. He had eaten egg both cooked and raw since he was 8 months old. His mother suffered allergic rhinitis. A month after the reaction, he resulted positive on SPTs for egg white (raw egg white prick-to-prick 5 mm, reagents Stallergenes 4 mm, Alk 2 mm; Histamine 5 mm) and, sIgE for egg white (1 kUA/l, class II). SPTs and sIgEs for egg yolk, ovomucoid were negative. Baseline serum tryptase was 2.8 μg/l. The patient underwent a food challenge to egg within 3 months, which was negative. From that moment on, he eats all forms of egg.

Discussion: To our knowledge, this is the second case of contact anaphylaxis to egg applied on burned skin, in a child who orally tolerates egg.

1118
Anaphylaxis induced by exercise, food dependent (rice) and facilitated by alcohol

Chivato, T[1]; Muñoz, M[2]; Santaolalla, M[3]; Fernández, M[4]; Mínguez, G[5]; Pérez Gordo, M[6]
[1]School of Medicine, Boadilla, Madrid, Spain; [2]HM Monteprincipe, Madrid, Spain; [3]HM Sanchinarro, Madrid, Spain; [4]HM Madrid, Madrid, Spain; [5]HM Madrid Torrelodones, Madrid, Spain; [6]IMMA, CEU School of Medicine, Madrid, Spain

Background: Prevalence of food allergy and anaphylaxis is increasing continously. Mechanisms involved in cases of anaphylaxis induced by exercise and dependent of intake of some foods and facilitated by

others factors (drugs or alcohol) had been previously described.

Method: Male of 23 years old, previously diagnosed of allergic rhino conjunctivitis due to sensitisation to pollens. Attend consulted for anaphylactic reaction (dizziness, urticaria and asthma) while was playing football. Previously had ingested rice and had consumed alcohol (gin). The anaphylactic reaction was treated at emergency room with intramuscular epinephrine, subcutaneous dexchlorpheniramine and intramuscular methyl prednisolone. The clinical picture described evolved favorably in a few minutes. In his medical history had never problems with physical exercise, food intake or alcohol.

Results: Skin tests were performed and were positive to pollens (grasses, *Cupressus arizonica* and olive) and negative to other pneumo allergens (dust house mites, animal dander and molds). Skin tests were performed with common foods and were positive to nuts (peanut and hazelnut), fruit (peach, apple), rice, corn and soybeans. The patient had previously tolerated all these foods.

Specific IgE was quantified. Different foods and pneumo allergens were positive: corn (0.85 kU/l), apple (14.4 kU/l), peach (5.21 kU/l), soybean (0.45 kU/l), groundnut (1.11 kU/l), hazel (0.49 kU/l), olive (6.66 kU/l), grasses (7.59 kU/l), arizonica (0.56 kU/l) and rice (1.08 kU/l).

Challenge test was performed with alcohol and was negative. Challenge test was performed with rice with negative results. Exercise test was conducted with negative results.

Conclusion: We described a case of anaphylaxis induced by exercise, food dependent (rice) and facilitated by alcohol.

1119
A hidden food allergen: soy

Ünsel, M[1]; Ardeniz, Ö[2]
[1]Izmir University Medical Faculty, Izmir, Turkey; [2]Ege University Medical Faculty, Izmir, Turkey

Background: Soy products are widely used in a variety of processed foods such as meet products, sausages, chocolate and breakfast cereals. The prevalence of soy allergy in adults is unknown. The development of soy allergy in the late adulthood is rare. In most of the reports birch pollen sensitisation was determined to be a risk factor for developing food allergy to soy due to cross-reactivity between the major birch polen allergen (Bet v 1) and soy allergen (Gly m4). A 38-year-old female referred to Allergy Unit with a history of oral allergy syndrome (OAS), itching of the scalp and palms, generalised urticaria, rhinitis and dyspnea necessitating intervention in the emergency department in few minutes after ingestion of meat with sauce and meatball at the restaurant for 10 years. She also reported to experience OAS after ingesting ready soup, chocolate, pastry and cookies. The personal history did not reveal allergic rhinitis, asthma, atopic dermatitis, drug or venom allergy.

Method: The skin prick test (SPT) with common food allergens and aeroallergens (Stallargenes, Paris, France) was performed. Soy-specific IgE was measured by using the CAP system (Phadia, Uppsala, Sweeden). Food challange test with soy was not carried out due to convincing anaphylaxis history.

Results: The repeated SPT with soy revealed a remarkable wheal which is greater than the positive control (soy; 7 mm, histamine; 5 mm). The SPT with aeroallargens including birch was found to be negative. The soy-specific IgE was noticeably positive (82.30 IU/l, normal range: 0–0.10 IU/l).

Conclusion: Here we present an adult case experiencing anaphylaxis to soy in the absence of birch sensitivity. Taking into consider the wide use of this protein it can be regarded as an hidden allergen responsible for food-related systemic allergic reactions or OAS in adults.

1120
Type I variant of Kounis syndrome secondary to Anisakis simplex allergy

Montagni, M; Olivieri, E; Bonzano, L; Melli, V; Martignago, I; Ridolo, E
Clinical and Experimental Medicine, University of Parma, Parma, Italy

Allergic reaction as a cause of acute coronary events was first described in 1950 and later called Kounis syndrome in 1991. Possible allergic trigger usually include drugs, contrast media, latex, hymenoptera sting and food. Type I Kounis syndrome presents as a coronary vasospasm with no atheromatous diseases. In our clinical case, type I variant of Kounis syndrome was induced by Anisakis simplex allergy. Anisakis simplex, a parasite in fish, is in fact able to sensitise humans via the alimentary tract causing immediate hypersensitivity reactions after consumption of parasitized fish.

Case presentation: A 57-years-old woman, with no risk factors for cardiovascular diseases, was admitted to the hospital for chest pain after running early in the morning. She was a fast from the night before, when she had eaten raw fish. The electrocardiogram revealed incomplete right bundle branch block and negative T waves for leads V1-V3, blood tests showed a peak in troponine I of 0.3 ng/ml. Echocardiography was normal and cardiac catheterisation demonstrated normal coronary arteries. Allergic pathogenesis was unforeseen.

A few month later she turned up the emergency room with chest pain, vomiting and flushing after the ingestion of cooked and raw fish. Troponine I was normal and the electrocardiogram did not detect any ischemic changes, but troponine I was 0.7 ng/ml. Cardiac Nuclear Magnetic Resonance was negative. A prick test gave highly positive results for Anisakis simplex and negative results for fish, the detection of specific IgE antibodies using ImmunoCAP system was positive (0.6 KU/l), thus confirming the causal agent. Basal serum tryptase was negative. This is the first case in letterature of Kounis syndrome caused by an emerging food allergen such as Anisakis Simplex.

1121
Anaphylaxis to multigrain bread

Mur Gimeno, P[1]; Martín Iglesias, A[1]; Lombardero Vega, M[2]; Cantero Arias, J[3]
[1]Allergy Unit, Santa Barbara Hospital, Puertollano, Spain; [2]ALK-Abelló S.A., R&D Department, Madrid, Spain; [3]Santa Barbara Hospital, Family Medicine, Puertollano, Spain

Background: We are attending to an increased use of plant seeds in 'natural diets' supplied for human consumption as a bakery additive and nutritional supplement because their content in high-quality proteins or omega-3 fatty acids. Flax (*Linum usitatissimum*) has been described as an unfrequent cause of anaphylaxis after ingestion of its seeds. We report a case report of a patient with oral sensitisation to flax.

Clinical case: A 50 year-old woman with intermittent mild rhinoconjunctivitis caused by mites and grass, sufferred an anaphylactic reaction 5 min after the ingestion of a piece of multigrain bread by first time. She started with pharyngeal and optical itching, nausea, vomiting, dyspnea that was followed by generalised urticaria and eyelid angioedema, requiring treatment at emergency room with full recovery in a few hours. The multicereal bread contained

wheat, roast barley and soy flour, yeast and sesame, poppy, millet and flax seeds. She tolerated wheat bread after.

Results: Prick test with a series of common inhalants and food allergens where positive for grass pollen, mites, linseed (20 × 20 mm) and negative for wheat, barley, maize, soybean and oat flour and sesame, millet and poppy seeds. Specific serum IgE by ImmunoCAP system was positive to phleum 0.57 kU/l (class 2), pteronyssinus 0.42 kU/l (class 2), linseed 2.07 kU/l (class 2) and millet 3 kU/l (class 2). Total IgE was 158 kU/l. We performed immunoblotting with flax and millet seeds. Oral food challenges were not performed because the patient did not give her consent.

Conclusion: The introduction of new food seeds in multigrain breads implies a growing risk of sensitising that must be taken into account for allergist. Since sensitising to flax seeds have been described by ingestion and inhalation, both oral and airborne exposure should be avoided.

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

Poster Session 42

Diagnosis of food allergy II

1122
Diagnostic performance of serum specific IgE for milk, egg, peanut, hazelnut, fish, shrimp, celery, apple and peach in the EuroPrevall cross-sectional study in allergy clinics

Barreales, L[1]; Vázquez-Cortés, S[2]; Bures, P[3]; Ballmer-Weber, B[3]; Clausen, M[4]; Gislason, D[4]; Jedrzejczak-Czechowicz, M[5]; Kowalski, ML[5]; Sinaniotis, N[6]; Papadopoulos, N[6]; Purohit, A[7]; de Blay, F[7]; Le, T-M[8]; Knulst, AC[8]; Belohlavkova, S[9]; Kralimarkova, T[10]; Popov, T[10]; Asero, R[11]; Dubakiene, R[12]; Mackie, A[13]; Del Prado, N[1]; Fernández-Pérez, C[1]; Mills, C[14]; Vieths, S[15]; Fernández-Rivas, M[2]

[1]IdISSC, Epidemiology Unit, Preventive Medicine Department, Hospital Clínico San Carlos, Madrid, Spain; [2]IdISSC, Allergy Department, Hospital Clínico San Carlos, Madrid, Spain; [3]Allergy Unit, Department of Dermatology, University Hospital of Zürich, Zürich, Switzerland; [4]Departments of Paediatrics, Allergy, Landspitali University Hospital, Reykjavík, Iceland; [5]Department of Immunology, Rheumatology and Allergy, Faculty of Medicine, Medical University of Lodz, Lodz, Poland; [6]Allergy Department, 2nd Pediatric Clinic, University of Athens, Athens, Greece; [7]Division of Allergy, Department Chest Diseases, University Hospitals of Strasbourg, Strasbourg, France; [8]Department of Dermatology/Allergology, University Medical Center Utrecht, Utrecht, The Netherlands; [9]Pediatric Department, Faculty Hospital Bulovka, Prague, Czech Republic; [10]Clinical Centre of Allergology, Alexandrovska Hospital, Sofia, Bulgaria; [11]Clinica San Carlo, Ambulatorio di Allergologia, Paderno Dugnano, Italy; [12]Vilnius University, Vilnius, Lithuania; [13]Institute of Food Research, Norwich Research Park, Norwich, United Kingdom; [14]Manchester Academic Health Science Centre and Manchester Institute of Biotechnology, University of Manchester, School of Translational Medicine, Manchester, United Kingdom; [15]Paul-Ehrlich-Institut, Division of Allergology, Langen, Germany

Background: Several studies have compared the serum food-specific IgE (sIgE) value with a food challenge reference standard in order to establish its diagnostic accuracy. The results varied from one study to another. The purpose of this analysis was to determine the diagnostic accuracy of sIgE measured by ImmunoCAP (Thermofisher Scientific) in predicting food allergy to hen's egg, cow's milk, fish, shrimp, peanut, hazelnut, apple, peach and celery.

Method: In the multi-centre cross-sectional study carried out in outpatient clinics within the EuroPrevall project, patients reporting immediate adverse reactions to nine specific foods were evaluated by means of sIgE to these foods. Clinical reactivity was assessed by double-blind placebo-controlled food challenges (DBPCFC). The ROC analysis was used to determine the optimal cut-off points, considering DBPCFC as gold standard. Sensitivity (Se) and specificity (Sp) were calculated at the optimal cut-off points of the ROC curve and at the standard cut-off of 0.35 kU_A/l.

Results: A total of 445 sIgE determinations were included. The analysis showed the following area under curve (AUC) and 95%CI values: 0.57 (0.41–0.72) for peanut, 0.55 (0.35–0.75) for hazelnut, 0.47 (0.21–0.72) for fish, 0.42 (0.24–0.61) for shrimp, 0.64 (0.47–0.82) for celery, 0.47 (0.28–0.66) for apple, 0.50 (0.28–0.71) for peach, 0.62 (0.48–0.76) for milk and 0.86 (0.76–0.96) for egg white. Considering Se and Sp values, the optimal cut-off points (kU_A/l) to classify patients as allergic to each food were: peanut 0.29, hazelnut 0.73, fish 0.24, shrimp 0.93, celery 0.20, apple 0.73, peach 0.88, milk 0.6, and egg white 0.23.

At the 0.35 kU_A/l cut-off point for egg white Se was 90.1% and Sp 65.2%. For the remaining foods Se varied from 51% to 83% and Sp from 14% to 63%.

At the optimal cut-off point found for egg white within this study ($\geq$0.23 kU_A/l), Se was 86.5% and Sp 72.2%. Hazelnut and celery showed good Se (83.6% and 83.3%) but very low Sp (40.0% and 53.3%). For the remaining foods Se ranged from 54% to 71% and Sp from 42% to 59%.

Conclusion: Se, Sp and AUC values for the optimal cut-off points of sIgE results were not high enough to consider this test as a good predictor of food allergy for milk, fish, shrimp, peanut, hazelnut, apple, peach and celery. An accurate cut-off point to predict food allergy could only be found for egg.

1123
IgE reactivity profile in patients with positive skin tests to shrimp

Martins, TF[1]; Mendonça, TN[1]; Moreno, AS[1]; Aragon, DC[2]; Arruda, LK[1]

[1]School of Medicine of Ribeirao Preto, University of Sao Paulo, Medicine, Ribeirao Preto, Brazil; [2]School of Medicine of Ribeirao Preto, University of Sao Paulo, Pediatrics, Ribeirao Preto, Brazil

Background: Tropomyosin is an important allergen in shrimp, and it has been implicated in IgE cross-reactivity among invertebrates. Our aims were to evaluate the role of IgE to tropomyosin in diagnosis of shrimp allergy and to investigate the implication of tropomyosin in cross-reactivity.

Method: Thirty-four patients (18–67 years-old, 14 male) who presented positive skin prick tests to shrimp, attending our Allergy Clinics, were evaluated by history, including detailed questions about symptoms upon shrimp ingestion, sensitisation to cockroach and mites, and IgE responses to shrimp and cockroach tropomyosins. Specific IgE to tropomyosins was quantitated by chimeric ELISA. IgE antibodies to shrimp tropomyosin (Pen a 1) were also measured by ImmunoCAP, as well as IgE to shrimp extract.

Results: Of the 34 patients who had positive skin tests to shrimp, 33 had detectable IgE to shrimp by ImmunoCAP (0.4–100 kIUa/l, Geometric Mean GM = 13.1 kIUa/l). Twenty-seven (79.4%) of 34 patients had IgE to shrimp tropomyosin by chimeric ELISA (GM = 22.7 IU/ml; range 1.8–62.0 IU/ml) and of these, 26 patients (76.5%) also had detectable IgE to Pen a 1 by ImmunoCAP (GM = 9.4 kIUa/l; range 1.9–100 kIU/l). There was a significant correlation of IgE to shrimp by ELISA and ImmunoCAP ($r = 0.94$, $P < 0.001$). In addition, there was an excellent concordance rate of results, with kappa value of 0.95. IgE to cockroach tropomyosin was detected in 27/34 patients (79.4%), with GM = 45.8 IU/ml, ranging from 1.9 to 192.8 IU/ml. There was an excellent correlation of IgE levels to shrimp and cockroach tropomyosin ($r = 0.92$, $P < 0.001$). There were 17 patients who reported reactions upon ingestion of shrimp, and 4 who could eat shrimp without any symtoms. Thirteen patients had never eaten shrimp and had positive skin tests to shrimp, cockroach and mite extracts. In this group, all patients had detectable IgE to shrimp, ranging from 0.4 to 100 kIUa/l. Seven non-allergic individuals without symptoms upon shrimp ingestion had undetectable IgE to shrimp and to shrimp tropomyosins.

Conclusion: Consistent with previous observations, shrimp tropomyosin was a major allergen among Brazilian patients. A strong correlation of IgE levels to shrimp and cockroach tropomyosin was found. Positive skin tests and serum IgE antibod-

ies to shrimp were detected in cockroach and mite-allergic patients who have never consumed this food. Our results suggest strong IgE cross-reactivity to tropomyosins of shrimp and cockroach.

1124
How stable are eliciting dose, severity of allergic reaction and reaction time when initial and 16-months oral re-challenge are compared in peanut allergic children?

Blumchen, K[1]; Trendelenburg, V[1]; Ahrens, F[2]; Gruebl, A[3]; Hamelmann, E[4]; Hansen, G[5]; Heinzmann, A[6]; Nemat, K[7]; Niggemann, B[1]; Beyer, K[1]
[1]Department of Pediatric Pneumology and Immunology, Charité University Medicine Berlin, Berlin, Germany; [2]Children's Hospital "Altona", Hamburg, Germany; [3]Department of Pediatrics, Technical University Munich, Munich, Germany; [4]Department of Pediatrics, Ruhr University Bochum, Bochum, Germany; [5]Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany; [6]Center for Pediatrics and Adolescent Medicine, University of Freiburg, Freiburg, Germany; [7]Department of Pediatrics, University Hospital Carl Gustav Carus, TU Dresden, Dresden, Germany

Background: In peanut allergic patients who show an allergic reaction at a higher eliciting dose at oral challenge one tend to play down the risk of accidental reaction to mute amount of peanut. However, this implies that eliciting dose stays stable over time.
Objective: To compare eliciting dose, severity of allergic reaction and time to objective reaction at repeated oral challenges in a prospective study in peanut allergic children.
Methods: Twenty-two peanut allergic children (median age: 7.4 years) with median peanut-IgE of 94 kU/l were recruited. They received a peanut challenge with maximum of eight, semi-log increasing titration steps of roasted peanut (12–18 000 mg equal 3–4500 mg peanut protein) every 2 h within 3 days until objective allergic reactions were observed. Severity of symptoms was graded in a scale of I–V. All children received an oral re-challenge after a median of 17 months (range: 13–19 months). Eliciting dose, severity of reaction and time to reaction under challenge were recorded and compared.
Results: Within the whole study population the median of the eliciting dose did not differ significantly between both challenges 17 months apart (median eliciting dose at first challenge: 260 mg, range: 12–4000 mg vs 120 mg, range: 12–19 000 mg whole peanut at second challenge, $P = 0.13$). However, comparing the eliciting dose intra-individually, three of 22 patients (14%) reacted to at least two dose steps lower at second challenge. Only 7/22 patients showed the same eliciting dose at both challenges. The median of the severity of reaction did not differ significantly between both challenges (median grade of severity at first challenge: III (range: I–IV) vs II (range: 0–IV) at second challenge, $P = 0.16$). However 4/22 patients (18%) showed an increase in severity of the allergic reaction at re-challenge. Notably, 17/22 patients reacted with immediate type symptoms at more than 30 min after ingesting their eliciting dose of peanut. There was no correlation intra-individually between reaction time comparing both challenges ($r_s = 0.29$, $P = 0.19$).
Conclusion: Although the majority of our study population stayed stable in regard to eliciting dose and severity, more than 10% showed much lower eliciting doses or more severe reaction at re-challenge when compared to their initial challenge. Thus, one should not play down the risk of accidental reactions to small amount of peanut if patients react to higher doses at oral challenge.

1126
Food allergy in a Middle Eastern country

Ehlayel, MS[1]; Bener, A[2]
[1]Weill Cornell Medical College-Qatar, Hamad Med Corp, Section of Ped Allergy-Immunology, Doha, Qatar; [2]Weill Cornell Medical College-Qatar, Hamad Med Corp, Medical Statistics & Epidemiology, Doha, Qatar

Background: Worldwide food allergy is relatively common among children. Medical literature is limited on studies on food allergy in many of the Middle East countries.
Objectives: This study was aimed at determining common clinical presentations, reactivity detection by vitro test and of skin prick test (SPT) to common food items, and type of food allergens.
Materials and methods: A retrospective analysis was conducted on 340 children with food allergy (aged 0–14 years) managed at Ped. Allergy-Immunology Clinics, Hamad General Hospital during 2008–2011.
Results: Data on 340 patients was analyzed. They were 231 males and 109 females. Their age (mean ± SD) was 43.1 ± 35.9 months. Co-existing other allergic diseases were observed in 46.7% of patients, and family history of allergies was positive in 75.6%. Consanguinity was noticed in almost 30% of cases. Commonest clinical presentations were acute urticaria (55%), atopic dermatitis (25.3%), anaphylaxis (8.8%), vomiting (5.6%), and failure to thrive (5%). Lab workup revealed mean WBC 4135.7 and AEC 430.6 cells/u, and total, IgE 258.8. Utilisation of diagnostic test for diagnosis of food allergy was 97.9% fusing food specific-IgE panel [RIDA3™] and 84.1% for SPT common foods. SPT added/picked 16% additional patients to those detected by food specific-IgE panel. Common food allergens were milk (24.8%), pistachio (16%), eggs (14.7%), cashew (14.5%), peanuts (13.8%), and wheat (11.2%).
Conclusion: Food allergy is common among children in Qatar. High index of suspicion should be raised in atopic children or family history of allergy. Milk, tee nuts, eggs and peanuts are very common allergens. The later food item may indicate a dietary shift in dietary habits of children in Qatar.

1127
Determination of egg specific IgE levels to predict clinical reactivity and tolerance according to the age groups by using the challenge tests in egg allergic children

Arik Yilmaz, E; Yavuz, ST; Cavkaytar, O; Buyuktiryaki, B; Sahiner, UM; Tuncer, A; Sackesen, C
Department of Pediatric Allergy, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Background: Food sIgE levels are the main tools to predict the clinical reactivity against foods. Previous studies determined cut-off values of egg sIgE levels for the childhood period with a wide range of age. The aim of this study is to determine the cut-off values of egg sIgE according to the different age groups to predict clinical reactivity and tolerance by using egg challenge test results and to investigate risk factors for anaphylaxis.
Method: IgE mediated egg allergy was diagnosed in the presence of positive skin prick test, specific IgE ≥ 0.35 kU/l and history of egg related symptoms or positive challenge tests. Open or DBPC challenge tests were performed in egg allergic children. The predicted probability curves of egg sIgE levels for clinical reactivity were determined by linear logistic regression analysis. Probability curves were created according to the age groups: <2, 2–4, ≥4 years of age. The risk factors for anaphylaxis were determined by logistic regression analysis.
Results: A total of 182 (50.3%) of 362 egg allergic children were underwent challenge tests [83 (45.6%) open; 99 (54.4%) DBPCFC]. Sixty-five (35.7%) out of 182 provocation tests were positive and 30 of those (46.2%) resulted in anaphylactic reaction. Egg sIgE levels showing clinical reactivity with 90% probability by using predicted probability curves were found 12.8 kU/l for all ages; 13.5 kU/l for <2 age; 9.7 kU/l for 2–4 age and 5.1 kU/l for ≥4 age. The initial egg sIgE level that dis-

tinguished between the persistence and development of tolerance with high sensitivity and specificity was found 6.2 kU/l by using roc curve analysis. The duration of egg allergy was longer in initial sIgE >6.2 kU/l than ≤6.2 kU/l according to the Kaplan-Meier analysis ($P < 0.0001$). Egg sIgE levels and the presence of gastrointestinal symptoms with egg consumption were determined as significantly risk factors for anaphylaxis by multivariate logistic regression analysis [(OR: 1.02, 95%CI: 1.01–1.04, $P = 0.004$) and (OR: 5.14, 95%CI: 2.53–10.46, $P = 0.000$) respectively].

Conclusion: Our results denoted for the first time the cut-off values of egg sIgE on different age groups in childhood with 90% probability. Our study may be a useful reference for the clinicians to test these cut-off values during follow-up of the tolerance development.

1129
Sensitisation profiles and clinical relevance of food allergy in patients sensitised to profilin

Alvarado, MI[1]; De la Torre, F[2]; Jimeno, L[2]; Rivas, B[1]; Lázaro, MJ[1]; Barber, D[2]
[1]Ciudad de Coria Hospital, Coria, Spain; [2]ALK-Abelló, Madrid, Spain

Background: Prevalence of food allergy increases in patients with pollinosis. Sensitisation to panallergens such as profilin and/or LTP is statistically associated to food allergy.

Objective: The objective of this study is to assess the relevance of profilin as severity marker of food allergy in a geographical area with high grass allergen pressure and where the prevalence of profilin sensitisation in pollen allergic patients is 65%.

Material and methods: Twenty-six patients with food allergy and with positive SPT to profilin and negative SPT to LTP were included in the study, 14 with moderate-severe clinic (cases). As controls were included 12 patients with mild food allergy (Oral Allergy Syndrome -OAS-). A double-blind placebo-controlled oral food challenge (DBPCOFC) with five different concentrations to purified profilin was performed to 18 patients. In all patients was determined the sensitisation allergen profile (sIgE-sIgG4) by ImmunoCAP-ISAC® (Thermo Scientific).

Results: All patients presented sensitisation to grass. The profile of sensitisation (sIgE) was profilin 92.3%, grass 84.6% and 69.2% recognised all allergens of grass.

The results of the DBPCOFC are presented in the next table (Type of reaction and number of patients):

Reaction	Controls (%)	Cases (%)
1-OAS	4 (50)	
2-OAS + digestive symptoms	1 (12.5)	2 (20)
1-OAS + aphta in oral mucosa	1 (12.5)	
3-OAS + espirometry alterations	1 (12.5)	4 (40)
3-OAS + systemic symptoms	1 (12.5)	
4-0AS + uvula edema		2 (20)
Uvula edema		2 (20)

DBPCOFC results.

These reactions were mild/moderate in 62.5%/37.5% respectively of controls and moderate/severe in 60%/40%.

Conclusion: Both in cases and controls, profilin may be considered as a marker of severity of the allergic reaction in grass pollen patients with food allergy and can induce more severe reactions than OAS, fulfilling the criterio to be considered a clinically relevant food allergen.

1130
Utility of allergen-specific IgE measurements for supporting the diagnosis of hen's egg and cow's milk allergy

Sato, S; Kutsuwada, K; Ebisawa, M
Department of Allergy, Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, Kanagawa, Japan

Background: Food allergy is increasing among children with two of the most common foods responsible being hen's egg (HE) and cow's milk (CM). The aim of this study was to compare probability curves derived from two serologic methods and to evaluate the application of these curves for diagnosis of food allergy in children.

Methods: Serum samples were collected from 204 patients suspected of having HE allergy (median age 2.6 years; age range 4-months–13.1 years) and from 185 patients suspected to have CM allergy (median age 2.7 years; age range 7 months–15.3 years). Final diagnosis of FA was confirmed by oral food challenge (OFC) and clinical history to the eliciting food. Acquisition of tolerance was defined as patients who had ingested heated-whole egg or 200 ml of CM without any symptoms. Serum samples were drawn before and after the food allergy (FA) diagnosis over a 6-month interval and then stored frozen. Allergen specific IgE (sIgE) testing for HE and CM was performed using the IMMULITE® 2000 XPi 3 gAllergy™ (3 g) and ImmunoCAP® (CAP) specific IgE assays. The relationship between 3 g and CAP was analyzed using correlation analysis and logistic regression to plot the predicted probability curves.

Results: Correlation was observed between 3 g and CAP for the EW and CM sIgE measurements (EW: $r = 0.936$, CM: $r = 0.864$). The EW sIgE values were approximately four times higher than CAP while the CM sIgE were about three times higher. In comparison to CAP, the 3 g assay detected seven out of nine patient samples with non-detectable (ND, <0.35 IUA/ml) sIgE to EW and four out of eight patient samples with ND sIgE to CM. The FA positive group had a significantly higher median value than the food tolerant group when measured by 3 g (IUA/ml): EW: 44.0 vs 8.68, CM: 14.2 vs 2.43, $P < 0.01$. The probability for failed challenge using EW IgE was 12% for <0.35 (IUA/ml) and 73% for >100 by CAP, whereas those of 3 g was 6% for <0.1 (IUA/ml) and 72% for >500. Against CM IgE, the probability using by CAP was 16% for <0.35 and 76% for >100, while, 3 g's was 5% for <0.1% and 88% for >500.

Conclusion: In the diagnosis of HE and CM allergy, measurement of IgE to EW and CM using 3 g is related to the OFC outcome when investigating children suspected of having FA and therefore may be useful in probability curve applications. However, it is important to note sIgE values are not interchangeable between serologic methods due to the differences observed.

1131
Preparation of the egg in the food challenge test: 'raw, medium or well done?'

Martelli, A[1]; Allievi, E[1]; Bianchi, R[2]; Origgi, D[1]; Piacentini, G[1]; Serradori, L[1]; Traina, G[1]; Vernich, M[2]
[1]Paediatrics, S. Corona Hospital, Garbagnate Milanese, Italy; [2]Paediatrics, Hospital Bollate, Bollate, Italy

Background: There is no agreement about egg preparation for oral provocation test in children with a history of IgE-mediated allergy reaction to hen's egg. Several different preparations have been proposed, raw egg, baked, lyophilised, but a shared standardisation has never been reached.

Methods: An open challenge with cooked egg was performed in 15 raw hen's egg-allergic children with IgE mediated reactions, nine males and six females, aged between 12 months and 9 years and 4 months; five children had a history of anaphylactic reactions. The whole egg was cooked for 5 min, with no added ingredi-

ents, prepared in an enamel pan, 3 mm thick, in order to guarantee heat uniformity. Egg doses were administered with the following schedule: labial contact without swallow, 0.1, 0.3, 1, 3, 10, 30 ml. Administrations were performed every 30 min. The total dose approximately corresponds to the average meal dose. Patients were kept under observation for 2 h after the last dose. The challenge was deemed concluded either when all the doses had been tolerated or in case of the appearance of a significant adverse reaction.

Results: Thirteen children out of 15 tolerated the cooked egg administration. One challenge was interrupted due to diarrhea and urticaria, and in another patient a skin rash appeared 90 min after the last dose administration. In this last case, the home introduction of cooked egg was suggested, with no further reactions. All the tolerant children were able to assume owen cooked eggs in the following months, while eliminating raw egg from their diet.

Conclusion: The vast majority of egg-containing market foods contain cooked egg. A raw egg challenge is not safe from a microbiological point of view and can cause severe reactions, while the baked egg challenge is more agreeable and allows the home introduction of cooked egg-containing foods with higher safety, once the test has proven negative. Therefore the intermediate egg cooking can be a suitable choice for egg challenge in children with egg-related allergies.

1132
Prospective validation and accuracy of Ara h2 in the diagnosis of childhood peanut allergy

Preece, KD[1,2]; Bhatia, R[1]; Belcher, J[1]; Patchett, K[3]; McElduff, P[4]; Mattes, J[1,2]
[1]Paediatric Allergy/Immunology, John Hunter Children's Hospital, Newcastle, NSW, Australia; [2]Hunter Medical Research Institute, Experimental &Translational Respiratory Group, University of Newcastle, Newcastle, NSW, Australia; [3]Immunology/Allergy, John Hunter Hospital, Newcastle, NSW, Australia; [4]Clinical Trials Unit, Hunter Medical Research Institute, University of Newcastle, Newcastle, NSW, Australia

Background: Peanut allergy is an increasing problem for children worldwide with huge disease burden on the community. Serum IgE levels against the peanut allergen Ara h2 have been shown in a number of retrospective studies to predict a clinical reaction at food challenge.

Method: Participants were recruited from a cohort of consecutive children who were scheduled for an open-labeled peanut food challenge (OFC) by their Paediatric Allergist for clinical indications. Participants attended a pre-challenge clinic in the week prior to food challenge and were assessed for coexisting atopic disease. All participants were assessed for skin prick test (SPT) sensitisation to whole peanut extract and serum was collected for whole peanut and Ara h2 specific IgE. We also measured exhaled nitric oxide and pulmonary function in all cooperative children. Participants underwent OFC and the medical supervising team was blinded to other test results. Evidence of reaction was assessed based on previously validated criteria.

Results: Of 65 families invited to participate, eight declined and one child was unwell with asthma exacerbations on two separate occasions. Three patients had equivocal challenge results and were excluded from analysis. Ara h2 specific IgE showed improved diagnostic accuracy when compared to SPT. Receiver operator characteristic curve analysis gave an area under the curve for Ara h2 sIgE of 0.84 (95% CI, 0.72–0.96). An Ara h2 cutoff of 0.67 kU/l gave 96.7% (95% CI, 82.8–99.9%) specificity with 73.9% (95% CI, 60–89.77%) sensitivity to predict clinical allergy. Specificity was improved to 100% (95% CI, 88.43–100.0%) if the cut off was increased to 1.39 kU/l. Interestingly, Ara h2 sIgE levels of 0.03 kU/l were required to discriminate peanut allergic patients with a sensitivity of >95%. We found no statistical difference between Ara h2 sIgE levels in those children who had anaphylaxis and those with any other clinical reaction. Combining a SPT range between 3.0 and 8.9 mm or an Ara h2 sIgE level of <0.7 kU/l as OFC inclusion criteria, 22% and 25% of the challenges respectively, would have been avoided. Only one patient misclassified as false positive.

Conclusion: Ara h2 specific IgE levels provide improved diagnostic ability in a paediatric population suspected to have peanut allergy when measured prospectively. Incorporation them into diagnostic screening algorithms can reduce the risk with food challenge and number of challenges required.

1133
Association of sensitisation to peanut allergen components with clinical reactivity

Svobodova, P; Malkusova, I; Panzner, P; Vlas, T
Department of Immunology and Allergology, The Faculty of Medicine in Pilsen, University Hospital in Pilsen, Charles University in Prague, Plzen, Czech Republic

Background: Peanut allergy is one of the most common food allergies and may cause severe reactions. However, many of sensitised patients tolerate it. Skin prick tests and specific IgE antibodies to peanut extract are not able to distinguish between symptomatic and asymptomatic patients. Oral food challenge is time-consuming and risk for patients. Reactivity to distinct peanut allergen components may draw attention to the potential risk of more severe reactions.

Method: We analyzed the group of 250 patients sensitised to peanut allergens: Ara h1, 2, 3 or 8. We used microarray ImmunoCAP ISAC for specific IgE detection. Patients were divided into eight groups according to their dominant clinical symptoms. Frequency of sensitisation to individual allergen components in each group was assessed.

Results:

1 Children, who do not eat peanuts because of low age (17 persons, avg. age 10 years): 12% sensitised only to Ara h1, 18% only to Ara h2, 41% only to Ara h8, 29% polysensitised.
2 Asymptomatic patients (180 pers., avg. age 29 years): 97% sensitised only to Ara h8, 1% to Ara h1, 1% to Ara h2, 1% polysensitised.
3 Oral allergic syndrome (15 pers., avg. age 26 years): 87% sensitised only to Ara h8, 13% polysensitised.
4 Atopic eczema flares (15 pers., avg. age 10 years): 60% sensitised only to Ara h8, 7% only to Ara h1, 12% to Ara h1, 2, remaining 21% to Ara h8 and one of the left components.
5 Asthma attack (2 pers., avg. age 13 years): 50% sensitised only to Ara h8, 50% polysensitised.
6 Digestive symptoms (3 pers., avg. age 22 years): 33% sensitised only to Ara h2, 67% to Ara h8.
7 Polysymptomatic patients (12 pers., avg. age 13 years): 25% sensitised only to Ara h2, 25% to Ara h8, 8% to Ara h1, 2, 17% to Ara h2, 8, 25% polysensitised.
8 Anaphylaxis (5 pers., avg. age 9 years): 100% were sensitised to Ara h2, 67% was polysensitised. No sensitisation to Ara h8 was seen in this group.

Conclusion: Asymptomatic patients were mostly adults and 99% of them were co-

sensitised to birch pollen, so that cross-reactivity is probably reason for their Ara h8 sensitisation (PR-10 group proteins). More severe reactions were observed in children. Sensitisation to allergen extracts in non-exposed children may be due to cross-reactivity or via breast feeding.

We confirmed the findings suggesting that allergy to seed storage proteins (especially Ara h2) is frequently responsible for the more severe allergy symptoms. Sensitisation to Ara h8 is frequently associated with mild or no symptoms.

1134
Clinical manifestations and conclusive diagnosis in cow's milk allergy

Lavrut, J[1]; Leonardelli, A[2]; Diaz, MC[1]; Slullitel, P[1]; Spinelli, S[1]; Silvestro, JAJ[3]
[1]Htal Gral de Niños P. de Elizalde, Unidad Alergia, Ciudad Autonoma de Buenos Aires, Argentina; [2]Htal Gral de Niños P. de Elizalde, Laboratorio Central, Sección Química Clínica, Ciudad Autonoma de Buenos Aires, Argentina; [3]Htal Gral de Niños P. de Elizalde, Capital Federal, Argentina

Background: Adverse reactions to cow's milk proteins (CMP) can be present from birth, even in exclusively breast-fed infants. We consider cow's milk allergy (CMA) using the term 'allergy' in accordance with the WAOs definition: a hypersensitivity reaction initiated by specific immunological mechanisms. Most children with CMA have immunoglobulin E (IgE) mediated allergy as a manifestation of their atopic constitution, with or without atopic eczema, asthma, or allergic rhinitis. A small group have non IgE- mediated (cell-mediated) CMA with mainly gastrointestinal symptoms.
Aim: The clinical symptoms and the usefulness of the analytical tests for confirmation of CMA in children with clinical suspicion.
Method: We evaluated clinically and immunologically 55 children (71% male, 29% female), aged between 1 month and 11 years. The methods used were Skin Prick test (SPT)/specific immunoglobulin E (sIgE) (cow's milk, α-lactalbumin, β-lactoglobulin, casein, soy, white and egg yolk) and oral provocation (OP) Test, considered as gold standard diagnostic tool.
Results: From the total population evaluated, the suspicion of CMA was confirmed in 33 patients (60%) via OP. Twenty of these children showed cutaneous symptoms, urticaria being the most relevant sign. Digestive symptoms were presented in 10 children, being diarrhea the most frequent finding, while the remaining three presented respiratory symptoms. The sensitivity achieved through the use of sIgE assay was 67% and a specificity of 81.5% for a breakpoint >0.35 KU/l when compared with PO. (PPV: 87%, NPV: 57.8%).

The sensitivity of SPT for a 3 mm cut-off point was 78.5% with a specificity of 71% compared with OP. (PPV: 78.5%, NPV: 71%). From the 20 children with cutaneous alterations, 12 were positive for SPT, sIgE and OP, while from the 10 children with digestive disorders, five were negative for sIgE and SPT, with positive OP.
Conclusion: Similar to findings reported in literature worldwide, in our population, clinical suspicion of CMA was greater than its diagnostic confirmation. Although SPT and sIgE tests possess an adequate sensitivity and specificity, OP remains the confirmation diagnostic tool.

1135
Clinical and immunological characteristics in children with walnut allergy

Lee, J-M; Koo, N-H; Jeong, K; Jeon, S-A; Lee, S-Y
Department of Pediatrics, Ajou University School of Medicine, Suwon, Korea

Background: Tree nut allergy is characterised by a high frequency of life-threatening anaphylactic reactions, and the walnut (WN) is the most common cause of tree nut allergy in western countries. However, studies of WN allergy are very rare in Asia. This study was designed to characterise clinical WN allergy and to identify the major allergenic components of WN, and co-sensitisation to other nuts in WN allergic children in Korea.
Method: We selected 31 patients (8–108 months) with clinical walnut allergy. Clinical data were collected from medical record and telephone survey. Sera from 26 patients were used for component-resolved diagnosis by protein microarrays (ImmunoCAP ISAC-CRD 103, Phadia).
Results: The median ages of first walnut exposure and reaction were around 28 months and 57% of patients were exclusively breast-fed during early infancy. Ninety-six percent of subjects had IgE antibodies to nJug r 1 and often presented with severe symptoms. Angioedema ($n = 12$) was the most common reaction, followed by urticaria (10) and anaphylaxis (9). In this study, nJug r 2, one of known major allergens was recognised only in two patients along with nJug r 1. Lipid transfer protein (LTP), nJug r 3 was detected in two patients along with nJug r 1. Most patients were sensitised to one or more nuts such as peanut (7), soy (7), hazel nut (6), cashew (4), sesame (3), brazil nut (3). A discrepancy was found between detecting antibodies of peanut by ImmunoCAP and ISAC. Twelve (40%) out of 30 were co-sensitised to peanut by ImmunoCAP. However, four out of 12 had no reactions to any of major peanut allergens, rAra h 1, rAra h 2, rArah 3, nAra h 6, nor LTP, nor the PR-10. Telephone survey was available with two patients. One had urticaria to peanut powder ingestion. The other had no previous history of peanut exposure.
Conclusion: Jug r 1 is the only major allergenic component in Korean children with clinical WN allergy, and 40% of patients were co-sensitised to various nuts.

1136
Clinical and immunological study of IgE-mediated barley allergy in Korean children

Lee, J-M; Jung, S; Jeon, S-A; Lee, S-Y
Department of Pediatrics, Ajou University School of Medicine, Suwon, Korea

Background: Barley is one of the most widely grown crops in the world and used as an ingredient in food processing but researches on adverse reactions to barley are unusual. To evaluate clinical characteristics of barley allergy and identify IgE binding patterns to commonly used barley in Korea, we underwent this study.
Method: Three patients with IgE-mediated barley allergy and two healthy controls were enrolled. Barley-specific IgE was measured by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). We made two kinds of barley extracts with covered and naked Hordeurn vulgare. Using our own made crude extract, IgE ELISAs were done. After SDS-PAGE separation of covered and naked barley extract, we underwent IgE immunoblot analysis with sera from three children with barley allergy and two control subjects.
Results: Three cases of IgE-mediated barley allergy were confirmed by detailed medical history and measurement of specific IgE by ImmunoCAP. An 11-months-boy experienced anaphylaxis by eating infant weaning food containing barley flour, and the serum barley specific IgE level was 35.2 kU/l. One patient had urticaria (specific IgE, 14.9 kU/l), and the other one had respiratory symptoms (59.6 kU/l) after exposure to barley containing foods. When the mean + 3SD was defined as the cut-off value for controls, all three patients with barley allergy had high serum-specific IgE antibody levels to two types of barley compared with controls. In all patients, the specific IgE levels were higher with covered barley than with naked barley. In addition, molecular weights of 25 and 41 kDa were strongly, and 14 kDa was weakly noted from both covered and naked barley

whereas molecular weight of 20 kDa was only detected from covered barley by IgE immunoblot.
Conclusion: We are reporting three cases of clinical barley allergic Korean children with detailed clinical characteristics immunological studies. Also, we revealed that the sensitisation to covered and naked Hordeurn vulgare are both important and the components of 14, 25, 40, 41 kDa would be important in Korean children with barley allergy.

1137
Plant food allergy exploration in adults: micro arrayed-based component-resolved diagnosis

Mailhol, C[1]; Apoil, PA[2]; Montaigne, E[1]; Schwartz, C[1]; Blancher, A[2]; Didier, A[1]
[1]Pneumology Allergology Department, Toulouse University Hospital, Toulouse Cedex, France; [2]Immunology Department, Toulouse University Hospital, Toulouse Cedex, France

Background: Immunocap® ISAC biochip is a biologic test using component microarray technique for detection of specific IgE (sIgE) toward 112 molecular allergens from food allergens and pneumallergens. The aim of this study was to determine the ISAC® profile of each patient and to correlate it with the clinical symptoms.
Method: We performed the test in a group of adults who claimed for clinical symptoms potentially in relation with a plant food allergy.
Results: Fifty-six patients who reported clinical symptoms of allergy to one or more plant food item were tested. Thirty-five patients were exclusively sensitised to one of the panallergen families explored by the biochip: seven to storage protein, four to LTP, 12 to PR10, 12 to profilins. Combined sensitisations were present in eight patients for profilins-PR10, two for LTP-profilins, two for LTP-storage proteins, four for LTP-PR10, two for LTP-PR10-profilins, one for LTP-PR10-storage proteins. Two were sensitised to all the panallergens families of plant food explored by this test. Storage proteins and LTP sensitisations were most often associated with severe clinical symptoms. PR10 sensitisation was associated with clinical manifestations that did not threatened life. However, in this particular group, symptoms intensity varied and some systemic manifestations have been reported. No clinical feature allowed identifying patients with PR10 sensitisation from the profilin-sensitised ones.
Conclusion: ISAC® biochip can be useful for plant food allergy diagnosis in adults. In only one test, the sensitisation profile is determined and the culprit molecular allergen(s) identified. This work also confirms the potential clinical implication of profilins in food allergies.

1138
Use of egg white specific IgE in clinical practice

Bruno Soares, J[1]; Marques, J[2,3]; Loureiro, V[4]; Matos, V[4]; Martins, P[2,3]; Leiria Pinto, P[2,3]
[1]Immunoallergology, Centro Hospitalar Lisboa Norte, Hospital Santa Maria – CHLN, Lisbon, Portugal; [2]Immunoallergology, Hospital Dona Estefânia – Centro Hospitalar Lisboa Central, Lisbon, Portugal; [3]CEDOC, Faculdade de Ciências Médicas, FCM, Universidade Nova de Lisboa, Lisbon, Portugal; [4]Hospital de Dona Estefânia, Centro Hospitalar de Lisboa Central, Clinical Pathology, Lisbon, Portugal

Background: Egg allergy is the second most frequent food allergy in children, being the egg white responsible for more severe reactions than yolk. As for other foods, the diagnosis is based on clinical history, skin prick tests and determination of specific IgE (sIgE). Ultimately, an oral food challenge (OFC) is needed to confirm either allergy or tolerance.
Aim: To determine the best threshold value for heated egg white sIgE associated with clinical tolerance.
Methods: All children submitted to heated egg white OFC from January 2010 to December 2012, for which specific IgE to egg white was determined in the 12 months prior to the OFC were included. ROC curves were calculated to determine the sensitivity (SE) and specificity (SP) of egg white specific IgE levels vs the OFC outcomes. IgE determination was performed using ImmunoCAP.
Results: A total of 48 children (60% males) were enrolled. Thirty tree children had a previous clinical reaction to egg and for the remaining fifteen avoidance was recommended due to positive skin prick test and high sIgE for egg white. Most reactions were cutaneous (urticaria/angioedema – 62%; eczema exacerbation – 14%) and gastrointestinal symptoms (17%). Two patients had anaphylaxis. The median age of the first symptoms was 1.0 year (P_{25}–P_{75}: 0.7–1.1 years) and OFC were performed at a median age of 3 years (P_{25}–P_{75}: 2–5 years). The determination of egg white specific IgE levels was moderatly useful in discrimination cases at risk for heated egg white allergy (AUC: 0.70). The best decision threshold for egg white specific IgE was 5.63 KU/l (sensitivity: 55.6%; specificity: 89.7%).
Conclusion: Our results differ from others previously reported to egg. Despite its limitations, egg white sIgE was a useful tool. The specific IgE threshold achieved could suggest heated egg white allergy. Although it doesn't replace OFC which still remains the gold-standard for food allergy diagnosis.

1139
Variability in judgement of symptoms during food challenge

van Erp, FC[1]; Meijer, Y[1]; Knulst, AC[2]; Gabriele, C[1]; van der Ent, CK[1]
[1]Pediatric Pulmonology and Allergology, University Medical Center Utrecht, Utrecht, The Netherlands; [2]Dermatology, University Medical Center Utrecht, Utrecht, The Netherlands

Background: Although current guidelines suggest how to perform and interpret food challenges, variability in judgement of symptoms exists. Until now, no data are available about the degree of variability in interpretation of food challenge outcome. This study aims to assess inter- and intra-observer variability of double blind peanut challenge test outcomes in children.
Method: All complete food challenge score sheets of double blind peanut challenges performed in 2008–2010 in our tertiary centre were included. Score sheets were reassessed by three clinical experts including double reassessment in a subset of score sheets. Inter- and intra-observer variability were evaluated using kappa statistics. Univariate associations between symptoms and the presence of agreement between observers were assessed.
Results: The three observers reassessed 191 food challenge score sheets including 48 duplicated forms. Symptoms were reported on 111 (58%) score sheets, in 55 (50%) of these cases observers fully agreed on food challenge outcome. Inter-observer variability varied from $k = 0.31$–0.46, indicating fair agreement. Intra-observer variability was relatively good and varied from $k = 0.50$–0.72. Oral allergy syndrome, abdominal complaints, food aversion and rash were significantly associated with disagreement; whereas nasal symptoms and urticaria were significantly associated with agreement between observers.
Conclusion: Food challenge outcomes are subjected to variability in interpretation between and within observers, especially when subjective symptoms occur. Focusing on more objective symptoms like nasal discharge and urticaria may be helpful to improve the reliability of food challenge test outcomes.

Poster Session 43

Novel aspects in allergy diagnosis

1140
Changes of allergen sensitisation during last 30 years in Korean respiratory allergic patients

Park, H-J[1]; Lim, HS[2]; Park, KH[1]; Lee, J-H[1]; Park, J-W[1]; Hong, C-S[1]
[1]Division of Allergy and Clinical Immunology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Seodaemun-gu, Korea; [2]Biomedical Research Center, Yonsei University College of Medicine, Seoul, Korea

Background: Finding the culprit allergen is important for diagnosis and management of the allergic diseases. Allergic skin prick test (SPT) has been widely used to find culprit allergen. The skin reactivity to allergens has been changed because of changes of lifestyle and global warming. So, we wondered what changes of allergen sensitisation happened in Korea.
Method: We enrolled 1135 patients of respiratory allergic diseases who were diagnosed at Severance Hospital from January 2010 to December 2011. The SPTs with inhalant allergens were practiced. And we reviewed our previous papers containing the results of the SPTs in the 1990s and 1980s.
Results: In cases of the 2010s, the SPT positive rate of rhino-conjunctivitis with or without asthma was much higher (75–95%) than any other allergic diseases. And the SPT positive rate was decreased by increment of the age group (P-value < 0.01). The skin reactivity to tree pollens has significantly increased to 36.4% in the 2010s from 19.0% in the 1990s and 8.8% in the 1980s. Among the tree pollens, the skin reactivity to oak (4.7 to >14.4%), birch (7.1 to >13.6%), alder (6.3 to >13.4%) and pine (2.9 to >14.3%) have significantly increased in the 2010s as compared with those of 1990s. The skin reactivity to grass pollens (20.3%) and weed pollens (40.9%) in 1990s were significantly increased to be compared with those of 1980s (10.4%, 25.6%), but significantly decreased in the 2010s (13.9%, 27.0%) to be compared with those of 1990s. The skin reactivity to house dust mites had no difference between 1990s (55.2%) and 2010s (55.6%). The skin reactivity to dog (27.3 to >20.7%) and cockroach (25.3 to >12.3%) have significantly decreased in the 2010s on comparison with those of 1990s.
Conclusion: With above results, we could find the changes of skin reactivity to inhalant allergens in Korean allergic patients in 2010s. Even though global warming is one of the causes of the change of skin reactivity, we need further study about environmental changes of indoor and outdoor and also ecological botany.

1141
Digestibility and IgE-binding of Maillard-treated cod fish parvalbumin

de Jongh, HH[1]; Lopez Robles, C[1]; Timmermans, E[1]; Nordlee, JA[2]; Lee, PW[2]; Baumert, JL[2]; Hamilton, RG[3]; Taylor, SL[2]; Koppelman, SJ[2]
[1]TI Food and Nutrition, Wageningen, The Netherlands; [2]University of Nebraska, Lincoln, NE, United States; [3]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States

Background: Food processing conditions may alter the allergenicity of food proteins by different means. We investigated the effect of the browning or Maillard reaction on the digestibility of codfish parvalbumin.
Method: Parvalbumin was digested and resultant peptides were analysed for apparent molecular weight and IgE-binding.
Results: It is shown that the kinetics of digestion, performed at various pHs ranging from 1.2 to neutral as relevant for conditions in the gastro-intestinal tract, was not substantially affected by the Maillard reaction. The peptides resulting from this digestion were similar in size for native- and Maillard-treated parvalbumin. However, the Maillard-treated parvalbumin had a strong propensity to form dimers and tetramers that express a pronounced IgE-binding. Although the peptides resulting from digestion were relatively large (up to 3–5 kDa), the IgE-binding was strongly diminished. We conclude that Maillard treatment of codfish parvalbumin does not affect the digestibility of parvalbumin, and that the peptides resulting from this digestion show low IgE-binding, regardless of Maillard treatment. Maillard treatment of parvalbumin induces the formation of higher order structures that are potentially potent IgE-binders.
Conclusion: Maillard treatment of codfish parvalbumin does not affect the digestibility of parvalbumin, and the peptides resulting from this digestion show low IgE-binding, regardless of Maillard treatment. Maillard treatment of parvalbumin induces the formation of higher order structures that are potentially potent IgE-binders and therefore, the allergenicity of Maillard-treated fish products should be monitored with great care in food processing and production.

1142
Performance characteristics of a third-generation chemiluminescent immunassay analyzer used for specific IgE measurements

Pinter, E[1]; Konderak, J[1]; Witos, Y[2]; Balo Banga, JM[3]
[1]Cental Laboratory Synlab Hungary, Clinical Biochemistry, Budapest, Hungary; [2]Hochschule Offenburg, Offenburg, Germany; [3]Dermatology, Military Hospital, Budapest, Hungary

Background: *In vitro* testing patients suffering from IgE-mediated allergy is an important tool. In addition to *in vivo* test procedures, the guideline of Clinical and Laboratory Standard Institute (CLSI) I/LA20-A2 demands the determination of performance characteristics of *in vitro* assay. Their measurement should focus on the laboratory's patient population.

The aim of this study was to evaluate the new IMMULITE 2000 allergen-specific IgE analyzer- the only one in Hungary- on our samples.
Method: Sera of 1463 patients suffering from immediate allergy were tested by 80 different inhalant and nutritive allergens, for specific and for total IgE (Cobas reagent, measured by Modular E analyzer). Precision, accuracy, Receiver Operating Characteristics curves; reportable, and reference ranges were determined according to well-established protocols.
Results: Within run imprecision coefficient of variation (CV) was measured using two different allergens ragweed (W1) and fruit mix (FP15) at low and high concentrations by IMMULITE 2000 analyzer. CV% were 6.2%, 4.8%; 3.9%, 3.72%, respectively. Between run imprecision was carried out in 30 replicates of two different allergen controls. CV was 3.73% and 5.25%, respectively. The IMMULITE 2000 has a broad working range of 0.1–100 KU/l. for specific IgE. Good linearity was found [$Y = 0.99x–0.4289$ $r^2 = 9988$.] For

comparative study, we used the determination of total and specific IgE in 1463 patients with primary allergy diagnoses. The Area Under the Curve was 0.83; sensitivity for total IgE at 92 KU/l cutoff value was 77%; specificity 74%; Negative Predictive Value: 90%. One hundred and seventy-two samples measured by IMMULITE were compared with HYCOR system: Concordance correlation coefficient were: 0.82, Pearson p precision: 0.84, accuracy: 0.98.

Conclusion: Analytical performance of the third-generation chemiluminescent immunoassay analyzer IMMULITE 2000 meets all the performance targets set for CLSI I/LA20-A2 evaluation criteria. It improved the workflow in the laboratory, resulting in faster turnaround time of results. This analyzer is a reliable tool to backup clinical practice. It is one of three methods accepted by Food and Drug Administration.

1143
An inhibition ELISA method for the determination of potency of tree pollen preparations

van Deursen, D; Peekel, I; van den Hout, R; Kerkvliet, E
Development, HAL Allergy BV, Leiden, The Netherlands

Background: Widely used potency assays with specific reagents such as biotinylated allergen extracts, or assays using the allergen cap system, are relatively expensive and contain reagents that cannot be characterised. We developed an IgE inhibition ELISA, using only well characterised components, which is able to determine the IgE potency of pollen preparations from separate as well as from mixed tree species.

Method: Ninety-six-well plates are coated with extracts from *Betula verrucosa*, *Alnus glutinosa* or *Corylus avellana* pollen, or with a mixture of these extracts. After blocking, different dilutions of pollen samples are added to the coated plates followed by adding a sera pool obtained from patients with established clinical allergy for spring tree allergens. The tree pollen specific IgE will bind to the coated allergens or to the tree pollen allergens in the solution (competition). After washing away all unbound antibodies and proteins, bound IgE is quantitatively stained with HRP-conjugated anti-IgE. An in-house reference preparation is included in the assay and parallel-line analysis is performed to calculate potency. The pollen extracts tested are drug substances from birch, hazel or alder, and drug products containing spring tree mixtures. The results were compared to potency data from commercially available assays using a biotinylated ligand or an allergen caps system.

Results: All samples tested showed linear inhibition curves with high correlations ($r > 0.99$) and reproducible potency data (CV $\leq$ 10%). Furthermore, the potency data were comparable with the potency data obtained with the commercially available assays (difference <20%).

Conclusion: The developed IgE inhibition ELISA is well suited for the use of potency determination of drug substances and products containing single species tree pollen preparations as well mixtures. Compared with the commercially available assays, the newly developed assay produces similar results and uses better characterised components.

1144
Comparison between serological methods for serum samples with high levels of specific IgE antibodies

Chiba, K[1]; Shimojo, N[1]; Morita, Y[1]; Nakano, T[1]; Inoue, Y[1]; Arima, T[1]; Kohno, Y[1]; Amano, N[2]
[1]Pediatrics, Graduate School of Medicine, Chiba University, Chiba, Japan; [2]Siemens Healthcare Diagnostics K.K., Tokyo, Japan

Background: Under the present situation in our medical facility, sera of a large number of patients with high specific IgE antibody (sIgE) levels need to be diluted for measurement by ImmunoCAP (CAP). However, the CAP values from diluted serum samples were sometimes not accurate. Recently, IMMULITE® 3 gAllergy™ (3 g) has launched an assay in Japan with a reportable range between 0.1 and 500 IU_A/ml, which can potentially reduce the number of sample dilutions. The purpose of the present study is to evaluate the correlation of values above 100 IUA/ml between 3 g and CAP.

Method: The subjects were infants and children who visited our hospital between December 2006 and October 2012. The serum sIgE levels over 100 IU_A/ml by CAP were assessed by 3 g without dilution. The 3 g and the dilution adjusted (neat) values by CAP were compared.

Results: The sIgE levels of 3 g to Japanese Cedar (T17), House Dust (H1), *Dermatophagoides farinae* (Df), and Ovomucoid (F233), were correlated with the neat values of CAP; T17 (Spearman's $r = 0.68$), H1 (Spearman's $r = 0.79$), Df (Spearman's $r = 0.85$), F233 (Spearman's $r = 0.91$). Overall, the mean neat values from CAP were higher than 3 g for airborne allergens, while reverse was the case for a food allergen, F233.

Conclusion: When comparing with the high titer CAP values with those by 3 g, the differences can be expected due to the assay principle. We assume that 3 g is more suitable for high sIgE in clinical settings.

1145
Distribution and regularity to common allergens in patients with allergic diseases

Peiyan, Z[1,2]; Sun, B[1]; Zeng, G[1]; Huang, H[1]
[1]Department of Allergy and Clinical Immunology, Guangzhou Institute of Respiratory Disease, Guangzhou, China; [2]The First Affiliated Hospital of Guangzhou Medical College, Guangzhou, China

Background: To investigate the types and distribution of allergens, and the responsiveness to these allergens as related to development of allergic disorders in a cohort of Guangzhou patients with allergies.

Method: Serum samples were obtained from a cohort of patients with allergic disorders ($n = 7047$) who visited the First Affiliated Hospital of Guangzhou Medical College between 2008 and 2011. The sera were subjected to analysis of 16 common allergens by using immune-capture approach. Chi-square test and linear regression were employed for data analysis.

Results: In the study population, the prevalence of aeroallergen-specific IgE was highest for house dusts (H2), followed by Dermatophagoide pteronyssinus (D1), Dermatophagoide farinae (D2), Blomia tropicalis (D5), Blattella germanica (I6), dog (E5), cat (E1) and mosquito (I71); the prevalence of food allergen-specific IgE was highest for milk (F2), egg (F252), wheat (F4), peanut (F13), crab (F23), shrimp (F24), soybean (F14) and codfish (F3). The subjects showed mild responses to all common aeroallergens except dust mites or dust mite-containing mixed allergens (house dusts) (D1, D2, D5 and H2). Similarly, the responsiveness was mild to eight types of tested food allergens. By age-group analysis, there were a peak of sensitisation to five types of aeroallergens (D1, D2, D5, E1 and H2) between 9 and 12 years of age, and to I6 and I71 between 15 and 18 years of age. For tested food allergens, the peak of sensitisation appeared before 3 years of age for milk, between 3 and 6 years of age for eggs (the detection rates for both decreased along with age), between 9 and 12 years of age for F13 and F14, and between 12 and 15 years of age for F23 and F24.

Conclusion: Knowledge concerning allergen characteristics at various age groups may be helpful for early diagnosis and intervention for allergies. H2, D1, D2, F2 and F252are major sensitisers responsible for common allergic disorders in Guangzhou. While F2 and F252 are major

sensitisers during early years of life, a subset of children may gain tolerance to both as their immunity becomes fully developed along with age. Moreover, mites may precede milk and eggs as prevailing allergens in some of these children as they grow up, potentially resulting from cross-reaction between certain allergens.

1146
The validity of an allergy screening method in elderly patients

Leto Barone, MS[1]; La Piana, S[2]; Gaia, LP[3]; Di Lorenzo, G[4]
[1]Dipartimento Biomedico di Medicina Interna e Specialistica (Di.Bl.M.I.S.), University of Palermo, Università degli Studi di Palermo, Palermo, Italy; [2]Dipartimento di Biopatologia e Biotecnologie Mediche e Forensi, Università degli Studi di Palermo, Palermo, Italy; [3]Dipartimento Biomedico di Medicina Interna e Specialistica (Di.Bi.M.I.S.), Università degli Studi di Palermo, Palermo, Italy; [4]Dipartimento Biomedico di Medicina Interna e Specialistica (Di.Bl.M.I.S.), University of Palermo, Palermo, Italy

Background: The screening test is a commercially available specific IgE qualitative serological assay to screen for allergic sensitisation.
Method: The study was permformed in order to investigate the accuracy of the screening test *in vitro*, in a random sample of elderly subjects. Two hundred and eighty-eight elderly took part in the study. Skin prick test to a panel of relevant aeroallergens in the studied area were employed as the reference diagnosic procedure (gold standard).
Results: Subjects with at least one positive skin prick test (≥3 mm, $n = 76$) were considered to have developed an allergic sensitisazion. The screening test correctly classified subjects as sensitised to an allergen or not-sensitised in 257 out of 288 cases (accuracy 88.9%, 95%CI 85.0–0.92.0%). The screening test sensitivity was 70.0 (95%CI 58.1–79.7) and specificity was 95.7 (95% CI 92.1–98.0), PPV was 85.4 (95% CI 85.1–93.4), NPV was 89.8 (95% CI 85.1–93.4), LR^+ was 16.5 (95% CI8.7–31.6), LR^- was 0.31 (95%CI 0.21–0.43) and the DOR was 52.2 (95% CI 21.5–133.6).

In the elderly subjects with respiratory symptoms, the qualitative assay correctly classified subjects as allergen-sensitised or non-sensitised in 81 out of 89 cases [accuracy 91.0 (95% CI 85.0–96.9)]. In this subgroup, the screening test sensitivity was 94.6 (95% CI 85.1–98.8), specificity was 84.8 (95% CI 68.1–94.9), PPV was 91.3 (95% CI 81.0–97.1) and NPV was 90.3 (95% CI 74.2–97.9). LR^+ was 6.2 (95% CI 3.0–14.2), LR^- was 0.06 (95% CI 0.02–0.17) and the DOR was 98.9 (95% CI 18.0–621.4).

Conclusion: The screening test is a valuable tool for the diagnosis of allergic sensitisation in a population of elderly subjects.

1147
Prevalence of sensitisation to inhalant and food allergens: a population-based study

Christoff, GC[1,2]; Karova, EG[3]; Stoeva, IL[4]
[1]Department of Health Economics, Faculty of Public Health, Medical University – Sofia, Sofia, Bulgaria; [2]Tokuda Medical Centre, Allergy Outpatient Clinic, Sofia, Bulgaria; [3]Department of Conservative Dentistry, Faculty of Dental Medicine, Medical University – Sofia, Sofia, Bulgaria; [4]Department of Allergology, Physiotherapy and Clinical Radiology, Faculty of Dental Medicine, Medical University – Plovdiv, Plovdiv, Bulgaria

Background: Atopy, the clinical definition of an IgE highresponder, can be documented either by the detection of IgE antibodies in serum or by a positive skin prick test (SPT). SPT is the standard for diagnosing IgE-mediated allergies. Many studies worldwide have reported prevalence of atopic sensitisation and its international variations. Such data however for Bulgaria are scarce. The objective of the present study is to assess prevalence and patterns of sensitisation to environmental and food allergens in a community sample.
Method: Patients, 225 men and women, age 4–81 years, consulting the outpatient clinic of a major private hospital in Sofia, because of a suspected IgE-mediated allergic disease were included. All participants completed a written questionnaire to define asthma and rhinitis prevalence, quality of life and use of health resources. Patients underwent SPT with 18 commercial inhalant and food allergen extracts (Alyostal Prick, 100IR-IC/ml Stallergenes, France; Cow milk prick 1000 BU/ml Bul Bio-NCIPD, Bulgaria). For each allergen a new lancet (Stallerpoint, Stallergenes, France) was used. All test procedures were in line with published practice guidelines, the EAACI Position Paper and the ISAAC phase II protocol. A test was regarded possitive if the wheal size was at least ≥3 mm and controls showed adequate reactions.
Results: Among 225 patients 142 (60.2%) were sensitised to at least one allergen, using cut-off level ≥3 mm. Proportions of positive SPT in the study group ranged depending on cut-off levels. For Der p from 16% when the allergen induced wheal size is at least 50% of that elicited by histamine to 17.8% at cut-off ≥3 mm; Der fr – 15.6% and 18.2% respectively; Cockroach – 13.8% and 16.4%; Cat – 14.2% and 15.6%; Dog – 11.1% and 12%; Aspergillius mix –4.9% and 6.2%; Penicillium mix – 9.3% and 10.7%; four cereals: 23.1% and 24%; 12 grasses – 25.8% and 26.2%; Fagaceae – 11.1%13.8%; Betulaceae – 9.3% and 11.1%; Milk – 8% and 9.3%; Egg whole – 8.4% and 10.7%. With regard to number of sensitisations per patient 8.5% of the participants have two sensitisations, 7.5–1%; 7.1–3%; 6.1–7%; 4.7–6% and 4.2% have 10 sensitisations.
Conclusions: In our study we find a high rate of overall sensitisation which is in line with the data for Europe (GA^2LEN SPT study). The pattern of sensitisation prevalence for our study centre is: 12 grasses at the top, followed by four cereals, Der p., Der fr., cockroach, cat, dog in descending order.

1149
Sensitisation to house dust mites and storage mites in an area with high exposure to both types of mites

Boquete, M[1]; Carballada, F[1]; Iraola, V[2]; Morales, M[2]; Leonor, JR[2]; Carnés, J[2]
[1]Hospital Xeral de Calde, Allergy Unit, Lugo, Spain; [2]Laboratorios LETI, R & D, Tres Cantos, Spain

Background: Previous studies demonstrated that in Spanish areas with high exposure to house dust (HDM) and storage mites (SM), is frequent to find mite allergic patients with positive skin prick test (SPT) to HDM and SM. The aims of this study were to determine the real sensitisation to both mite -groups, and the allergens involved.
Method: Serum samples from 43 consecutive patients with positive SPT to any HDM (*Dermatophagoides pteronyssinus*, *D. farinae*, *E. maynei*) and at least to one SM (*Lepidoglyphus destructor*, *Glycyphagus domesticus*, *Tyrophagus putrescentiae* and *Acarus siro*) were taken from an area with high exposure to mites (Galicia). Specific IgE (sIgE) levels to HDM and SM, as well as Der p 1, Der p 2 and Der p 10 were determined by CAP system. Immunoblot and immunoblot-inhibition analysis using *D. pteronyssinus*, *L. destructor* and *T. putrescentiae* in solid phase and as inhibitors were done.
Results: Most patients (93%) had specific IgE to HDM and SM. sIgE to *D. pteronyssinus* was significantly higher than those observed in other species. The second highest value of sIgE, *D. farinae*, was significantly greater than to the SM, with exception for *L. destructor*. The most frequent recognised *D. pteronyssinus* allergens were 14.5 and 27 kDa bands, whereas for *L. destructor* and *T. putrescentiae* were bands at 14 and 15 kDa. Immunoblot inhibition showed that no extracts were able to inhibit completely the others. The highest inhibition was obtained with *D. pteronyssinus* to *T. putrescentiae*. On the other hand,

D. pteronyssinus and *L. destructor* showed a low capacity of inhibition with each other, specially in the 14–15 kDa bands, which may correspond to group 2 allergens.

Conclusion: In our area, most of patients with positive SPT to HDM had sIgE to both mite groups. *D. pteronyssinus* and *L. destructor* were the main sensitising species, being the sensitisation specific for allergens of these species (co-sensitisation), while sensitisation to *T. putrescentiae* may be affected by cross-reactivity. Group 2 allergens seem to be the main allergens involved in the specific sensitisation to SM.

1150
Predictive value (tolerance induction) of IgG and its subclasses in patients allergic to common mite

Aldrey, O[1]; Fabiano, F[2]; López, M[2]; Lista, D[2]; Rodríguez, I[2]

[1]Inmunología, Instituto Médico La Floresta, Caracas, Venezuela; [2]Inmunología, Instituto Docente de Inmunodiagnóstico, Caracas, Venezuela

Background: Allergic reactivity to common mite represents a public health's problem in Europe and Latino America. It is important to evaluate the possible role of specific IgG and its subclasses in the diagnosis and course of illness.

Method: A transversal study was done with 129 allergic patients and 29 healthy individuals, among 0–45 years old. Patients with allergic rhinitis (AR), bronchial asthma (BA), atopic dermatitis (AD) and/or immunodeficiency, who attended the consultation of allergy, among January to May 2012 were included. International criteria were applied during clinical trials. An automated ELISA instrument was used to determine specifics IgE, IgG and IgG4. A comparative study was done using McNemar method.

Results: Among all allergic patients (76 women, 53 men), prevalence of BA was 24%, AR 36%, BA + AR 57%, BA + AD 23%, BA + Immunodeficiency + AR 20.9%. BA+ AD+ atopic march 11.3%; while the prevalence of BA+AR+AD+ atopic march was 18%. To determine specific IgG and IgG4 against mite, 51.9% of allergic patients showed high IgG levels, while 5.4% of them showed high levels of IgG4. There was significant differences respect to healthy individuals ($P = 0.05$), 20.6% of them showed high levels IgG and none of them showed high levels of IgG4. Thirty individuals showed high levels of specific IgE and IgG, while a slightly higher percentage had high IgG levels with normal IgE levels ($P = 0.016$). Only two allergic patients showed high IgE and IgG4 levels, while 41 of them showed high IgE levels and normal IgG4 levels ($P = 0.001$).

Conclusion: The sensitivity and specificity of IgG analysis is 49.59% and 42.30%, respectively. While the sensitivity and specificity of IgG4 analysis is 3.17% and 100%, respectively. This results showed that biomarkers are more specifics and less sensible for diagnosis and follow of the allergic pathology and have a predictive value (tolerance induction) in the course of illness.

1151
Diagnostic performance of the atopy patch test with inhalant allergens

Fuiano, N[1]; Incorvaia, C[2]; Diddi, G[1]

[1]ASL Fg Pediatric Allergy Service, Torremaggiore, Italy; [2]Allergy/Pulmonary Rehabilitation, ICP Hospital, Milan, Italy

Background: Recent studies suggested the utility to perform the atopy patch test (APT) not only in patients with atopic dermatitis (AD) but also in patients with respiratory allergy, especially when caused by house dust mites. We tested a large group of patients suffering from AD with or without respiratory symptoms (rhinitis, asthma) by the APT and compared its results to those of skin prick test (SPT) and *in vitro* IgE measurement.

Methods: The study was conducted on 521 children and adolescents (292 males, 229 female, median age 6 years, age range from 0.5 to a 18 years), who had AD and respiratory allergy with different clinical presentations: current AD, 47 pts (group A), current AD and respiratory symptoms, 72 pts (group B), past AD and respiratory symptoms, 69 pts (group C) and only respiratory symptoms, 280 pts (group D); 53 healthy subjects served as control group (E). All subjects underwent SPT by allergen extracts from Stallergenes (Milan, Italy), APT by allergen extract from Chemotecnique (Malmö, Sweden) and CAP/RAST by material from Phadia (Milan, Italy) using the most important inhalant allergens occurring in our geographical area (grass pollen, cypress pollen, Parietaria pollen, Compositae pollen, house dust mites, Alternaria tenuis, and cat epithelium).

Results:

In group A, the APT was positive in 15%, SPT in 2%, and CAP/RAST in 6% of patients;

in group B, the APT was positive in 67%, SPT in 22%, and CAP/RAST in 22% of patients; in group C, the APT was positive in 59%, SPT in 25%, and CAP/RAST in 38% of patients;

in group D, the APT was positive in 48%, SPT in 29%, and CAP/RAST in 30% of patients;

in group E, APT was positive in 2%, SPT in 6%, and CAP/RAST in 4% of subjects, this corresponding to a specificity of 96.2% for APT, 88.4% for SPT, and 92.5% for CAP/RAST.

Conclusions: These findings indicate that in subjects sensitised to inhalant allergens, not only with AD but also with respiratory symptoms, the APT has a diagnostic performance higher than SPT and *in vitro* IgE measurement. In particular, APT showed a superior specificity.

1152
Nasal vs bronchial provocation in diagnosis of rhinopathia/asthma bronchiale

Wenzel, MD[1]; Riffelmann, FW[1]; Dellweg, D[2]

[1]Allergology, Krankenhaus Kloster Grafschaft, University of Marburg, Schmallenberg, Germany; [2]Pneumology, Krankenhaus Kloster Grafschaft, University of Marburg, Schmallenberg, Germany

Background: Nasal and bronchial Provocation (NP/BP) are helpful to judge about the clinical relevance of cutaneous sensibilisation with an inhalative allergen. BP is usually performed as a stationary procedure and thus is more expensive than an ambulant nasal provocation. We have compared both examinations in a head to head prospective comparison with 31 parallel examinations.

Method: Seven NP-BP comparisons were conducted in patients with asthma alone (group I) and 24 comparisons in patients with both, asthma and pollinosis/chron. rhinopathia (group II). Tested allergens: 14 × pollen, 11 × mites, 3 × fungi, 2 × animals. If the results of NP and BP was discordant, the result of BP was suspected as correct.

Results: In the seven patients without rhinopathia (group I) there were already three pos. nasal reactions (43%), which were confirmed in the later bronchial provocation. Three other nasal provocations of these seven patients showed a negative result, but a positive BP, one patient had a negative NP and BP. So the lack of rhinopathia was not an argument against a positive nasal provocation.

NP and BP neg in $n = 4$, IgE-level of these patients were negative.

NP neg and BP pos in $n = 16$, IgE-level: 10.2 IU/ml (mean), EAST-class 2,5. Twenty-five percent (four of 16) had normal IgE-level.

NP pos and BP neg in $n = 1$

NP and BP pos in $n = 10$, IgE-level in the mean 12.2 IU/ml, EAST-class 3,4, in two of 10 (20%) normally.

Discussion: Relying on NP only would have caused a wrong negative result in 16 of 31 tests (52%). One NP (3.1%) had no correlate on BP and has to be regarded as false positive. It seems to be negligible for nasal provocations, if the asthmapatient suffers from a rhinopathia as well. IgE-levels alone are not reliable since 25% of patients with a positive BP were IgE negative. Studies with larger patient numbers are needed.

Conclusion: The indication to proceed bronchial provocations should be used liberally, if an IgE-mediated sensibilisation with clinical symptoms is present in the absence of a positive nasal provocation.

1153
Allergen cross reactivity among pollen allergens from India and Europe

Singh, AB
Allergy and Immunology, Institute of Genomics and Integrative Biology, Delhi, India

Background: The term 'Cross-Reactivity' when used to describe allergens means that the allergen specific IgE antibodies recognise and binds to similar amino-acid sequence in different substances to which patients are exposed, wheather these substances are allergens or not, even if the proteins are present in two unrelated sources. However, if these proteins are sensitising proteins they are referred to as cross-allergenic proteins. The cross-reactivity among sensitising allergens (of IgE antibodies) is of interest due to different reasons. A vast variety of different molecular structures referred to as allergenic epitopes have been shown to be able to induce IgE mediated hypersensitivity reactions. However, a high structural homology between phylogenetically conserved allergenic proteins present in different and and even in unrelated sources of exposure, seems to play an important role in IgE mediated polysensitisation.

Material and methods: To study cross allergenicity pollen and other plant materials established as allergens in patients from India and from Europe were selected. Blood samples from patients with positive SPT to respective allergens from Euphorbiaceae and Urticaceae members was collected and sera separated. ELISA and blott inhibition studies were carried out to quantify and identify cross reactive allergens.

Results: Cross-reacting allergens from different pollen allergens from Euphorbiaceae and Urticaceae members from India and Europe have been studied. Based on Clinical and immunological studies we observed cross reacting allergens from Holoptelia from India and Parietaria from Europe belonging to family Urticaceae and Ricinus communis from India and, Mercularis annua and Heavia brassilensis from Europe. As all the organisms have originated from common biological ancestors, there is possibility that these allergens may have common proteins with sequence similarity and similar properties.

Conclusions: This possibility of having similar sequences throughout the plant kingdom or at least in the taxonomically same families is quiet high. But complete cross-reactivity (100%) never occurs. Advantages of Cross-reactivity in clinical practice helps in replacement of traditional diagnostic procedures by new techniques approach. Thus, cluster of cross-reactive allergens may simplify diagnostic procedures and therapeutic regimens in different Eco-geographical regions.

1154
Detection of clinical relevant monosensitisation to Can f 5 component in dog allergic patient using molecular allergy test

Rudenko, M[1]; Boyle, R[1]; Burova, K[1]; Seneviratne, S[1]; Egner, W[2]; Bhayat-Cammak, A[2]; Bradshaw, N[3]
[1]London Allergy and Immunology Centre, London, United Kingdom; [2]Protein Reference Unit Sheffield Teaching Hospitals, Sheffield, United Kingdom; [3]Thermo Fisher Scientific ImmunoDiagnostics, Milton Keynes, United Kingdom

Background: Recent developments in immunology have started to influence clinical allergy by introducing a promising approach of molecular allergy (allergology) – ImmunoCap ISAC.

Aim: We present a unique case of severe allergic rhino-sinusitis in a 39 y.o. female patient that was diagnosed using ISAC.

Method: ISAC is designed to detect specific IgE antibodies to a large number of allergenic epitopes – components of known allergens from a single blood test.

Results: The patient (smoker) complained on a variety of symptoms that were bothering her for more than 3 years: headaches, significant fatigue, nasal blockage, breathlessness on exercise and occasionally at rest. There were no ocular symptoms. She had several courses of antibiotics during this period with slight improvement. Careful history revealed progressive deterioration in her condition; there was no difference in her symptom pattern through the season although she noted that she had more sinus pains in winter. There was a dog, but no dampness in the house. There was no personal or family history of atopy. Anterior rhinoscopy had appearance of acute inflammation with no structural abnormalities. Spirometry showed FEV1 95%, FVC 92%, peak flow 85% predicted. Skin prick test with valid controls, was negative to a standard panel of aeroallergens including dog. Immunocap ISAC test was offered to broaden the spectrum of allergens tested. This showed – mono-sensitisation to Dog rCan f 5 Arginine esterase 1.5 ISU and negative to all other components.

Conclusion: The spectrum of usually tested dog allergens appears incomplete: Two lipocalins, Can f 1 and Can f 2, and serum albumin, Can f 3, have been characterised in detail but do not fully account for the IgE antibody-binding activity in all dog-allergic patients. Allergen activity has previously been detected in dog urine Can f 5, believed to be produced only in male dogs. ISAC was very beneficial in this case and opened a new insight on the diagnostic process.

1155
Immediate-type allergy caused by polyvalent sensitisation to fish food components – a case report

Rauschenberg, R; Abraham, S; Spornraft-Ragaller, P; Beissert, S; Bauer, A
Department of Dermatology, University Hospital Carl Gustav Carus, TU Dresden, Dresden, Germany

Background: A variety of arthropod species, such as Artemia (brine shrimp) und Daphnia, are commonly used as fish food in aquaculture. Their major allergens have not been characterised yet. Cross-reactivity with other kinds of crustaceans and chironomid larvae/midges is assumed. We report about a 37-year-old woman, who repeatedly suffered from conjunctivitis, dyspnea and rhinitis after feeding her aquarium fish with crustaceans like brine shrimp and Daphnia as well as chironomid larvae, sludge worms etc. Eating shellfish has never resulted in any allergic symptoms.

Method: Specific IgE antibodies against Daphnia were measured using UNICAP (Phadia, Uppsala, Sweden). Furthermore, a prick test including Daphnia, Artemia salina, krill, Cyclops, sludge worm (Tubifex) and several mosquito larvae was performed.

Results: Specific IgE antibodies against Daphnia were found (CAP 4). The prick test showed a well-marked urticarial reaction to Daphnia (10/30), Artemia salina (11/40), krill (5/30), Cyclops (8/30), sludge worm (Tubifex 3/25) and mosquito larvae (red 10/25; black 10/30, white 10/27). Due to underlying symptoms and test results we diagnosed a type I allergic response to several fish food components.

Conclusion: An immediate-type allergy caused by sensitisation to fish food components is a potential differential diagnosis for asthma and rhino-conjunctivitis in patients involved in aquaculture. Allergen avoidance is strongly recommended. Rescue medication must be available.

1156
Peach allergy: sensitisation profile based on component resolved diagnostics. Case reports

Korovkina, ES
Allergological Department, Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia

Background: Clinical and molecular patterns of Rosaceae fruit allergy are different: oral allergy syndrome (OAS) is mainly related to birch pollen Bet v 1 and profilins, and systemic symptoms, more frequently due to lipid-transfer proteins (LTP) in association with a peach allergy. Component resolved diagnostics (CRD) allows the simultaneous assessment of specific IgE (sIgE) antibodies to a vast range of molecular allergens and can give us a better comprehending of the above patterns. The aim of this study has been to evaluate the efficiency of this method in peach allergic patients and can be used for monitoring of some effective allergen-specific immunotherapy (ASIT).

Two case reports of peach allergy:

A 7-year-old boy with symptoms of allergic rhinoconjunctivitis in the April–May period, and a lip angioedema after ingestion of the peach, apple, cherry, hazelnut, which he has had since the 3-year-old age. *In vitro* tests for sIgE determinated by CRD had positive results to allergens related to the PR-10 proteins group: Aln g 1, Pru p1, Mal d1, Cor a1.0401. The Pru p1 is a pathogenetically important protein of Group 10, allergic reactions to this component are not dangerous in terms of an urgent condition development. Moreover, the birch pollen ASIT contributes to reduction of OAS on Rosaceae fruit.

A 39-year-old woman, with no symptoms of an allergic disease, reported the case of acute generalised urticaria, laryngeal oedema immediately after peach consuming. Soon after taking antihistamine, the symptoms were decreasing the next 2 h. A few months later urticaria appeared after eating a peach-pie. sIgE was measured by CRD; positive levels to peach allergens Pru p3 and other family proteins nsLTP – rCor a8 and nArt v3, were identified. Therefore, clinical manifestations of this patient's allergy are not associated with sensitisation to pollen allergens.

Conclusion: Peach allergy seems to be predominantly mediated by Pru p3 associated with clinical severity, although sensitisation to mugwort and hazelnut LTP's should be considered as a risk factor for the development of severe systemic reactions. Patients with birch-pollen allergy reported only mild local symptoms, probably due to the cross-reactivity of PR-10 proteins. CRD is helpful in comprehending of the molecular background behind sensitisations and is necessary to be provided before ASIT by pollen allergens in order to avoid unresponsiveness to this kind of therapy.

1157
Respiratory allergy related to accidental exposure to carnation (dianthus caryophyllus) in a healthy non atopic patient

Brinia, A[1]; Vovolis, V[1]; Tsiougkos, N[1]; Petrodimopoulou, M[2]; Kompoti, E[1]
[1]Allergy Department, Laiko General Hospital, Athens, Greece; [2]Laiko General Hospital, Athens, Greece

Background: Carnation (dianthus caryophyllus) is a decorative plant that belongs to the family of Caryophlilacea. It has seldom been mentioned to be involved in occupational allergy, in which manifestations were mainly respiratory via an IgE-dependent mechanism. Respiratory allergy to carnation has not been reported so far in individuals without occupational exposure.

Method: Our case regards a 32-year-old non smoker male, presenting rhinitis and asthma symptoms (sneezing, nose and eye itching, watery rhinorrea, dyspnoea, dry coughing, chest tightness and wheezing), while entertaining himself at greek nightclubs (bouzoukia), during the past 2 years. A detailed history revealed that symptoms were mainly induced while he participated in throwing carnations, an activity usually taking place in greek nigthclubs as a manifestation of joy. Contact with carnation was not necessary for symptoms to be elicited. Blood counts and biochemistry were normal, as were urine analysis and chest x ray.

To determine the contribution of carnation to the patient's symptoms skin prick tests (SPTs) with fresh three different parts of the plant (petals, stamens and stem) as well as with boiled petals were performed.

SPTs with major aeroallergens as well as with an unpurified extract from fresh *Gypsofila paniculata*, a plant belonging to Caryophillacea, were also conducted.

The same extracts were tested on five control subjects.

Result: Our patient had no atopy profile, as all SPTs to majors aeroallergens were negative. SPTs with all three parts of the plant were positive. SPT with boiled petal extract was positive too. Additionally, SPT with gypsophfila paniculata was positive. Control group provided negative SPTs to carnation and gypsophila paniculata.

Conclusion: Our patient was proved to be sensitised to a thermoresistant allergen existing in all parts of the plant (petal, stems, stamen). Culprit allergen was shown to be common in all members of the same plant family, carnation and gypsophila. Of major significance was the fact that even off distance exposure to carnation could induce respiratory symptoms, implying that the responsible agent was an inhalant plant material. Clinicians should always bear in mind that rhinitis or asthma may in fact be attributed to rare allergens not included in common diagnostic screening panels.

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

Poster Session 44

Bacterial and viral infections in allergy

1158
Prevalence of tuberculosis infection estimated by skin testing with recombinant protein CFP10-ESAT6 among hospital workers in Moscow

Slogotskaya, L[1]; Bogorodskaya, E[2]; Litvinov, V[1]; Kudlay, D[3]; Seltsovsky, P[1]; Nikolenko, N[3]; Ivanova, D[1]
[1]Scientific and Clinical Antituberculosis Center, Clinical Research, Moscow, Russia; [2]Scientific and Clinical Antituberculosis Center, Moscow, Russia; [3]OJSC "Pharmstandard", Moscow, Russia

Background: Transmission of tuberculosis (TB) to health care workers (HCWs) is global issue, though effective infection control measures are expected to reduce nosocomial TB. The discovery of Mycobacterium tuberculosis specific antigens, lacking in BCG and most environmental mycobacteria, led to the development of *in vitro* assays based on antigen-stimulated interferon-g (IFN-g) production. Many healthcare institutions are switching from Tuberculin Skin Test (TST) to Interferon-gamma release assays (IGRAs).

A preparation for skin testing called DIASKINTEST® (DST), which represents recombinant protein CFP10-ESAT6, produced by *Echerichia coli*, has been developed in Russia. Aim is to compare the prevalence of positive responses to the DST in healthcare workers (HCWs) of tuberculosis and non-tuberculosis institutions as markers of latent tuberculosis infection (LTBI).

Method: We recruited a cohort of HCWs and performed skin tests (DST) in 1037 workers tuberculosis and 53 psychiatric institutions (nurse, doctor, clinical staff, lab staff or non clinical support staff). Any size induration was considered to be positive DST reaction. All were divided into groups with duration of working at least 5 years and more than 5 years.

Results: The prevalence of DST positivity was: in all types workers tuberculosis institutions 14.5% (95%CI 12.3–16.6%) (150/1037); psychiatric hospital 0 (0/53), $P = 0.0029$;in all types workers tuberculosis institutions with duration of working <5 years 11.5% (45/391), >5 years −16.3 (105/646) ($P < 0.05$).

DST positivity depending on the type of work with duration of working in a tuberculosis institutions was: clinical doctors with duration <5 years 17.0% (7/41), >5 years −15.3% (27/176); nurses with duration <5 years 12.6% (31/246), >5 years −15.3% (27/176); all clinical staff with duration <5 years 13.2 (38/287), >5 years 17.8% (90/506); all lab staff with duration <5 years 0% (0/25); >5 years 12.5% (8/64); non clinical support staff with duration <5 years 13.9% (7/79); >5 years 14.4% (7/76) – the differences were not statistically significant.

Conclusion: Our study demonstrated the high prevalence of LTBI estimated by DST positivity among all types of HCWs in tuberculosis institutions and associated with duration of working while there was no case of positive reactions among workers of psychiatric hospital which corresponds to the degree of danger of infection (statistically significant). Infection control program should be reinforced.

1159
The biological peculiarities of *Staphylococcus* collected from children's lesion skin with atopic dermatitis

Kudryavtseva, A[1]; Fluer, F[2]; Maximushkin, A[2]; Morozova, O[3]
[1]I.M. Sechenov First Moscow State Medical University, Pediatrics Hospital, Moscow, Russia; [2]Gamaleya Research Institute of Epidemiology and Microbiology, Moscow, Russia; [3]Laboratory of Clinical Microbiology, I.M. Sechenov First Moscow State Medical University, Moscow, Russia

Background: Nowadays the number of patients with severe atopic dermatitis (AD) is steadily increasing. The study of possible causes for AD acute forms will help reduce AD progression.

Method: This study researched into the peculiarities of staphylococcal enterotoxins SEA, SEB, TSST-1 production by coagulazopositive (CPS) and coagulazonegative (CNS) staphylococcal strains taken from children's lesion skin with AD. It enrolled 34 children with AD. SCORAD lower than 30 was regarded as mild, from 30 to 60 as moderate and over 60 as severe AD. 35.7% of the enrolled children were identified as patients with mild AD, 21.4% with moderate and 42.8% with severe AD. The patients were averagely aged 4.9 ± 1.6 years (from 6 month to 13.2 years old). The skin smear seeding was performed on blood agar. The isolates obtained were identified according to conventional microbiological tests. The staphylococcal toxins were estimated in the supernatant by ELISA.

Results: *S. aureus* was detected in 18 patients, whereas half of them were with severe AD. Twenty-three coagulazonegative staphylococcus strains such as *S. epidermidis*, *S. haemolyticus* и *S. warneri* from the lesion skin were isolated in 21 cases. CNS and CPS associations were detected only in four patients with severe AD. Fifteen out of 19 *S. aureus* strains produced toxins, where 12 strains did it in association with SEA, SEB and TSST-1 (six strains taken from severe AD patients with the average SCORAD 67.8 ± 3.75 and four strains from moderate AD patients with the average SCORAD 36.5 ± 3.99). Out of six *S. warneri* strains, one strain had SEA, SEB and TSST-1, one strain had SEA and SEB and the other had SEA. Eight out of 12 S. epidermidis strains produced various toxin associations. Two out of five *S. haemolyticus* strains also revealed enterotoxin activity, where one of them simultaneously produced three toxins in a patient with moderate AD and SCORAD 36. When studying the interaction between toxin production and SCORAD values, it was distinctly established that a higher index value depended on more toxin number produced by various staphylococcus strains (one toxin – 18 ± 6; two toxins – 36.6 ± 10.9 and three toxins – 54.8 ± 5.35 ($P < 0.05$).

Conclusion: Our study revealed the correlation between AD aggravation and SEA, SEB and TSST-1 production by staphylococcus. CNS may produce different toxin associations and negatively affect disease progression. This problem calls for further research of the biological properties of this bacterium.

1160
Immune system and infectious diseases in hospitalised by multidrug-resistant Enterobacteriaceae: clinical impact and microbiological aspects

Nenna, S[1]; Carbone, L[1]; Leuci, D[1]; Del Gaudio, T[2]
[1]UOC Internal Medicine Unit, "L. Bonomo" Hospital, Andria (BT), Azienda Sanitaria Locale BT, Andria, BT, Italy; [2]UOC Clinical Pathology Unit, Microbiology Service, "L. Bonomo" Hospital, Andria, Italy

Background: Inadequate antibiotic therapies result in increased costs of hospitalisation and increased mortality. Reduced immune and increased resistance of enterobacteriaceae to carbapenems constitute a major welfare problem because often the only effective therapeutic option. We investigate the clinical relevance of reduced susceptibility to antibiotics in patients admitted with infectious diseases by multidrug-resistant enterobacteriaceae (EM).
Methods: Patients admitted to Internal Medicine Unit were evaluated. Samples of whole blood, urine and bronchial aspirate were analyzed. Antibiotic sensitivity testing was performed. C-reactive protein (CRP) and white blood cell (WBC) count were evaluated as indices of Systemic Inflammatory Response Syndrome (SIRS). The Procalcitonin (PCT) as an index of bacterial sepsis. The dosage of immunoglobulins IgA, IgG, IgM was performed to evaluate the immune system. Molecular methods and microbiological test were performed to determine factors related to the mechanisms of the antibiotic resistance. The production of enzymes beta-lactamase extended spectrum (ESBLs), AmpC beta-lactamases (AmpC) and carbapenemases (KPC) were characterised.
Results: One thousand three hundred seventy patients (about 73 years of average) were evaluated. Three hundred and eighty-six pts (28%) presented SIRS with increase of WBC (average 16 000 cells/mmc) and PCR (mean: 73 mg/l). Four percent presented sepsis with PCT increase (mean: 12 μg/ml). No immune deficiency was highlighted. Isolates strains of E. coli, Klebsiella spp. and Proteus spp. were collected. Three wild strains of *E. coli* were also recovered. Fifteen isolates of EM were recovered: eight *E. coli* (53%) four $EBSL^+$, one $penicillinase^+$; three Proteus mirabilis (20%) two $cAMP^+$, one $cAMP^+$ + $EBSL^+$; four *Klebsiella pneumoniae* (27%) all KPC^+. High resistance toward antibiotics tested was highlighted including carbapenems. The average length of stay of patients with sepsis was 13 days: 10 days for *E. coli*, 12 days for *P. Mirabilis*.; 18 days for *K. pneumoniae*.
Conclusions: Twenty-eight percent of admitted showed SIRS and 4% of these revealed sepsis by EM. CRP, WBC and PCT showed good correlation with the outcome. The dosage of PCT allowed the early diagnosis of sepsis in the course of SIRS and monitoring of antibiotic treatment. The infection by *Klebsiella* KPC^+ was associated with poor outcome; this outbreak increase in the stay and proved to be the leading cause of death in patients with sepsis. This study showed the emergence of infections by EM in patients hospitalised in Internal Medicine.

1161
Periodontal disease in patients with respiratory allergy

Yáñez-Pérez, V; González-Díaz, SN; Arias-Cruz, A; Galindo-Rodríguez, G; Gallego-Corella, C; Calva-Mariño, M; Mejía-Salas, KY; Domínguez-Sansores, L; Hernández-Sanchez, H; Rangel-Garza, L
Centro Regional de Alergia e Inmunología Clínica, Hospital Universitario 'Dr. José Eleuterio González', Universidad Autónoma de Nuevo León, Monterrey, Mexico

Background: 'Hygiene hypothesis' states that patients with early exposure to infectious agents are less prone to develop allergic diseases due to early development of the immune system. Periodontal disease results from exposure and accumulation of bacteria at the gingiva and teeth. Our hypothesis is that patients with less periodontitis are more susceptible to respiratory allergy due to the 'hygiene hypothesis'. The aim of this study is to determine if there is a relationship between periodontal disease and respiratory allergy.
Method: A cross-sectional comparative study was performed in patients seen at the Allergy Clinic at Monterrey, Mexico. Thirty randomised patients with any kind of respiratory allergy (Group A) were compared to 30 healthy subjects (Group B). Both groups underwent clinical assessment with validated tools for asthma, allergic rhinitis and allergic rhinoconjunctivitis (ISAAC questionnaire). Also, all of the patients were evaluated with skin test for 36 common aeroallergens. Finally, both group patients were assessed by qualified Periodontists to determine the degree of periodontal disease using a clinical classification (I–IV grades).
Results: Sixty patients were evaluated. Group A mean age was 29.3 years with a sex distribution of 63% male and 37% female; while Group B mean age was 30 years with a sex distribution of 60% male and 40% female. Allergic diseases of Group A were as follow: allergic rhinitis 100%, asthma 67%, atopic dermatitis 33% and allergic rhinoconjuntivitis 13%. All patients in Group A had at least one positive skin test for the 36 aeroallergens evaluated. After oral examination, we found that all of the patients in both groups had some degree of periodontal disease. Group A showed periodontal disease grade I in 13% ($n = 4$), grade II in 0%, grade III in 70% ($n = 21$), and grade IV in 17% ($n = 5$). On the other hand, Group B revealed periodontal disease grade I in 0%, grade II in 0%, grade III in 80% ($n = 24$), and grade IV in 20% ($n = 6$). Severe periodontal disease (grade III and IV) was seen in all the patients in Group B compared to Group A which showed 87% of severe periodontal disease.

Table 1 Periodontal disease grade among groups

	I *n* (%)	II *n* (%)	III *n* (%)	IV *n* (%)	Total *n* (%)
Group A	4 (13)	0 (0)	21 (70)	5 (17)	30 (100)
Group B	0 (0)	0 (0)	24 (80)	6 (20)	30 (100)

Conclusion: Due to the high prevalence of periodontal disease in both groups, we were not able to establish a correlation between prevalence of periodontal disease and respiratory allergic conditions.

1162
Staphylococcus aureus and hand eczema: to treat or not to treat

Grivcheva-Panovska, V
Dermatology Unit of Allergology and Clinical Immunology, Univerisity Sts Cyril and Methodius, Skopje, FYR Macedonia

Background: Despite several studies indicating the role of *S. aureus* in atopic dermatitis, not much was said on *S. aureus* in adult patients with hand Eczema (HE).
Method: Bacterial swabs at baseline and three different visits from nose and hand eczema target lesion in patients without clinical signs of secondary bacterial infection of HE; Spa typing of *S. aureus* subtypes; Hand Eczema severity index and physician assessment; Patients were divided to group 1: placebo ointment (petrolatum), for 5 + 14 days and group 2: placebo ointment for 5 days and clobetasol propionate for 14 days (double-blinded); ANOVA statistical analysis.
Results: *S. aureus* was found on the hands in 37 patients with HE and four controls ($P < 0.001$); presence of *S. aureus* was related to increased severity of the Eczema ($P < 0.001$). All patients were with identical *S. aureus* types on the hands and in the nose which further on decreased to 83%. Eight different CC were identified, no association of CC type with HE severity was

found and toxin-producing strains were not more frequent in HE patients.

Conclusion: *Staphylococcus aureus* was present on hands in 54.4% of all patients with HE, and was significantly related to the severity of the disease. *S. aureus* could be important cofactor for persistence of HE including virulence factors pertinent to bacterial survival in the host, such as cytolysins and hemolysins (α, β, γ and δ) and panton-valentine leukotoxin. Treatment of *S. aureus* in allergic diseases typically characterised by tissue eosinophilia might lead to more efficient control of hard to treat chronic hand Eczema.

1163
Unexpected cavitary pulmonary lesions in a patient with severe asthma treated with omalizumab

Guida, G[1,2]; D'Urso, A[3]; Bosco, F[1]; Negro, C[1]; Rus, C[1]; Pagliaro, M[1]; Borin, F[1]; Fora, R[4]; Bigo, P[1]
[1]ASLTO2 Ospedale Birago di Vische, Medicina Interna II, Torino, Italy; [2]ASLTO2, Ambulatorio di Allergologia, Torino, Italy; [3]ASLTO2 – Ospedale Amedeo di Savoia, S.C. Pneumologia, Torino, Italy; [4]ASLTO2 – Ospedale Amedeo di Savoia, S.C. Prevenzione Rischio Infettivo, Torino, Italy

Background: Long-term treatment with systemic steroids exerts immune-suppressive effects on the cellular immune system and increase the susceptibility to intracellular infections. Corticosteroid-dependent asthma may be a condition at risk of fungal pulmonary colonization or infection or rarely to mycobacteria. Moreover rare vasculitic pulmonary complication have been described in patients with severe asthma receiving omalizumab.

Method: We describe the clinical case of a 55 years old woman affected by corticosteroid dependent severe asthma and chronic rhinosinusitis with nasal polyps, who underwent omalizumab treatment for recurrent asthma exacerbations. After 1 year of treatment with Omalizumab she complained worsening of dyspnoea and wheezing and deterioration of bronchial obstruction at spirometry, without improvement after taking systemic corticosteroid. Blood samples showed neutrophilic leukocytosis, no eosinophilia and high reactive C protein. The screening for autoimmune autoantobodies, including ANCA, resulted negative. On X ray and HRCT of Chest, multiple cavitary pulmonary lesions were demonstrated.. Bronchoalveolar lavage (BAL) showed a high neutrophils count, no eosinophils and resulted negative for cancer cells. Culture from BAL were negative for mycobacteria or aspergillum while resulted positive for *Stafilococcus aureus* and *Candida Albicans* colonization.

Results: A diagnosis of multiple pneumatocele due to *Stafiloccoccus aureus* infection and Candida colonization was postulated. The patient was treated with piperacilline/tazobactam, levofloxacine and fluconazole resulting in rapid clinical and Spirometric improvement and progressive resolution of cavitary lesions. After 1 month Omalizumab treatment was restarted without complication.

Conclusion: Patients with severe Corticosteroid-dependent asthma must be carefully monitored when clinical exacerbation occurs. Even if Omalizumab therapy have been seldom associated to vasculitic lesions, other explanations of pulmonary deterioration must be ruled out.

1164
Pharmacological characteristics of *Gynocardia odorata* R.Br. and its application in antimicrobial potencies: randomised controlled trial

Mollik, MAH[1,2]
[1]Peoples Integrated Alliance, Biological Sciences, Dhaka, Bangladesh; [2]Biotech Concern, Research and Development, Dhaka, Bangladesh

Background: Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development. Approximately 80% of the world inhabitants rely on traditional medicine for their primary healthcare and play an important role in the healthcare system of the remaining 20% of the population. The potential of higher plants as a source of new drugs is still largely unexplored. *Gynocardia odorata* R.Br. belongs to the plant family Achariaceae Harms and is widely distributed throughout the Bangladesh. Traditionally the seeds of the plant have been used as leprosy, lupus, scrofula, and many skin diseases along with for its wound healing properties, but to date there is no documented evidence corroborating its antimicrobial potencies, it is an attempt to investigate.

Method: The studies were conducted to determine the antibacterial and antifungal potencies of *Gynocardia odorata* R.Br. seeds extract on methanol against both bacteria and fungi using the cup-plate method. In the studies antibacterial potencies on methanol extract of *Gynocardia odorata* R.Br. seeds extract were tested against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Staphylococcus aureus*, and *Vibrio cholerae* at the concentrations of 500, 1000, 1500, and 2000 μg/ml as well as zone of inhibition compare with standard antibiotic chloramphenicol. On the other hand, antifungal potencies on methanol extract of *Gynocardia odorata* R.Br. seeds were carried out against Candida albicans, and Candida tropicalis as well as compared with the standard drug fluconazole.

Results: *Gynocardia odorata* R.Br. seeds extract on methanol were observed that the highest antibacterial potencies were shown against *Staphylococcus aureus* while it was lowest active against Vibrio cholerae and also active against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Shigella flexneri*. The antifungal potencies of *Gynocardia odorata* R.Br. seeds extract on methanol against *Candida albicans*, and *Candida tropicalis* in addition to compare with the standard drug fluconazole.

Conclusion: The indigenously available medicines and technologies can prove an asset in the tropical and developing countries of the world. At the same time developed countries also can be benefited because of safety profile of the plant extracts.

1165
Immune response to human parainfluenza-3 infection in human nasal epithelial cells

Lewandowska-Polak, A[1]; Brauncajs, M[2]; Paradowska, E[3]; Jarzebska, M[1]; Kurowski, M[1]; Moskwa, S[1]; Lesnikowski, ZJ[3]; Kowalski, ML[1]
[1]Department of Immunology, Rheumatology and Allergy, Medical Univeristy of Lodz, Lodz, Poland; [2]Department of Microbiology, Immunology and Laboratory Medicine, Medical University of Lodz, Lodz, Poland; [3] Laboratory of Molecular Virology and Biological Chemistry, Polish Academy of Science, Lodz, Poland

Background: Viral infections may cause acute respiratory illness and exacerbate chronic inflammatory diseases of the upper and lower airways. Human parainfluenza Virus 3 (PIV3), belonging to the family Paramyxoviridae, may be a cause of pneumonia and bronchiolitis in infants and children, and is involved in asthma exacerbations in adults. The role of PIV3 in the upper airway diseases has not been well defined. The aim of this study was to evaluate the effect of PIV3 infection on the immune response in the cultured upper airway epithelial cells.

Method: Nasal epithelial RPMI 2650 cells were cultured into confluence and than were infected with PIV3 (MOI of 0.1, 0.01, 0.001). The virus cytophatic effect and viability of infected cells were assessed 24, 48 and 72 h after infection. TNF-alfa, IL-10, TSLP, IL-8, RANTES, eotaxin, GM-CSF and IFN-gamma levels in supernatants were measured by ELISA. Expression of cytokines mRNA in RPMI 2650 cells was evaluated after reverse transcription, with real-time polymerase chain reactions.

Results: Cytopathic effect of PIV3 in RPMI 2650 cultures was observed after 72 h. A significant increase of IFN-gamma protein level and mRNA expression were observed 24 h after infection. The increase in the released IFN-gamma protein was virus load-dependent (10.2 ± 3.3 pg/ml for 0.001 MOI, 12.4 ± 2.3 pg/ml for 0.01 MOI and 14.5 ± 3.3 pg/ml for 0.1 MOI as compared to 3.2 ± 1.3 SEM pg/ml for uninfected cells). At 72 h after infection both RANTES mRNA expression and RANTES protein levels were significantly increased (mean protein concentration: 3.5 ± 1.4 pg/ml for 0.001 MOI, 10.8 ± 4.6 pg/ml for 0.01 MOI and 61.5 ± 18.4 pg/ml for 0.1 MOI as compared to 2.4 ± 1.3 pg/ml for uninfected cells). No measurable concentrations of TNF-alfa, IL-10, IL-8, eotaxin, GM-CSF and TSLP were detected in virus infected cells supernatants.
Conclusion: PIV3 induces both Th1 and Th2 cytokines expression in cultured human nasal epithelial cells.

1166
Immune system disregulation during chronic herpes virus infection in childhood

Stamenkovic, HM; Stankovic, T; Saranac, LJ; Brankovic, LJ; Milicevic, R; Kamenov, B
Clinic of Pediatric, Clinic Center Nis, Immunology and Allergology, University of Nis, Nis, Serbia

Background/Introduction: An important characteristic of the herpes group viruses (HSV, CMV, EBV) is their ability to persist in the tissues of their hosts for many years after initial infection as intracellular viruses. Characteristic life of virus (hronic persistent and ciclic replication) in organisms is often followed by immune dysregulation (Th1 or Th2 tip immune response). Chronic stimulation of immune system and immunodeficienty are development by virus persisting in organisms.
Materials and methods: Clinically manifestations in patients with herpesvirus infections were examined. We analysed: white blood cell count, hemoglobin level, serum immunoglobulins level, enzymes of cell destruction (LDH, CPK, AST and ALT), oxidative metabolism of the peripherial blood phagocytes as ability of NBT reduction, ELISA test of antibody for one of the viruses: HSV, CMV and EBV. Serum level of IFN-g, IL-4 and DHEAS, cortisol were mesured by ELISA test.
Results: Our patients had and all of them had positive ELISA test on one of viruses (CMV, HSV or EBV). This were initial parametars for separate our patients in our analysis. Our parameters approved low level of hemoglobin, monocytosis, lymphocytosis, virocytosis and leukopenia. Our patients had high level LDH, CPK, low ability of NBT reduction and hypergamaglobulinemia. High levels of IFNg (70%) followed high levels of LDH, CPK, GOT and GPT. Decrease levels of DHEAS and cortisol opposite control grupe were evident.
Conclusion: Chronic activation of immune system is background of patogenetic mechanisms during herpes virus infection. Different level of DHEAS and cortisol are part of regulatory mechanisms of immune response across endocryne system. Increase levels of DHEAS in our patients can display chronic inflammation. Absence of increase level of cortisol may suggestion that our patients had a little 'acute' fase of infection opposite a lot of chronic disorders. Increase level of IFN-g can suggestion on dominant Th1 response in our patients. Analyse of immunoregulatory mechanisms is essential to order level and place of damage cells, tissue and organs. It is important for therapy and prognosis of disease.

1167
Interferon- and immunotherapy in the treatment of immunocompromised children with recurrent acute respiratory infections associated with different recurrent and latent herpesviral infections

Nesterova, IV[1]; Kovaleva, SV[2]; Chudilova, GA[2]; Lomtatidze, LV[2]
[1]Allergy and Immunology, Russian University of Friendship of People, Moscow, Russia; [2]Clinical Immunology, Kuban State Medical University, Krasnodar, Russia

Background: Children, suffering from recurrent (from 4–6 to 16–24 episodes a year) acute viral respiratory infections (ARVI) and different recurrent and latent herpesviral infections (HVI), are imunocompromised and have different disturbances of the immune system (IS) and interferon (IFN) system (I.Nesterova et al., 2005).
Methods: We had studied 27 children (both sex, age 5–8 years) who suffered from recurrent ARVI (from 4–6 to 16–24 episodes a year) and different herpesviral infections (HVI). We had investigated: clinical and anamnestic features, PCR and sulfur diagnostics for detection of herpesviruses, antiviral immunity T chain, neutrophls (NG), natural killers (NK) and IFN status.
Results: All patients had disturbance of IFN status (100%) and immunodeficiencies (89.1% of cases). A program of interferon- and immunotherapy was developed: (i) Viferon (system and local), using differential doses of IFNα2 – 3 months. Isoprinosinum was applied to recover the T chain. Licopid was used to correct the defects in NK and/or NG. High clinical effect was received: the level of ARVI was decreased in 5.2 fold, duration of AVRI was decreased in 1.6 fold, duration of the period clinical free from ARVI was increased from 171 days till 309 days, the number of acute episodes of HSV1/2 was decreased in 3–5 fold; the reconstruction of immune system had 85% of patients, IFN system-100% of patients.
Conclusions: Created program of combine interferon- and immunotherapy for immunocompromised children who suffer from recurrent acute viral respiratory tract infections associated with recurrent and latent herpesviral infections had demonstrated high clinical and immunological effects in 87.5% of cases.

1168
The efficacy of methylprednisolone pulse therapy in children with severe community-acquired pneumonia

Shin, YH[1]; Kim, JH[1]; Yoon, JW[1]; Choi, SH[2]; Kim, HY[3]; Jee, HM[1]; Han, MY[1]; Kim, JT[4]
[1]Pediatrics, CHA University School of Medicine, Seoul, Korea; [2]Pediatrics, Kyung Hee University College of Medicine, Korea; [3]Pediatrics, Bundang Jaesaeng Hospital, Seongnam, Korea; [4]Pediatrics, School of Medicine, The Catholic University of Korea, Seoul, Korea

Background: Community-acquired pneumonia is a very common disorder in pediatric populations. The mainstays of treatment for community-acquired pneumonia are early diagnosis and initiation of appropriate antibiotic therapy. On occasion, progression to severe pneumonia occurs despite appropriate therapy. The benefit of systemic steroids as adjunctive treatment in patients with severe community-acquired pneumonia (CAP) remains uncertain. The aim of the present study was to determine the efficacy of methylprednisolone pulse therapy on childhood community-acquired pneumonia whose symptoms deteriorated despite appropriate treatment.
Method: We retrospectively evaluated the effect of methylprednisolone pulse therapy in 18 children with pneumonia whose clinical and radiographic course deteriorated despite broad-spectrum antibiotics.
Results: The mean (±SD) age was 38.4 ± 35.7 months, and 10 were boys. All children had received appropriate antibiotics at presentation, but they had persistent fever and progressively worsening radiographic findings. In addition to broad-spectrum antimicrobial therapy,

methylprednisolone (1–2 mg/kg for 3 days, then tapered over 7 days) was administered on day 3 (±1.5 days) of admission. Thirteen children became afebrile within 24 h, and their clinical status and radiographic findings improved over several days. Their clinical status and radiographic findings improved within several days. The white blood cell count at presentation was $7976 \pm 3555/mm^3$. C-reactive protein at presentation was 2.9 ± 4.9 mg/dl.

Conclusion: Methylprednisolone pulse therapy appeared to be effective in reducing morbidity and is associated with clinical and radiographic improvement. Therefore, methylprednisolone treatment may be helpful for reducing morbidity in children with antibiotics-nonresponsive severe pneumonia.

1169

Case report of human immunodeficiency virus (HIV)-1 positive patient on 3rd line antiretroviral therapy in MSF clinic, Mumbai, India

Andries, AI[1]; Khan, S[1]; Verma, R[1]; Fernandes, D[1]; Isaakidis, P[1,2]
[1]Médecins Sans Frontières Belgium Trust India – Mumbai Project, Mumbai, India; [2]Operational Research Unit, Médecins Sans Frontières, Luxemburg, Luxembourg

Background: In 2011, it was estimated that 2.39 million people were living with HIV in India. It is the third largest in the world in number of people living with HIV, with adult HIV prevalence of 0.31% (0.25–0.39%). Mumbai is the most populous city in India, with around 14 million inhabitants. Medecins Sans Frontieres (MSF), an international non-government organization, started HIV/AIDS comprehensive care project in Mumbai in February 2006. The clinic functioned as a referral center for patient with suspected 2nd line failure and patient in need of 3rd line antiretroviral therapy (ART) which the medicine could not be accessed free of cost in public sector. The aim of this study is to describe lessons learned from a case of patient on 3rd line ART.

Method: We report a case of WHO Stage IV HIV infection on 3rd line ART. The study is a prospective observational cohort of the patient in MSF Clinic Mumbai, India.

Results: Patient, Mr. JP, 42 years old first came on 3 February 2010. The patient was referred by private practitioner for suspected failure of 2nd line ART. Patient was first diagnosed in private sector as HIV positive in 2002 and received various ART regimens. On admission to our facility, the patient was confirmed to have HIV-1 infection by Western Blot. Baseline CD4 was 61/μl and viral load was 278 000 copies/ml. The patient received 2nd line ART Tenofovir, Zidovudine, Lamivudine, and Ritonavir boosted Lopinavir while improving the adherence and waiting for 3rd line antiretroviral to be available. On 7 June 2011, patient received 3rd line ART Raltegravir, Abacavir, and Ritonavir boosted Darunavir based on previous antiretroviral exposure and genotyping result.

Conclusion: After improving the patient's adherence and introducing the 3rd line ART, the patient's immunological status was improved. The recent CD4 taken on June 2012 was 283/μl and viral load was 62 copies/ml. Good adherence is important before introducing the new regimen to ensure the regimen work effectively. Access to viral load testing is very essential, since by monitoring the viral load, early adherence problem could be detected before further resistance happened. Moreover, 3rd line antiretroviral should be made available for the patient who has failed on 2nd line ART.

1170

Asthma and atopic sensitisation are associated with refractory *Mycoplasma pneumoniae* pneumonia in children

Shim, JY; Shin, JE; Shim, JW; Kim, DS; Jung, HL; Park, MS
Kangbuk Samusung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea

Background: Nationwide outbreak of *Mycoplasma pneumoniae* pneumonia (MP) refractory to macrolide antibiotics occurred in Korea from June to November in 2011. Refractory MP has been reported to be efficacious and well-tolerated to steroid therapy in children. We compared clinical and laboratory characteristics between children with refractory MP requiring steroid treatment and those without and evaluated risk factors for refractory MP.

Method: MP was diagnosed when serologic titer of antimycoplasma antibody increased more than four times to the initial level or the first assay of antimycoplasma antibody titer was over 1:1280. Steroid therapy was started when clinical and radiographic course worsened despite appropriate antibiotics treatment.

Results: Total 203 children were diagnosed as MP, and 26 children were treated with steroid. Mean duration of steroid therapy was 4.3 days and all of the children became afebrile within 24 h after steroid therapy. The prevalence of refractory MP was higher in children with pleural effusion, lobar pneumonia with more than two lobes, higher serum LDH levels, oxygen requirement, and a longer duration of hospitalisation and fever after admission. Atopic sensitisation and history of asthma were associated with refractory MP after adjustment for age, sex, and family history of allergic diseases.

Conclusion: Atopic sensitisation, history of asthma, and high serum LDH level can be risk factors for refractory MP requiring steroid therapy in children.

1171

A case of toxocariasis masquerading as liver metastatic nodules in a patent with breast cancer

Lee, SP[1]; Park, S[2]; Kang, SM[1]; Kim, YJ[1]; Kyung, SY[1]; Park, J-W[1]; Jeong, SW[1]; Lee, SM[1]
[1]Gachon University Gil Medical Center, Department of Medicine, Incheon, Korea; [2]Department of Pathology, Mokdong Hospital, Ewha Womans University, Seoul, Korea

Background: Eosinophilic liver abscess can be confused with primary liver cancer or metastasis especially in patients with preceding cancer, however there were only a few reports in which eosinophilic liver abscess was confused with, or had to be distinguished from neoplasm. We report a case of breast cancer in which we had to differentiate eosinophilic liver abscess from liver metastasis of breast cancer.

Case report: A woman aged 55 years whose case was diagnosed as breast cancer, infiltrating ductal carcinoma, and invasive apocrine carcinoma, underwent a modified radical mastectomy 20 months ago, and received adjuvant chemotherapy including fluorouracil, doxorubicin, and cyclophosphamide three times. Then, she had received tamoxifen for 17 months. She ate raw bovine liver 6 months ago, and suffered from febrile sensation 1 month ago, and abdominal discomfort 2 weeks ago. Serial abdominal computed tomographies (CT) showed migrating irregular-shaped low attenuated lesions. Peripheral blood eosinophil, serum total IgE, and eosinophilic cationic protein (ECP) were markedly elevated (1220/μl, 684 U/ml, and 88 μg/l, respectively). Stool examination did not show any ova of parasite, and IgG antibodies for cysticercus, sparganum, paragonimus, and clonorchis which were most prevalent parasites in Korea were all negative. She underwent liver biopsy, and eosinophilic liver abscess was observed. We suspected the infection of Toxocara canis which is common parasite causing peripheral blood eosinophilia and eosinophilic liver abscess in Korea, and measured the specific IgG antibody for Toxocara canis using Enzyme-linked immunosorbent assay (ELISA). The result was positive, and we prescribed albendazole 400 mg twice daily

for a week, and methylprednisolone 12 mg once daily for 2 weeks. She did not complain of febrile sensation or abdominal discomfort any more, and peripheral blood eosinophil was normalised to 439/μl and multiple low attenuated hepatic lesions observed on previous CT disappeared on follow-up CT 2 months later.

Conclusion: We must consider the possibility of eosinophilic liver abscess or parasitism when peripheral blood increases, and abdominal image shows single or multiple hepatic lesions in patients who have history of raw food ingestion, even though he or she has previously diagnosed cancer.

1172
A case of apparently allergic asthma actually caused by Toxocara infection

Qualizza, R[1]; Incorvaia, C[1]; Grande, R[2]
[1]Istituti Clinici di Perfezionamento, Milan, Italy; [2]IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Background: Infection from Toxocara canis may cause a number of clinical symptoms due to potential involvement of any organ or tissue. Here we present a case of asthma presenting with the characteristics of an allergic form but actually caused by Toxocara.

Methods: The patient was a 22-year old male who had been suffering from seasonal rhinoconjunctivits and asthma caused by sensitisation to grass pollen for 3 years. He was referred to our Allergy service because of perennial nasal obstruction and cough for 1 year. The patient was not a smoker and had no pets at home. Skin prick tests (SPT) with inhalant allergens confirmed the sensitisation to grass pollen and showed a positive result also to house dust mites. To blood examination, a mild increase of C reactive protein (CRP) and eosinophils were found. Following dust mite control measures at home, an improvement of nasal obstruction but no change in cough was reported. Therefore, specific subcutaneous immunotherapy (SCIT) with a dust mite abstract was planned. At the first SCIT visit, the patient reported a recent episode of generalised urticaria unrelated to drug assumption of to other possible causes. SPT with food extracts were negative. SCIT was not initiated and additional laboratory examination was done. All of them were negative except IgG antibodies to Toxocara, detected by Western blotting. Anthelmintic therapy was prescribed using mebendazole (one 100 mg tablet b.i.d. for 3 days), repeated after 20 days up to three times, each cycle being followed by the assumption of the probiotic *Lactobacillus reuteri* for 10 days.

Results: After the second cycle of mebendazole treatment, cough completely disappeared and was accompanied by a normalisation of CRP (from 0.8 to 0.2) and eosinophils (from 9.8% to 3.7%). Of note, the patient no longer presented asthma during the pollen season and had a clear improvement also of nasal symptoms, with only occasional need to use antihistamines. After 1 year, the Western blot for antibodies to Toxocara turned negative.

Conclusion: This case confirms the recent literature on the ability of Toxocara infection to present as apparent allergy. The correct diagnosis allows to cure the disease by anthelmintic therapy.

1173
Fungal contamination of premises as a risk factor for respiratory allergy

Maslova, L[1]; Novikova, TP[2]
[1]Belarusian Medical Academy of Postgraduate Education, Minsk, Belarus; [2]Allergology, Byelorussian Postgraduate Academy, Minsk, Belarus

Background: Currently, moulds are among the unconditional risk of allergic rhinitis and asthma, in closed rooms. The inhabitants of modern cities, especially children, spend a significant part of their time indoors. Our objective was to determine the structure of mycobiota indoor and its impact on respiratory allergy development.

Method: Questionnaire to assess the living conditions of patients, suggest fungal infection, visual examination of lesions of microscopic fungi, mycological examination of premises, sampling locations of the alleged mold damage, the selection of fungi in culture. Evaluation of 174 patients living in the fungal contamination included a medical history of life and disease, allergic history, physical examination, skin allergotesting, blood samples for determination of specific IgE to the major inhalation allergens.

Results: Mycological analysis of the isolates showed a high degree of microscopic fungi spores sample contamination, dominated genera Penicillium, Cladosporium, Aspergillus, Ulocladium. A total of 174 people living in the areas affected by fungi, 59 of them (33.9%) have a sensitisation to fungal allergens, including thresholds specific Ig E. Almost half of the sensitised patients (16.7%) were sensitised to several species of fungi. One hundred and seventy-four residents of the surveyed areas were affected by the fungus, and 33 people (19%) had a diagnosis of asthma. Asthma fungal installed in 21 patients, six of whom had isolated fungal sensitisation, 15 – sensitisation to fungal allergens combined with sensitisation to house dust mite allergens.

Conclusion: Among the patients living in a residential area where the growth of fungi took place, there were increased sensitivity to fungal allergens in isolation or in combination with other types of sensitisation. There is a need of residential patients with fungal sensitisationis inspection, the development of measures for the indoor fungi elimination. When moulds induce the early type of hypersensitivity specific immunotherapy should be used as the primary method of pathogenetic therapy.

1174
Polimorphonuclear neutrophil antigen FcγRI (CD64) over expression in pregnancy is a marker of innate immune activation

Ierullo, AM[1]; de Amici, M[2]; Perotti, F[1]; Torre, C[2]; Spinillo, A[1]
[1]Fondazione I.R.C.C.S Policlinico San Matteo, Department of Obstetrics & Gynecology, Fetal Medicine Unit, Pavia, Italy; [2]Fondazione I.R.C.C.S. Policlinico San Matteo, Department of Paediatrics and Neonatology, Pavia, Italy

Background: Normal pregnancy produces inflammatory changes in peripheral blood leukocytes. FcγRI (CD64) expressed on polimorphonuclear neutrophils (PMNs) play a central role during an immune response. We aimed to test the hypothesis that FcγRI (CD64) antigen on neutrophil expression (PE molecules) and cross-linking sites may be elevated during pregnancy as a measurable manifestation of physiologic activation of the immune response.

Method: Transactional study in healthy women with singleton uncomplicated pregnancies ($n = 102$) and non pregnant fertile controls ($n = 41$). Maternal FcγRI (CD64) was determined by flow cytometry measured between 8 and 40 weeks of gestation and in non-pregnant women. FcγRI (CD64) on neutrophils was measured with a FAC Scan flow cytometer (Becton-Dickinson®, San Jose, CA, USA) using an FL3 threshold to analyse leucocytes only. Analysis was conducted with non-parametric statistics. A P-value <0.05 was considered statistically significant.

Results: The FcγRI (CD64) percentage (mean; SD) on neutrophils was significantly different between pregnant patients and non-pregnant [(33.7;20.8) vs (15;7.7) $P < 0.0001$]; number of PE molecules (mean; SD) in the pregnant group was significantly higher than in the non-pregnant controls [(2247;745) vs (1652.7;624) $P < 0.0001$].

The cut-off value of 2081 of FcγRI number of PE molecules discriminate pregnant

women from non-pregnant with a sensitivity of 88% and a specitivity of 75%.
Conclusion: In normal pregnancy CD64 antigen on neutrophil PE molecules and cross-linking sites were elevated supporting the hypothesis that pregnancy is a state of physiological activation of the immune response.

1175
The program of immunorehabilitation, including local and systemic interferon- and immunomodulating therapy, created for immunocompromised patients with allergic rhinitis and asthma

Nesterova, IV[1]; Sepiashvili, RI[2]
[1]Allergy and Immunology, Russian University of Friendship of People, Moscow, Russia; [2]Allergology and Immunology, Russian University of Friendship of People, Moscow, Russia

Introduction: Recurrent acute respiratory viral infections (ARVI) can provoke the development of frequent exacerbations of allergic rhinitis (AR) and atopic asthma (AA) (Papadopoulos N.G. et al., 2006). In patients with recurrent ARVI the IFN system and antiviral mechanisms of immune system may be defective (I. Nesterova, 2007–2010]).
Methods: We examined 54 patients of both sex at the age of 18–55 years with perennial AR, moderate AA and symptoms of recurrent ARVI: 8-to 12 ARVI cases annually. The level of total IgE was significantly elevated as compared to normal level. All patients were sensitised to domestic allergens; 31.48% had food allergy and were sensitised to mycelial fungi. Complex investigation of anti-viral state of immune system and interferon status was carried out.
Results: All patients had secondary ID (various disorders of T-cell, NK, phagocytes) and defects of IFN system in different combinations. The program of immunorehabilitation was created:

1 selective β_2 agonists inhalation combined with topic steroids Fluticasone in the adequate doses;
2 nasal Fluticasone irrigation in the adequate doses;
3 elimination regime for allergens and antigens;
4 ntioxidants;
5 sanation of chronic niduses of infection;
6 local and systemic recombinant IFNα2 (Viferon));
7 immunomodulation: Imunofan- for T cell chain correction; for restoration of phagocyte system, NK – Likopid.

Positive clinical and immunological effects became in 83.33% patients: AR and AA exacerbations were absent for 3 months, no episodes of ARVI were registered during this period.
Conclusion: High clinical and immunologic efficiency of the program of immunorehabilitation, including local and systemic interferon and immunomodulating therapy, creating for immunocompromised patients with AR and AA, was shown.

1176
Factors of the innate and of adaptive immunity in newborn with herpes virus and chlamydial, mycoplasmal intrauterine infection

Belovolova, R; Voronenko, I; Dolgopolova, O; Tumasova, M
Rostov State Medical University, Rostov-on-Don, Russia

Background and method: The study involved 57 children of the 1st month of life with intrauterine infection (IUI), herpesvirus and chlamydial-micoplasmic etiology confirmed by the detection DNA and the genome of HSV, CMV, EBV, Chlamidia Trachomaticus, Mycoplasma Gominis in the blood, urine, or mucosal scrapings of tissues by PCR examination and the presence of IgM antibodies or low avidity IgG for CMV, EBV, HSV (types 1 + 2), IgM antibodies to Chlamydia, Mycoplasma by ELISA diagnostics.
Results: Clinical manifestation of IUI herpesvirus etiology with damage of central nervous system (CNS) in 75% of children, with metabolic abnormalities – 59%, jaundice – in 35%, pneumonia – 24%, intrauterine growth retardation – in 24% of children. Eighty percent of children with micoplasmic-chlamydial etiology of IUI had damage of CNS, metabolic disorders – 85%, pneumonia – 35%, intrauterine growth retardation in 30%, jaundice – in 20%, impaired cardiovascular system in 30% of children. General regularity immune system reactions to an infectious agent has been reduction of metabolic activity of neutrophils, disimmunoglobulinemiya with increasing levels of IgM and the imbalance of peripheral blood lymphocyte subsets. With IUI herpesvirus etiology imbalance lymphocyte subpopulations occurs mainly reduced content of CD8+ -, CD16+ – lymphocytes, increases levels of serum IgM. It was found inhibition of metabolic activity of neutrophils, particularly in the stimulated NST test. With IUI Chlamydia and Mycoplasma etiology noted decrease of CD4+ lymphocytes, increase in serum IgM and reducing the IgG, more prominent reduction of metabolic activity of neutrophils.
Conclusion: Detection of markers of herpes virus infection, the presence of dysfunction of the immune system against clinical manifestations of intrauterine infection in children 1 month of life is an indication for inclusion in the complex therapy of antiviral, and containing interferon immunocorrecting drugs. With Chlamydia and Mycoplasma IUI effective was complex antibiotic and immunomodulatory drugs.

1177
Anaphylaxis after the probable rupture of a hepatic hydatid cyst

Zambonino Carreiras, MA[1]; Garcia Menaya, JM[1]; Bobadilla Gonzalez, P[1]; Corrales Vargas, SI[1]; Maheche Garcia, AK[1]; Cordobes Duran, C[2]
[1]Infanta Cristina Hospital, Allergy Service, Badajoz, Spain; [2]Merida Hospital, Allergy Service, Merida, Spain

Background: Echinococcosis or hydatid disease (HD) is a zoonosis caused by the larval stages of taeniid cestodes belonging to the genus Echinococcus. HD is usually asymptomatic for a long period of time, since cyst growth is commonly slow – growth rate in the liver is variable, ranging from 1 to 5 mm in diameter per year.

We present a case of anahylaxis after the probable rupture of a hepatic hydatid cyst.
Method: A 45-year-old woman with a history of anaphylactic reaction in 1998 – developed after the intake of strawberries (previously well tolerated) – experienced heat, dizziness, sweating and loss of consciousness this year immediately after the intake of sausages, bread and chips (previously well tolerated). She was transferred to our hospital after intravenous administration of dexchlorpheniramine, metilprednisolone and physiological serum. During physical examination, erythema in the left hemiabdomen and in the proximal third of the left thigh was detected. She was observed overnight in the hospital and returned home without any symptoms. After that she ate bread and sausages with no reaction. Three days after the episode, she experienced erythema and angioedema 8 h after having eaten potatoes, avoiding them since then. She avoids strawberries too. We realised skin test, some blood analysis and abdominal ultrasound.
Results: Skin prick test with commercial extracts of cat, profiline, LTPs, anisakis, latex and a standard battery of foods (egg, milk, cereals, nuts, fish, fruits, meats, molluscs, crustaceans and vegetables) including strawberries and potatoes were negative.

Prick-prick with potatoes and strawberries were negative. Blood count showed a 22.4% eosinophils (2100 e/mm^3). Serum tryptase was normal. Total IgE was high (1.786 IU/ml). Specific echinococcus IgE (>100 kU/l) and specific ascaris IgE (1.78 kU/l) were high too. Specific IgE for anisakis, potatoes and strawberries was negative. Hydatid serology was positive (1/12 800). Abdominal ultrasound showed a 5.5 cm hepatic cyst compatible with hydatic cyst.

Conclusion: We present a case of a patient with anaphylaxis due to the probable rupture of a hepatic hydatid cyst masked with a food allergy history (with negative tests). Hepatic equinococcis is not an infrequent disease in our area, so particular caution should be taken in this regard in every case of anaphylaxis with a high level of eosinophils or with incompatible anamnesis.

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

Poster Session 45

Up-to-date information on hereditary angioedema

1178
Analysis of characteristics associated with reinjection of icatibant: results from the Icatibant Outcome Survey

Longhurst, H[1]; Aberer, W[2]; Bouillet, L[3]; Caballero, T[4]; Fabien, V[5]; Zanichelli, A[6]; Maurer, M[7]
[1]Bart's Health NHS Trust, Department of Immunology, London, United Kingdom; [2]Department of Dermatology and Venerology, Medical University of Graz, Graz, Austria; [3]National Reference Centre for Angioedema, Internal Medicine Department, Grenoble University Hospital, Grenoble, France; [4]Hospital La Paz Health Research Institute (IdiPaz), Biomedical Research Network on Rare Diseases (CIBERER, U754), Madrid, Spain; [5]Shire Human Genetic Therapies, Eysins, Switzerland; [6]Department of Clinical Science "Luigi Sacco", University of Milan, Milan, Italy; [7]Department of Dermatology and Allergy, Allergie-Centrum-Charite, Charite – Universitätsmedizin Berlin, Berlin, Germany

Background: Icatibant is a selective bradykinin B2 receptor antagonist approved for treatment of acute attacks of hereditary angioedema (HAE), a rare autosomal dominant disorder caused by decreased levels (type I) or function (type II) of C1-inhibitor. HAE is a debilitating disease characterised by recurrent and unpredictable attacks of swelling of the face, upper airway, extremities, or gastrointestinal tract. Phase III clinical trials with icatibant have shown that the treatment of the majority of HAE attacks requires only one subcutaneous injection of icatibant 30 mg. Here we report an analysis of the characteristics of HAE attacks associated with reinjection during real-world use of icatibant.
Method: The Icatibant Outcome Survey (IOS) registry [Shire HGT, Eysins, Switzerland (NCT01034969)] is an international, prospective observational study designed to monitor the safety and efficacy of icatibant during long-term treatment. Descriptive analyses of reinjection data obtained from July 2009 through October 2012 were performed. A total of 516 attacks occurred in 155 patients with HAE type I or type II.
Results: The majority of attacks (92.6%, $n = 478$) were treated with a single injection of icatibant. Most patients (85.8%, $n = 133$) treated each attack with one injection. In case of reinjection, patients administered the 2nd injection at a median time of 11.75 h after the first. There were no differences in the number of injections used to treat HAE attacks by administrator type [self- vs healthcare professional (HCP)-administered], attack severity, or attack frequency (in patients up to two attacks per month): 93.3% ($n = 278$) and 91.0% ($n = 183$) of attacks treated by self-administration and HCP, respectively, used a single injection. Ninety-two percent of severe attacks were treated with a single injection of icatibant ($n = 311/338$). In patients with <20 ($n = 102$) or ≥20 ($n = 37$) attacks per year, 84.3% vs 86.5% of patients, respectively, treated their attacks using a single injection.
Conclusion: These real-world observational outcomes show that the majority of HAE type I or II attacks were successfully treated with a single injection of icatibant. Overall, administrator type, attack severity, and frequency did not impact the need for reinjection.

1179
Adverse effects of danazol prophylaxis in female patients with hereditary angioedema due to C1-INH deficiency (HAE-C1-INH)

Zotter, Z; Czaller, I; Csuka, D; Szabó, E; Kőhalmi, KV; Varga, L; Farkas, H
Department of Internal Medicine, Semmelweis University, III, Budapest, Hungary

Background: HAE-C1-INH is a rare autosomal dominant disorder characterised by paroxysms of edema-formation in the subcutis and/or the submucosa. Its management comprises two stages – that is, the therapy of overt attacks, and the prevention of their recurrence. Danazol (an attenuated androgen), commonly used – among other agents – for long-term prophylaxis, has diverse side effects. This study intended to investigate the virilizing effects and the adverse effects of danazol on serum lipid profile, and hepatic function in our female HAE-C1-INH population.
Method: Lipid profile (total cholesterol, HDL, LDL, triglycerides), and hepatic function (bilirubin, GOT, GPT, gamma GT, total protein, ALP, LDH) were compared between patients treated ($n = 31$), or not treated ($n = 41$) with danazol. The 31 danazol-treated patients were interviewed individually, about the type and severity of virilizing effects (using a four-grade severity score system), as well as their satisfaction with danazol therapy (according to a 10-grade scale). Physical examination was also performed.
Results: The duration of danazol treatment was 10.31 years [2–23] and a daily dose of 131.7 mg [33–250] was administered. The most frequent adverse effect were hirsutism ($n = 13$), weight gain ($n = 12$), menstrual disturbances ($n = 7$), and diaphoresis ($n = 7$). The mean number of adverse effects was 2.9 per patient. There were no significant differences in the severity of danazol adverse effects in relation to either the duration of dosing, or daily drug dose. The mean level of satisfaction with treatment was 8.47 on a 10-grade scale. We performed multiple logistic regression analyses adjusted for age, drug dose, and treatment duration, to evaluate effects of danazol on lipid profile and liver function. HDL levels were lower ($P = 0.033$), in danazol-treated patients, compared with those not treated with danazol. No significant differences were found between the two groups as regards total cholesterol, triglyceride, LDL, VLDL, Lp(a) levels, and liver function parameters.
Conclusion: Our findings indicate that long-term treatment with danazol – using the lowest effective dose (max. 250 mg/day) – has a mild virilizing effect. So far, however, this has not led to the discontinuation of danazol therapy in our female patients. Danazol treatment does not alter the lipid profile and liver function of females with HAE-C1-INH.

1180
Attacks following tooth extractions in patients with hereditary angioedema: retrospective study

Baysan, A; Gulec, M; Kartal, O; Yesillik, S; Demirel, F; Musabak, U; Sener, O
Division of Immunology and Allergic Diseases, Department of Internal Medicine, Gulhane Military Medical School, Ankara, Turkey

Introduction: Tooth extraction may trigger attacks in patients with hereditary angioedema (HAE). Our study aims to determine frequency and characteristics of HAE attacks occurred after tooth extractions.
Method: Standardised questionnaires constructed for this purpose are administered either by phone or face-to-face interview to

HAE patients, who are followed by our clinic.

Results: One hundred and twenty-seven tooth extractions of 26 patients, in 92 sessions, are evaluated. In 33 (% 35) sessions, patients are known to be HAE. Among these patients only five have used danazol and one has used tranexamic acid as long-term prophylactic treatment. Before tooth extraction short-term prophylactic treatment is applied only to two patients (danazol for one patient, C1 inhibitor concentrate for another). Fourteen of 92 sessions were ended with attacks. Five of these attacks were facial, three of them were laryngeal and six of them were facio-laryngeal. All attacks had occurred in 6 h following tooth extraction. Fresh frozen plasma was used for only one attack. Others had resolved spontaneously.

Conclusion: There is a risk of life-threatening laryngeal edema induced by tooth extractions in patients with HAE. Early diagnosis and increased awareness can decrease the risk of laryngeal attacks in patients with HAE following tooth extractions.

1181
Acute oesophageal necrosis and sudden death in a man with C1 inhibitor deficiency hereditary angioedema

Gonzalez-Quevedo, T[1]; Piñero-Saavedra, M[1]; Leguísamo-Milla, S[1]; Borrero, JJ[2]; Member of GEAB
[1]Allergology Unit, Universitary Hospital V del Rocío, Seville, Spain; [2]Pathology Deparment, Universitary Hospital V del Rocío, Sevilla, Spain

Background: Acute esophageal necrosis or 'black esophagus' is a very rare clinical entity that shows high rates of mortality and sex and age predilection (4:1 men: women; age average = 68 year). It could be an incidental finding diagnosed at post-mortem examination or during an upper gastrointestinal endoscopy. The most common presenting symptom is gastrointestinal bleeding. Its etiology in unknown but it is thought to occur as a vascular or ischemic phenomenon (ischemia-reperfusion injury). It has been suggested associated to comorbidities as cardiovascular risk factors (dyslipemia, high blood pressure, diabetes mellitus), anticardiolipin antibody syndrome, microbial infection, herpes simplex esophagitis, or Stevens-Johnson Syndrome.

Method: A 67 year old man, with very frequent attacks of gastrointestinal angioedema and diagnosed of C1-Inhibitor mutation: Leu273Arg and splicing in exon 5, Hereditary Angioedema (HAE) type 1, and since November 1989 on a low dose androgens maintenance therapy (danazol 100 six times weekly, or stanozolol 2 mg last 2 years). He never needed C1-Inhibitor concentrate as emergency therapy. He suffered from depression and was having amitriptilina 25 mg and potassium cloracepate 25 mg daily. Cholelithiasis was ultrasonographically detected in 2003 and was referred to digestive surgery on 2007 but he rejected intervention. He had a routine clinical exam and ultrasound only some days before his death. Neither revealed any abnormalities but cholelithiasis. Patient was received in the emergency unit of our hospital after 7 days of nausea and vomiting. An emergency ultrasound exam detected an intense distension in stomach and inmediately after that he had his first hemorrhagic vomit and died.

Results: Histologically necrotic debris, absence of viable squamous epithelium, necrosis of esophageal mucosa and submucosal inflammatory infiltration was present. Not thromboembolism signs. Liver: percistical very much increased fibrosis and steatosis. Brain: signs of acute cortical infarction and multiple little focus of temporal hemorrhages.

Conclusion: To our knowledge it is the first case report about black esophagus in HAE patients. This event allow us to suggest that, as Peliosis hepatis, long-term androgens therapy or HAE itself could be a comorbidity to have in account for this type of evolution.

1182
Retrospective analysis of emergency surgery in patients with hereditary angioedema

Baysan, A; Gulec, M; Kartal, O; Yesillik, S; Demirel, F; Musabak, U; Sener, O
Department of Internal Medicine, Division of Immunology and Allergic Diseases, Gulhane Military Medical School, Ankara, Turkey

Introduction: Undiagnosed patients with hereditary angioedema (HAE) may undergo unnecessary operations due to gastrointestinal system (GIS) attacks. Moreover, any operation performed with general anesthesia may trigger attacks in these patients. In our study, we aim to determine the frequency of unnecessary operations and laryngeal attacks occurred after intubations.

Method: Standardised questionnaires are administered via either by phone or face-to-face interview to HAE patients, who are followed by our clinic.

Results: Thirty-three operations were analyzed. Ten of these were done before diagnosis of HAE and due to possible misdiagnosis of GIS attack as acute abdomen (seven appendectomy, two laparotomy, one cholecystectomy). Six of 33 operations were performed in diagnosed HAE patients; two of six were with long-term danazol prophylaxis only, other two were with long-term danazol prophylaxis plus short-term prophylaxis with C1 inhibitor concentrate, one was with short-term C1 inhibitor prophylaxis only and the last one was without prophylaxis. No attacks were occurred any of these patients. Laryngeal attacks occurred in four of 27 operations of undiagnosed HAE patients in 6 h postoperatively and recovered spontaneously.

Discussion: Frequency of unnecessary operations is so high among patients with HAE. Although laryngeal attack frequency due to general anesthesia is not high, short-term prophylaxis should be given preoperatively to all HAE patients, as they are at risk of developing life-threatening laryngeal attacks. Early diagnosis and increasing awareness of HAE will result decreased rate of unnecessary operations and also decreased risk of laryngeal attacks that can be prevented by short-term prophylaxis.

1183
Children with hereditary angioedema due to C1 inhibitor deficiency respond better to tranexamic acid than adults

Djenouhat, K[1]; Gharnaout, M[2]; Ibsaine, O[3]
[1]Institut Pasteur d'Algérie, Biological Investigations Center, Algiers, Algeria; [2]CHU Beni Messouseni Messous, Pneumophysiology, Algiers, Algeria; [3]CHU Parnet, Pediatric Department, Algiers, Algeria

Background: Although rarely mentioned in literature, tranexamic acid has demonstrated its relative effectiveness in the treatment of number of patients with hereditary angioedema due to C1 inhibitor deficiency.

Method: Sixteen patients among our families with antigenic deficiency of C1 inhibitor were regulatory followed by physicians. Our population is divided to two groups, the first group is composed of four children (3–12 years), while the second one is composed of 12 adult patients with ages ranging from 19 to 39. Plasma C1 INH, C3 and C4 were assayed by nephelometer (Dade Behring, Germany) and C1 IHN activity was determined by chromogenic assay (Immunochrom C1- INH, Immunodiagnostic, Baxter, Wien). All sixteen patients were treated with Tranexamic acid at different doses depending of disease severity.

Results: Results of our preliminary study showed that only one child had one mild attack during that period, no severe crisis was observed in our pediatric series. However, three of the 12 (25%) adult patients have between 2 and 5 severe crises vary in

that year. We considered the efficiency of treatment according to number of attacks developed over 12 months.
Conclusion: Regardless of the dosage of tranexamic acid, we concluded that this drug is more effective in children than in adults with hereditary angioedema due to C1 inhibitor deficiency. This could be explained in part by lower factors triggering in children compared to adults, as the states of stress, estrogenic impregnation and others.

1184
Hereditary angioedema with normal C1Inhibitor and factor XII mutation: a French cohort

Boccon-Gibod, I[1,2]; Bouillet, L[1,2]; Gompel, A[2,3]; Fain, O[2,4]; Du-Thanh, A[2,5]; Raison-Peyron, N[2,5]; Ollivier, Y[2,6]; Gayet, S[2,7]; Pralong, P[8]; Djenouhat, K[9]; Kirk, R[10]
[1]Internal Medicine, Grenoble University Hospital, Grenoble, France; [2]National Reference Centre for Angioedema, Grenoble, France; [3]Port Royal Cochin, APHP, Paris, France; [4]Jean Verdier Hospital, APHP, Bondy, France; [5]Saint-Eloi University Hospital, Montpellier, France; [6]Côte de Nacre University Hospital, Caen, France; [7]Conception Hospital, ap-hm, Marseille, France; [8]Hopital de Lyon sud, Lyon, France; [9]Institut Pasteur d'Algérie, Alger, Algeria; [10]Department of Child Health, Princess Alexandra Hospital NHS Trust, Harlow, United Kingdom

Background: Hereditary angioedema (HAE) with normal C1 Inhibitor (C1Inh) was first described in 2000. Mutations in the coagulation factor XII (FXII) gene have been identified in a minority of affected HAE families with normal C1Inh. In France, since 2006, the national reference centre of angioedema, a French multisite network of physician experts has collected data and established a cohort of those patients. The aim of this study is to describe the clinical aspects of this cohort.
Method: We have conducted a retrospective, observational, cohort study of 27 patients diagnosed with normal C1 Inh with FXII mutation, from 15 families. We collected 18 patients completed records from six different sites of reference center. We reported clinical features: family history, age, sex, onset of symptoms, attacks (sites, duration, frequency, treatment) and oestrogen sensitivity.
Results: One hundred percent of patients are female with mean age of 36 years old. Fifty-three percent of them have family history of angioedema. Mean onset of symptoms is 22 years old. One hundred percent of patients experienced face attacks, 73% upper airways attacks, with 60% of laryngeal attacks. Abdominal attacks were observed in 80% of patients and peripheral attacks in 87%. Bladder attacks were described for 20% of them. Over the last year, the mean occurrence of attacks was 7.2. Regarding oestrogen sensitivity, 80% of patients worsened under oestrogenic pills or pregnancy, 13% have had symptoms only with oestrogenic pills or during pregnancy and 7% reported no oestrogen influence. Twenty-seven percent of patients were treated at least for one attack with Icatibant, and 7% with C1Inh plasma-derived concentrate. Thirty-three percent received long term prophylaxis with tranexamic acid and 7% with C1Inh plasma-derived concentrate.
Conclusion: On a small number of HAE patients with normal C1Inh and FXII mutation, all collected inside the national reference center of angioedema, we report specific clinical features, compared to HAE patient with C1 Inh deficiency. We want to focus on the fact that in this cohort only women are symptomatic and 93% of them have oestrogen sensitivity. A study with more patients is needed to confirm those data.

1185
Effect of C1-inhibitor concentrate in the treatment of acute cutaneous attacks of hereditary angioedema

Bork, K[1]; Staubach, P[1]; Bernstein, J[2]; Feuersenger, H[3]; Machnig, T[3]; Craig, TJ[4]
[1]Department of Dermatology, Johannes Gutenberg-University, Mainz, Germany; [2]Medical Center and Bernstein Clinical Research Center, University of Cincinnati, Cincinnati, OH, United States; [3]CSL Behring GmbH, Marburg, Germany; [4]Penn State University College of Medicine, Hershey, PA, United States

Background: Treatment with plasma-derived C1 esterase inhibitor (C1-INH) concentrate is well established for acute hereditary angioedema (HAE) attacks. However, data on the efficacy of C1-INH for cutaneous edema of HAE compared to placebo is limited, therefore the effect of C1-INH in cutaneous edema in the I.M.P.A.C.T.2 study was compared to historical untreated controls.
Method: Data from 36 HAE patients treated for cutaneous edema (peripheral and facial location) in the I.M.P.A.C.T.2 study were compared to 45 patients with cutaneous edema from historical control data from a hospital database of HAE patients. 'Average total attack resolution time' (TRT) was the endpoint of the comparison between the 38 patients with 288 attacks treated with 20 IU/kg body weight C1-INH in I.M.P.A.C.T.2 and the 46 patients of the historical control with 8404 untreated attacks. Differences were assessed by a Wilcoxon test and a log-rank test; TRT was analyzed descriptively and graphically by Kaplan-Meier curves.
Results: The C1-INH treated patients are comparable to the historical untreated controls regarding the baseline demographic characteristics age, sex and HAE type. The historical control group has a mean TRT of 3.74 days [90% CI (3.31; 4.17); median: 3.00 days]. In comparison, treated cutaneous attacks have a mean TRT of 2.04 days [90% CI (1.16; 2.93); median: 1.37 days]. This difference is highly significant ($P < 0.0001$, Wilcoxon test and log-rank test). Kaplan–Meier graphs confirm this finding of a significant difference between treated and untreated cutaneous attacks.
Conclusion: Based on these pooled results, treatment of peripheral HAE edema attacks with body-weight adjusted C1-INH concentrate provided faster attack resolution compared to no treatment.

1186
Treatment of hereditary angioedema type I or II attacks: use of icatibant in a phase III controlled setting compared with real-world outcomes

Maurer, M[1]; Longhurst, H[2]; Li, HH[3]; Fabien, V[4]; Lumry, W[5]
[1]Department of Dermatology and Allergy, Allergie-Centrum-Charite, Charité – Universitätsmedizin Berlin, Berlin, Germany; [2]Department of Immunology, Bart's Health NHS Trust, London, United Kingdom; [3]Institute for Asthma and Allergy, Chevy Chase, MD, United States; [4]Shire Human Genetic Therapies, Eysins, Switzerland; [5]AARA Research Center, Dallas, TX, United States

Background: The For Angioedema Subcutaneous Treatment (FAST)-3 study (NCT00912093) was a multicentre, randomised, double-blind, placebo-controlled, phase III study in patients with hereditary angioedema (HAE) type I or II and demonstrated that icatibant significantly reduced median time to ≥50% reduction in symptom severity and almost complete symptom relief. In contrast, the Icatibant Outcome Survey (IOS) registry (NCT01034969) is an international, prospective, observational real-world study sponsored by Shire HGT. We have evaluated whether icatibant used in a real-world setting demonstrates similar efficacy as in a controlled trial setting by comparing the efficacy of almost complete symptom resolution (FAST-3) with complete symptom resolution (IOS).
Method: In FAST-3, patients received icatibant 30 mg administered subcutaneously by healthcare professionals (HCP) within 6 h of non-laryngeal attacks becoming moderate in severity and within 12 h of attack onset. In the IOS study, patients with HAE treated mild, moderate, and severe attacks upon recognition of the symptoms of an attack, and patients could self-administer icatibant 30 mg subcutaneously or have it administered by a HCP. Median time to treatment, time to resolution [almost complete resolution (FAST-3)

or complete resolution (IOS)], and attack duration were compared. Data are presented for non-laryngeal attacks treated <12 h from attack onset.

Results: Data from 85 patients (243 attacks) from IOS and 43 patients (43 attacks) included in FAST-3 were compared. Median times from symptom onset to treatment with icatibant were shorter in IOS compared with FAST-3 [1.50 h (n = 85) vs 6.45 h (n = 43); $P < 0.001$]. For patients who received icatibant within 12 h for non-laryngeal attacks, the median time from treatment to complete attack resolution in IOS was shorter than the median time to almost complete resolution in FAST-3 [4.00 h (n = 77) vs 8.00 h (n = 43); $P < 0.0001$]; median attack duration also was substantially reduced in IOS compared with FAST-3 [7.50 h (n = 77) vs 16.92 h (n = 43); $P < 0.0001$].

Conclusion: In the IOS registry, a real-world setting with minimal restrictions for treatment, a majority of HAE type I or II attacks were treated considerably earlier with icatibant when compared with phase III controlled trial data. Based on these observational IOS data, icatibant significantly shortened the time from treatment to attack resolution and the duration of attacks.

1187
Life-threatening oedema following cardiac catheterization in a 84-year-old patient with mild hereditary angioedema

Aygören-Pürsün, E; Schwabe, D
Pediatric Clinic, University Hospital Frankfurt, Frankfurt, Germany

Background: Hereditary angioedema (HAE), caused by deficiency in C1-inhibitor (C1-INH), can lead to unpredictable oedema of the subcutaneous tissues or mucous membranes with potentially fatal complications. Surgery or dental work may trigger oedema episodes. Therefore, current guidelines recommend pre-operative prophylaxis with C1-INH or attenuated androgens in patients with HAE undergoing surgery.

Method: Case report on a 84-year-old female patient with diagnosis of type I HAE, who had not suffered HAE attacks over the past years.

Results: The patient was admitted to our the hospital with acute myocardial infarction. She underwent immediate cardiac catheterizsation and received two cardiovascular stents. As the patient had not experienced HAE attacks over the past years, no attention was paid to the diagnosis of HAE on the part of the patient. Thus, no pre-procedural prophylaxis using human pasteurised C1-INH concentrate was administered in this emergency situation. The procedure was complicated by a false aneurysm at the site of arterial catheterizsation. The situation caused severe distress in the patient, in consequence of which a severe laryngeal oedema developed rapidly. Intubation was exceptionally difficult and extubation could be performed only after administration of human pasteurised C1-INH concentrate.

Conclusion: Given the unpredictable nature of acute HAE attacks, the case underlines both the awareness of treating physicians of HAE diagnosis before procedures are undertaken and the importance of having a readily available supply of acute treatment, like human pasteurised C1-INH concentrate, in the emergency setting. Even patients with mild or asymptomatic HAE have a risk of peri-operative edema; and even minor surgery and associated mental stress situations can lead to potentially life-threatening edema.

1188
Successful sigmoid resection with C1 concentrate prophylaxis in a patient with hereditary angioedema

Baysan, A[1]; Gulec, M[1]; Kartal, O[1]; Demirel, F[1]; Yesillik, S[1]; Ersoz, N[2]; Musabak, U[1]; Sener, O[1]
[1]Division of Immunology and Allergic Diseases, Department of Internal Medicine, Gulhane Military Medical School, Ankara, Turkey; [2]Department of General Surgery, Gulhane Military Medical School, Ankara, Turkey

Introduction: Operations with general anesthesia may trigger laryngeal attacks in patients with hereditary angioedema (HAE). Short-term prophylaxis and close follow up post-operatively for possible laryngeal attacks are very important.

Case: A 60 year-old male patient has had HAE attacks since childhood, but he was diagnosed just 10 years ago and given danazol for long-term prophylaxis. His attacks improved 60–70% with danazol. One year ago, he referred to our clinic for severe facio-laryngeal attack and was treated with C1 inhibitor concentrate infusion. Upon investigation for bloating and constipation complaints, he was diagnosed a sigmoid mass. Due to his laryngeal attack history, the patient was given 1000 Ü C1 inhibitor concentrate 2 h preoperatively and 500 Ü C1 inhibitor concentrate intraoperatively. Cuff leak pressure measured serially during surgery. In a 5-h operation, he was underwent sigmoid resection and terminal colostomy. Pathological examination of the resection material reported as mucinous adeno carcinoma. During postoperative period, sufficient C1 inhibitor concentrate was available in intensive care unit and the patient was monitored closely. No HAE attack was seen postoperatively.

Discussion: It is important to state that the HAE patient with sigmoid cancer has used high dose danazol, which might be cause of the liver cancer, for 10 years. Nevertheless, no data was found in the literature about casual relationship between long-term danazol usage and sigmoid cancer. General anesthesia in HAE patients should be planned together with allergist and anesthesiologist. Taking patient's earlier attacks into consideration, proper doses of C1 inhibitor concentrate should be given preoperatively; moreover, sufficient amount of C1 inhibitor concentrate should be available for instantaneous intra- and post-operative use.

1189
Recombinant human C1 inhibitor for treatment of acute attacks of hereditary angioedema: a randomised, double-blind, placebo-controlled clinical trial

Riedl, M[1]; Moldovan, D[2]; Levy, R[3]; Baker, J[4]; Obtulowicz, K[5]; Grivcheva-Panovska, V[6]; McNeil, D[7]; Andrejevic, S[8]; Reshef, A[9]; Farkas, H[10]; Li, H[11]; Bernstein, J[12]; Davis, A[13]; Lockey, R[14]; Lumry, W[15]; Wedner, HJ[16]; Craig, T[17]; Gower, R[18]; Shriov, T[19]; Relan, A[20]; Cicardi, M[21]
[1]University of California, Los Angeles, CA, United States; [2]University of Medicine and Pharmacy, Tirgu Mureş, Romania; [3]Family Allergy & Asthma Center, Atlanta, GA, United States; [4]Baker Allergy Asthma Dermatology, Lake Oswego, OR, United States; [5]Szpital Uniwersytecki w Krakowie, Wewnętrznych, Poland; [6]PHI University Clinical Centre Skopje, Skopje, FYR Macedonia; [7]Ohio State University, Columbus, OH, United States; [8]Clinic Center Serbia, Belgrade, Serbia; [9]Sheba Medical Center, Ramat Gan, Israel; [10]Hungarian Angioedema Center, Semmelweis University, Budapest, Hungary; [11]Institute for Asthma and Allergy, P.C., Chevy Chase, MD, United States; [12]University of Cincinnati, Cincinnati, OH, United States; [13]Medical Research of Arizona, Scottsdale, AZ, United States; [14]University of South Florida, Tampa, FL, United States; [15]University of Texas Southwestern, Dallas, TX, United States; [16]Washington University, St. Louis, MO, United States; [17]Pennsylvania State University, Hershey, PA, United States; [18]Marycliff Allergy Specialists, Spokane, WA, United States; [19]UMHAT 'Tsaritsa Yoanna – ISUL' EAD, Sofia, Bulgaria; [20]Pharming Technologies, Leiden, The Netherlands; [21]Università degli Studi di Milano, Milan, Italy

Rationale: Hereditary angioedema (HAE) is generally caused by a deficiency in functional C1 esterase inhibitor and characterised by recurrent attacks of tissue swelling affecting multiple anatomic regions. Recombinant human C1 esterase inhibitor (rhC1INH) was previously shown effective for the treatment of angioedema attacks in patients with HAE. The current study evaluated safety and efficacy of rhC1INH (50 U/kg to maximum 4200 U) in a larger patient population.

Methods: Patients experiencing eligible angioedema attacks were randomised (3:2) to intravenous rhC1INH or saline (placebo).

The primary endpoint was time to onset of symptom relief at the primary attack location, assessed as time from start of study drug infusion to onset of sustained beneficial effect, defined by patient responses demonstrating improved symptoms on a Treatment Effects Questionnaire (TEQ) at consecutive time points. For comparability with prior studies, symptoms were additionally assessed using a Visual Analogue Scale (VAS). In time to event analyses, comparisons were conducted via log-rank test, stratified by primary attack location. Safety was also evaluated.

Results: Patients [$N = 75$; age 40 ± 14 years (mean $\pm$ SD), 63% female] with peripheral (45%), abdominal (37%), facial (11%), or oropharyngeal-laryngeal (7%) angioedema attacks were randomised (44 rhC1INH; 31 saline). Based on the TEQ, median (95% CI) time to onset of symptom relief at the primary attack location was 90 min (61, 150) in rhC1INH-treated vs 152 min (93, not calculable) in saline-treated patients ($P = 0.031$). As assessed by a persistent decrease $\geq$20 mm on the VAS, median time to onset of symptom relief was 75 min (60 105) for rhC1INH-treated vs 303 min (81, 720) for saline-treated patients ($P = 0.003$). Median time to minimal symptoms (all VAS scores < 20 mm) was 240 min (177, 270) in rhC1INH-treated vs 362 min (240, not calculable) in saline-treated patients ($P = 0.005$). Overall, rhC1INH was safe and well tolerated. Adverse events (within 72 h of infusion) occurred in 7% of patients (4/56) who received rhC1INH (as randomised treatment or rescue medication) compared with 22% patients (4/18) who received saline. No thromboembolic events, anaphylaxis, or neutralising antibodies to C1INH were observed.

Conclusions: Consistent with previous studies, administration of rhC1INH resulted in significantly faster improvement of angioedema attacks in HAE patients compared with saline, with an overall positive safety profile.

1190
Hereditary angioedema in the emergency department – time to drug administration

Branco Ferreira, M[1,2]; Paulos Viegas, L[1]; Spínola Santos, A[1]; Pereira Barbosa, M[1,2]
[1]Immunoallergology, Hospital Santa Maria, Lisbon, Portugal; [2]Clinical Immunology, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

Background: Hereditary Angioedema (HAE) is a rare, autosomal-dominant disorder, characterised by recurrent episodes of cutaneous angioedema, abdominal pain or laryngeal edema. These episodes can be life-threatening and are medical emergencies requiring prompt and effective therapy.

We aimed to evaluate, in known HAE patients presenting with severe episodes, the time delay between emergency department (ED) admission and injection of effective therapy (icatibant or C1 inhibitor concentrate (C1INH)).

Method: Retrospective review of our Hospital's ED clinical records of known HAE adult patients admitted in our hospital's ED with a severe HAE episode between November/2009 and May/2011 and who received treatment with icatibant or C1INH. All patients had with themselves at all times a patient's card identifying both the disease and the most adequate therapies. To be included in the study, patient's clinical records had to clearly indicate the location and characteristics of the attack as well as the time of ED admission and time of administration of icatibant or C1INH. We analyzed demographic and clinical data as well as time of admission and time of drug injection.

Results: In this 18 months' period 20 patients received icatibant or C1INH for a severe HAE attack in our hospital's ED. Mean delay from admission to injection was 2.74 ± 2.87 h [median 2.06; (0.25–10.67)]. Icatibant administration had a mean delay of 2.3 h vs 3.2 h in C1INH ($P = 0.161$). Abdominal attacks had a statistically significant greater delay: 4.55 ± 3.65 h in abdominal attacks ($n = 8$) vs 1.98 ± 1.63 h in pharyngo-laryngeal attacks ($n = 7$) vs 0.91 ± 0.35 h in attacks involving the face ($n = 5$). Gender, age and HAE type (I or II) did not present significant differences in the time delay before icatibant or C1INH administration.

Conclusion: Even in already diagnosed patients and even in patients with a hospital card identifying the disease and the best therapeutic options there is considerable in-hospital delay in the ED before effective therapy is administered, especially when patients present abdominal attacks. Our data reinforces the usefulness of home treatment schedules, in order to reduce the unnecessary suffering of many HAE patients during a severe attack.

1191
Retrospective evaluation of surgical operations in patients with hereditary angioedema

Baysan, A; Gulec, M; Kartal, O; Yesillik, S; Demirel, F; Musabak, U; Sener, O
Division of Immunology and Allergic Diseases, Department of Internal Medicine, Gulhane Military Medical School, Ankara, Turkey

Introduction: Undiagnosed patients with hereditary angioedema (HAE) may undergo unnecessary operations due to gastrointestinal system (GIS) attacks. Moreover, any operation performed with general anesthesia may trigger attacks in these patients. In our study, we aim to determine the frequency of unnecessary operations and laryngeal attacks occurred after intubations.

Method: Standardised questionnaires are administered via either by phone or face-to-face interview to HAE patients, who are followed by our clinic.

Results: Thirty-three operations were analyzed. Ten of these were done before diagnosis of HAE and due to possible misdiagnosis of GIS attack as acute abdomen (seven appendectomy, two laparotomy, one cholecystectomy). Six of 33 operations were performed in diagnosed HAE patients; two of six were with long-term danazol prophylaxis only, other two were with long-term danazol prophylaxis plus short-term prophylaxis with C1 inhibitor concentrate, one was with short-term C1 inhibitor prophylaxis only and the last one was without prophylaxis. No attacks were occurred any of these patients. Laryngeal attacks occurred in four of 27 operations of undiagnosed HAE patients in 6 h postoperatively and recovered spontaneously.

Discussion: Frequency of unnecessary operations is so high among patients with HAE. Although laryngeal attack frequency due to general anesthesia is not high, short-term prophylaxis should be given preoperatively to all HAE patients, as they are at risk of developing life-threatening laryngeal attacks. Early diagnosis and increasing awareness of HAE will result decreased rate of unnecessary operations and also decreased risk of laryngeal attacks that can be prevented by short-term prophylaxis.

1192
Hereditary angioedema type III or without deficiency of C1INH. Clinical characteristics of six families

García-Moral, A; Sala-Cunill, A; Luengo, O; Labrador-Horrillo, M; Cardona, V; Guilarte, M
Allergy Section, Internal Medicine, Hospital Vall d' Hebron, Barcelona, Spain

Background: Hereditary angioedema (HAE) type III, or without C1INH deficiency, is a recently described, rare entity that is often underdiagnosed. It may be classified in two subtypes depending on the presence of F12 gene mutations (HAE-FXII and HAE-unknown). The aim of the

study was to describe the clinical characteristics of patients with HAE type III.
Methods: Retrospective study of clinical characteristics and triggers of angioedema attacks in patients with HAE type III. Index cases were included and a family study was performed in all the relatives that could be recruited, including F12 gene mutations.
Results: Six women with a median age of 30 (20–43 years) were included as index cases; the family study increased the number to 18 diagnosed individuals (15 woman/three men). F12 gene mutations were identified in all of them.

C1INH levels and function were normal in 17/18 (C1INH function was 60% in one patient). Thirty-eight percent of patients, three men and four women, were asymptomatic. The majority of the patients (88%: 16/18) suffered an angioedema (AE) attack related to hyperestrogenic situations. One patient had an attack after a trauma and the other one had an attack without any identified trigger. All symptomatic patients had developed AE when they were on oral contraceptives. Of those patients that had been pregnant, only 3/10 (33%) had attacks during pregnancy. ACE-inhibitors were involved in two AE attacks (one coinciding with a dental extraction). Regarding the localisation, most attacks were located in face and lips, 3/11 patients had an upper airway attack and three had an abdominal attack.

Two attacks were treated with tranexamic acid and one with plasma-derived C1INH with partial response. Two patients received icatibant for the treatment of a face and laryngeal attack respectively, with onset of relief of symptoms in <1 h and complete remission of symptoms in 6 h.
Conclusion: In our population, F12 gene mutations were detected in all patients and hyperestrogenic states, such as the use of contraceptive treatments or pregnancy, were the precipitating factor in most cases.

1193
Lack of evidence for increased thrombotic risk with high doses of C1-esterase inhibitor concentrate in a non-clinical model of venous stasis and arterial thrombosis

Herzog, E; Schuermann, D; Raquet, E; Zollner, S; Dickneite, G; Pragst, I
CSL Behring GmbH, Preclinical Research & Development, Marburg, Germany

Background: It has been suggested that administration of C1-esterase inhibitor (C1-INH) concentrate may be associated with an increased risk for thromboembolic complications, but the supporting data available are limited and conflicting.
Method: The pharmacodynamic activity and thrombotic safety of plasma-derived, C1-INH concentrate (Berinert®, CSL Behring, Marburg, Germany) was assessed following intravenous (IV) application to rabbits. Non-clinical investigations included activity measurements of C1-INH, coagulation factors XI (FXI) and XII (FXII), evaluation of thrombus formation after induction of venous stasis and arterial thrombosis, and monitoring of surrogate markers of prothrombotic risk, i.e. thromboelastographic parameters, thrombin generation, platelet aggregation, activated partial thromboplastin time (aPTT), prothrombin time (PT), thrombin-antithrombin (TAT) complexes, and prothrombin fragments F1 + F2.
Results: The IV administration of up to 800 IU/kg C1-INH concentrate resulted in a dose-dependent inhibition of C1-esterase, FXI and FXII. While in the venous stasis model, C1-INH concentrate did not induce or increase thrombus formation, an inhibition of arterial occlusion was observed compared to placebo treatment following induction of arterial thrombosis. This observation was corroborated by increased aPTT, decreased thrombin generation, inhibition of platelet aggregation, and absence of TAT or F1 + F2 fragments.
Conclusion: High doses of C1-INH concentrate (up to 800 IU/kg) were associated with antithrombotic effects in a non-clinical model of arterial thrombosis and did not increase the prothrombotic risk in an established animal model of venous stasis.

1194
How does life change in patients with hereditary angioedema after approval of icatibant for self administration

Wiednig, M
Dermatology, Medical University of Graz, Graz, Austria

Background: The hereditary angioedema (HAE) is a rare, lifethreatening disease that affects 1 of 10 000 to 1 of 50 000 individuals due to deficiency of C1-Inhibitor. It is characterised by unpredictable, recurrend subcutaneous or submucosal swellings involving the skin, upper respiratory tract and gastrointestinal tract. Beside an autosomal dominant disorder spontaneous mutations were seen in 20%. Until 2009 treatment of acute attacks was done with plasmaderived C1-Inhibitor (pd-C1-Inh) concentrate had to be administered intravenously and required the help of HCP for administration. Then treatment was revolutionized by the approval of Icatibant, a bradykinin-B2-receptor antagonist for subcutaneous treatment of acute attacks. Since April 2011 Icatibant is approved for selfadministration in Austria.
Method: We report the course of disease in two of our patients before and after the possibility of selftreatment. Both were suffering from very frequent attacks. Both female patients are suffering from HAE type 1. Both experience very frequent attacks. The intensity ranges from moderate to severe. Mostly they have subcutaneous and abdominal swellings, whereas the abdominal pain is severe in most cases and accompanied by vomiting, diarrhea and circulatory collaps. Both have very severe venous access problem. Before Icatibant was available most attacks remained untreated. Severe attacks restricting their daily life and work occurred frequently. Since Icatibant was approved for selfadministration more attacks were treated and no severe attacks could develop.
Results: Quality of life improved in both patients because they are able to travel, doesn't miss working days, no intravenous injection is required, no fear a HCP is out of reach. Treatment is available in every situation of life now. Side effects comprised only mild erythema which resolved without any treatment.
Conclusion: The possibility for selfadministration results in remarkable decreased number of severe attacks which leads to improved quality of life. And it is also an important economic factor that patients have less missing days at work.

1195
Hydatidosis as cause of anaphylaxis and recurrent urticaria and angioedema

Raducan, I; Martorell, C; Ortega, S; Morales, C; Peláez, A
Hospital Clinico Valencia, Alergia, Valencia, Spain

Background: In 2012 a 56-year-old female patient had an episode of palmar-plantar itching followed by generalised urticaria, facial and lingual angiodema, tachycardia and dyspnoea. The clinical case was solved in a matter of hours applying the treatment established at the Emergency Department (adrenaline, corticoids and antihistamines). During the last 10 years the patient had suffered six episodes of urticaria and facial-lingual angiodema which subsided in a matter of hours after administering corticoids and antihistamines. No relation to drugs, food or other factors. None of the episodes had coincided with consumption of fish. Personal history: surgical intervention on hepatic hydatid cysts 22 years ago.
Method: The patient underwent the anaphylaxis diagnostic protocol: thorough physical examination, complete

blood-count, biochemistry, serum tryptase, stool examination for parasites, skin prick tests with aeroallergens, foods and *Anisakis*. total serum IgE, specific IgE to *Ascaris*, *Echinoccus* and those allergens that were positive in the skin tests (ImmunoCAP ThermoScientific) were quantified. Thoracoabdominal CT scan was carried out in view of hepatic hydatidosis history.

Results: Physical examination was normal. Blood count – 380 eosinophils/ml, biochemistry and serum tryptase were normal, stool examination for parasites was negative. Skin tests with aeroallergens and foods – negative. Skin tests with Anisakis +++. Total serum IgE- 4934 kUA/I. Specific IgE (KUA/I) to Echinnoccus – 86, Anisakis – 0.8, Ascaris – 1.6. Thoracoabdominal CAT scan – hepatomegaly, presence of three cystic lesions in right hepatic lobe parenchyma, the largest of which is 57 × 62 × 62 mm in segment IV B associating images compatible with daughter cysts on the inside.

Conclusion: Relapsing hydatidosis with urticaria/angiodema and anaphylaxis was diagnosed. The patient underwent surgical intervention successfully without any postoperative complications. Since surgery she has had no other episodes.

Sensitisation towards *Anisakis* and *Ascaris* was detected, which could be due to a possible cross reactivity with *Echinoccus*.

Although presently hydatidosis is not a frequent cause of anaphylaxis in our country, it must be considered in cases of recurring anaphylaxis/urticaria, especially in middle-aged patients if there has existed any risk of infestation or hydatidosis history.

Poster Session 46

Mechanisms of atopic dermatitis

1196
Toll-like receptor gene polymorphisms are associated with atopic dermatitis in Volga-Ural region of Russia

Gimalova, G[1]; Prans, E[2]; Karunas, A[1]; Fedorova, Y[1]; Gumennaya, E[3]; Levashova, S[4]; Etkina, E[4]; Kõks, S[2,5]; Khusnutdinova, E[1,6]
[1]Institute of Biochemistry and Genetics, Ufa Science Centre, Russian Academy of Sciences, Human Molecular Genetics, Ufa, Russia; [2]Department of Physiology, University of Tartu, Tartu, Estonia; [3]Dermatovenerologic Dispensary of Bashkortostan Republic, Ufa, Russia; [4]Bashkir State Medical University, Ufa, Russia; [5]Centre of Molecular and Clinical Medicine, University of Tartu, Tartu, Estonia; [6]Department of Biology, Bashkir State University, Ufa, Russia

Background: Atopic dermatitis (AD) is the common chronic inflammatory disorder with cutaneous hyperreactivity to environmental triggers. It is known that pattern-recognition receptors, namely toll-like receptors (TLR) play a crucial role in innate immunity. Recent studies have shown an association between TLR genes variations and allergic diseases development.
Method: We have screened four SNPs in TLR1 (rs4833095, rs5743604, rs5743571, rs2101521) and six SNPs in TLR10 (rs4331786, rs4129009, rs11096957, rs11466617, rs10004195, rs4543123) genes, both map closely together on chromosome 4. The AD group consisted of 335 AD patients from Volga-Ural region of Russia (104 Russians, 62 Tatars, 10 Bashkirs and 159 individuals of mixed origin). The control group included 330 non-atopic individuals (91 Russians, 75 Tatars, 37 Bashkirs and 127 individuals of mixed origin). Genomic DNA was isolated by phenol-chloroform extraction. The genotyping of SNPs was performed by real-time PCR.
Results: The AD patients of total group have significantly higher TLR1 gene polymorphisms alleles rs5743604*A and rs5743571*C frequencies when compared with control group ($P = 0.022$, $P = 0.0008$ respectively). The frequencies of the TLR10 gene variant alleles rs10004195*T and rs4543123*A were also significantly higher in AD patients than in healthy donors ($P = 0.0325$ and $P = 0.0426$). The haplotype analysis revealed statistically significant difference in frequencies of TTATAC haplotype (rs11466617-rs10004195-rs4543123-rs4833095-rs5743604-rs5743571) between patients and controls (72% and 64% respectively; $P = 0.0124$). Besides, AD patients have significantly lower TAGCGT haplotype frequency ($P = 0.0062$) compared with controls.

Individuals with AD of Russian ethnic origin have significantly higher frequency of TLR1 gene polymorphism allele rs5743571*C comparing with controls ($P = 0.0004$). Besides, AD patients have higher frequency of rs11466617*T of TLR10 gene ($P = 0.046$). The haplotype analysis revealed the most prevalent haplotype TTATACG with frequency significantly higher in AD patients than in controls (78% and 66% respectively, $P = 0.0141$). The AD patients have also significantly higher TAGCGCA and lower TAGCGTA haplotype frequencies ($P = 0.004$ and $P = 0.0204$, respectively). There weren't any differences between AD patients and controls of Tatar and Bashkir ethnic origin found.
Conclusion: The results of our investigation show that TLR1 and TLR10 genes polymorphisms are important risk factors of atopic dermatitis in the Volga-Ural region of Russia.

1197
Cytokine status in children with different types of immune responses in atopic dermatitis

Vorozhko, IV; Sentsova, TB; Revyakina, VA; Denisova, SN; Monosova, OY; Kirillova, OO; Timopheeva, AG
Allergy Department, Research Institute of Nutrition RAMS, Moscow, Russia

Background: To estimate the process of immunoregulation in infants aged 0–36 months with atopic dermatitis (AD) we have examined serum levels of cytokines and selectines in 89 infants with AD of different severity.
Method: The serum concentration of total IgE was determined by the immunoenzyme method. For quantitative determination of the allergen-specific IgE and IgG to cow milk protein the non-concurrent immunoenzyme analysis was used. The serum levels of IFN-γ, IL-2, IL-4, IL-5, IL-12, IL-13 was determined by the ELISA – enzym-linked immunosorbent assay.
Results: This study showed the role of cellular immunity in the development of allergic inflammation and humoral specific response with significant role of the local immune response, in AD infants with predominantly IgE response. In patients with mixed type of immune response (IgE + IgG) we revealed increased serum concentration of IL-4, IL-5, IL-12 and IL-13 compared with control group. We revealed high levels of leukocyte migration factors: ICAM-1 ($P < 0.05$) and E-selectine. The trend to decrease level of IL-2 and increase levels of IFN-γ and TNFα in patients with mixed type of the specific immune response was revealed. The level of IL-5 was significantly higher in patients with mixed type of the immune response compared to other patients. In patients with isolated IgG immune response the level of IL-12 was higher compared to infants with lack of specific response. In patients with isolated IgE-specifis immune response the level of TNFα was significantly higher compared to infants with other types of immune responses.
Conclusion: Obtained data of the interrelation of cytokines, molecules of the intercellular adhesion in infants with AD showed the participation of allergenspecific IgE and IgG antibodies in the pathogenesis of AD with equal activity of non-specific immune inflammation and objective signs of the disease.

1198
Serum levels of total IgE correlate with sCD25, sCD30, SCORAD and age of children with atopic dermatitis

Rosińska-Więckowicz, AM[1]; Więckowicz, M[2]; Czarnecka-Operacz, M[1]
[1]Department of Dermatology, Poznań University of Medical Sciences, Poznań, Poland; [2]Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences, Poznań, Poland

Background: Elevated serum level of total IgE (tIgE) is considered one of Hannifin and Rajka's criteria for atopic dermatitis (AD). Previous studies suggested that evaluation of serum levels of tIgE may correlate with disease extent and severity in patients with atopic dermatitis (AD). Our aim was to investigate the relationship between evaluated tIgE levels and soluble forms of other immune parameters, consid-

ered to be useful in evaluation of disease extent and severity of patients with AD.
Method: We evaluated 102 patients with AD: 77 children (mean age 5.1 ± 4.1 years) and 25 adults (mean age 23.8 ± 4.4 years). Disease extent and severity in the group of adults (A-AD) and children with AD (CH-AD) was measured with SCORAD index. Serum levels of tIgE were determined using ImmunoCAP. Serum levels of the soluble form of CD25 (sCD25) and soluble form of CD30 (sCD30) were determined in both investigated groups using ELISA. The statistical analysis was performed using STATISTICA version 8.0 (Stat Soft Inc. 2007).
Results: In the CH-AD group statistically significant, positive correlation was observed between serum levels of tIgE and age (Rs 0.265, $P = 0.020$), as well as SCORAD values (Rs 0.659, $P = 0$). In A-AD group correlation of serum levels of tIgE with age was insignificant (Rs −0.112, $P = 0.591$), however significant positive correlation between serum levels of tIgE and SCORAD was observed (Rs 0.445, $P = 0.025$). Moreover, in the CH-AD group serum levels of tIgE correlated inversely both with serum levels of sCD25 (Rs −0.273, $P = 0.016$), as well as sCD30 (Rs −0.304, $P = 0.007$). In the A-AD group mutual correlations between serum levels of tIgE and sCD25 and sCD30 were insignificant (respectively: Rs −0.301, $P = 767$ vs Rs 0.428, $P = 0.052$).
Conclusion: Although recently emphasis is put on novel markers used to monitor activation of the immune system in patients with AD, in our opinion evaluation of serum levels of tIgE remains the most useful marker of disease severity both in adult patients, as well as children with AD. Moreover, positive correlation between serum levels of tIgE and age, observed in children with AD, may be considered a good marker of the progressive allergisation of the immune system, which translates into increased level of disease extent and severity in patients with AD.

1199
The potential role of $TRPV_1$ in the induction of substance P-expression in murine dermal neurons after allergic skin inflammation

Sander, S[1]; Heck, S[2]; Werfel, T[3]; Le, D-D[2]; Al Kadah, B[4]; Bals, R[2]; Welte, T[5]; Fischer, A[1]; Dinh, QT[2]
[1]Clinical Unit of Allergy, Department of Pediatric Pneumology and Immunology, Medical University Berlin, Charité – Campus Virchow, Berlin, Germany; [2]Pneumology and Experimental Pneumology and Allergology, Saarland University Medical Center and Saarland University Faculty of Medicine, Homburg, Germany; [3]Medizinische Hochschule, Immundermatologie und Experimental Allergologie, Hannover, Germany; [4]Department of Otorhinolaryngology, Saarland University Medical Center, Homburg, Germany; [5]Respiratory Medicine, Centre for Internal Medicine, Hannover Medical School, Hannover, Germany

Background: The neuropeptide substance P, which is a member of the tachykinine family, plays an important role in the modulation of allergic skin inflammation. However, the regulatory mechanisms of substance P-expression and -release are unknown. Because of the neurogenic inflammation reaction caused by the lipophilic alkaloid capsaicin, which binds to $TRPV_1$-receptor, and the co-localisation of substance P and $TRPV_1$, there is evidence, that $TRPV_1$ plays a triggering role in the expression level of substance P.
Methods: We studied the influence of $TRPV_1$ on the expression level of substance P in murine model of allergic skin inflammation. $TRPV1^{-/-}$ mice were treated with ovalbumine (OVA) by using intraperitoneal injection for allergic sensitisation on days 1, 7, 21 and subcutane scapular allergic provocation with OVA on day 27. Control mice received phosphate buffer saline (PBS) and OVA provocation. Skin tissues and dorsal root ganglia (Th1-5) were stained with hematoxylin/eosin and double-fluorescence immunohistochemistry for PGP 9.5 and substance P. Dermal neurons were identified by neuronal tracing using the dye hydroxystilbamidine (Fluoro-Gold™) for retrograde-labeling.
Results: Staining with hematoxylin/eosin showed no significant increase of inflammatory cells in skin tissues of OVA-sensitised/-provocated and PBS-sensitised and OVA-provocated animals. There was no significant difference in substance P-positive, retrogradely-labeled neurons of OVA-sensitised/-provocated $TRPV_1$-knockout mice if compared to the (PBS-treated) control $TRPV_1$-knockouts (*t*-test: $P = 0.24$; OVA: 12.28 ± 1.64%, PBS: 10.11 ± 0.42%). In contrast to wild-type control mice (data not shown), there are no changes in the substance P-expression in overall neurons of $TRPV_1$-knockout mice after OVA-sensitisation and provocation in comparison to the $TRPV_1$-knockout controls (*t*-test: $P = 0.79$; OVA: 7.4 ± 0.28% and PBS: 7.3 ± 0.15%).
Conclusion: The steady level of substance P-expression in $TRPV_1$-knockout mice after OVA-sensitisation/-provocation suggests a triggering role of $TRPV_1$ on the induction of substance P-biosynthesis in dermal neurons during allergic skin inflammation.

1200
Comparison of selected clinical and immunological parameters in children and adults with atopic dermatitis

Rosińska-Więckowicz, AM[1]; Więckowicz, M[2]; Czarnecka-Operacz, M[1]
[1]Department of Dermatology, Poznań University of Medical Sciences, Poznań, Poland; [2]Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences, Poznań, Poland

Background: Atopic dermatitis (AD) is a chronic and relapsing skin disease, which usually appears in infancy, but may persist to adulthood. Our aim was to investigate the differences in the course of AD in children and in adults on the basis of selected clinical and immune parameters.
Method: One hundred and two patients with AD: 77 children (mean age 5.1 ± 4.1 years) and 25 adults (mean age 23.8 ± 4.4 years). Disease extent and severity in adults (A-AD) and children with AD (CH-AD) was measured with SCORAD index. Serum levels of total IgE (tIgE), soluble form of CD25 (sCD25) and soluble form of CD30 (sCD30) as well as serum levels of interleukin 13 (IL-13) were determined in both investigated groups using ELISA. Statistical analysis was performed using STATISTICA version 8.0 (Stat Soft Inc. 2007).
Results: Serum levels of tIgE were significantly higher in A-AD group in comparison with CH-AD group ($P = 0.006$). There was no significant difference in SCORAD values between the two investigated groups ($P = 0.108$). Serum levels of sCD25 and sCD30 were significantly higher in CH-AD group in comparison with A-AD group (respectively $P = 0$ and $P = 0$). In both investigated groups, serum levels of IL-13 were lower than the threshold level indicated by the kit manufacturer.
Conclusion: According to our expectations, serum levels of tIgE were significantly higher in adults than in children with AD, which may be related with progressive allergisation to external allergens during the lifetime. Moreover, we expected to observe significantly higher values of SCORAD in A-AD group compared to CH-AD group, as clinical signs of chronic inflammatory state in the skin in adults with AD influence the final score of SCORAD index, however such relationship was not observed. Although some authors consider IL-13 to be a useful marker of chronic inflammation in skin in AD patients, this was not observed in our group of AD patients. Significantly higher serum levels of sCD25 and sCD30 in CH-AD group may be related with more intense response of the immature immune system in children, however further studies are necessary.

1202
Correlation between serum 25-hydroxyvitamin D levels and severity of atopic dermatitis in pediatrics

Kim, SW; Sung, SM
St. Mary's Medical Center, Pediatrics, Busan, Korea

Background: Many studies have been the association between vitamin D and asthma, atopic dermatitis, allergic disease; but, is ambiguous and unclear. In this study, the associations of 25-hydroxy vitamin D levels with atopic dermatitis and with allergic lab finding were evaluated.
Method: The study was conducted on 159 atopic dermatis children ageded 1–18 years. Using the SCORAD index, we evaluated the severity of disease and Serum 25-hydroxy vitamin D3 were determined. The relationship between serum vitamin D levels, SCORAD index, ECP, eosinophil count and total IgE were examined in atopic dermatitis children.
Results: We found severe, moderate and mild AD in 43 (27%), 59 (37%) and 57 (36%) patients, respectively. In atopic dermatitis childrens, 25-hydroxy vitamin D levels had direct and significant correlations with both SCORAD index ($P = 0.001$) and eosinophil counts. ($P = 0.004$) There were no associations between vitamin D levels and total IgE, ECP, sex, age. ($P > 0.05$).
Conclusion: These data showed that serum 25-hydoxy vitamin D levels were inversely associated with atopic dermatitis and suggest that vitamin D deficiency may related to the severity of atopic dermatitis.

1203
Bronchial hyperresponsiveness to methacholine in children with atopic dermatitis

Kim, S; Park, G
Busan St. Mary's Medical Center, Busan, Korea

Background: The methacholine challenge test (MCT) is commonly used to assess abronchial hyperresponsiveness (BHR). Many studies have shown the importance for BHR in children with bronchial asthma, but BHR in children with atopic dermatitis (AD) is poorly defined. The aim of this study is to identify BHR in children with AD and investigate the relationship between degree of BHR and severity of AD.
Method: MCT were performed in 105 children with atopic dermatitis (mean age, 12.08 years). Children with history of bronchial asthma were excluded. SCORAD score, MCT results, skin prick test results, and blood tests (total IgE, eosinophils count, neutrophil count, ECP) were analyzed. Positive MCT is defines as PC20 (the provocative concentration of methacholine that results in a 20% drop in FEV1) ≤8 mg/ml.
Results: Positive MCT was observed in 39 (38.6%) children. Children with positive MCT demonstrated significantly higher levels of eosinophil count compared with the children with negative MCT (605.64 ± 56.79 vs 450.32 ± 32.21 K/μl, $P = 0.012$). No significant differences were observed in SCORAD score, total IgE, and ECP, house dust mite (HDM) sensitisation. Significant association was found between severity of BHR and neutrophil count ($r = 0.497$, $P = 0.001$). But such relationship was not observed for SCORAD score, total IgE, and ECP.
Conclusion: BHR to methacholine was detected in children with AD. No significant relationship was observed between degree of BHR and severity of AD.

1204
Increased serum periostin levels in children with atopic eczema

Baek, H-S[1]; Lee, S-Y[2]; Lee, H-R[2]; Lee, H-B[3]; Oh, J-W[3]; Izuhara, K[4]
[1]Hallym University College of Medicine, Seoul, Korea; [2]Department of Pediatrics, Hallym University College of Medicine, Anyang, Korea; [3]Hanyang University College of Medicine, Seoul, Korea; [4]Saga Medical School, Saga, Japan

Background: Recently it has been found that periostin, a matricellular protein, sets up a vicious circle that links Th_2-type immune responses to keratinocyte activation and plays a critical role in the amplification and chronicity of allergic skin inflammation. The aim of this study was to investigate the relationship between periostin levels and the presence of atopy.
Methods: Seventy atopic dermatitis (AD) children aged 1 month to 10 years were included in our study. Subjects were characterised as having atopic eczema (AE; $n = 55$) or non-atopic eczema (NAE; $n = 15$). Serum periostin levels, blood eosinophil counts, eosinophilic cationic protein (ECP), serum total and specific immunoglobulin E (IgE) levels were measured. Serum specific IgE were measured with the Immuno-CAP system and microarrays (ISAC®). Atopy was defined as the presence of at least one positive allergen-specific IgE test result (IgE ≥ 0.35 kU/l) or positive skin prick test finding.
Results: Serum periostin levels were significantly higher in children with AE than in children with NAE (82.98 ± 22.35 vs 68.40 ± 14.13 ng/ml; $P = 0.020$). However, serum periostin levels in children with atopic dermatitis were not significantly correlated with disease severity, blood eosinophil counts, ECP and serum total IgE levels.
Conclusions: Serum periostin levels were increased in children with AE. It is suggested that periostin may play a role in the pathogenesis of AD by mechanisms related to atopy.

1205
Staphylococcus aureus colonisation on cheeks at 6 months of age is associated with atopic dermatitis and egg white sensitisation at 1 year of age: a birth cohort study in Chiba, Japan

Shimojo, N[1]; Ozawa, N[1]; Nakano, T[1]; Morita, Y[1]; Inoue, Y[1]; Arima, T[1]; Takemori, T[2]; Kohno, Y[1]
[1]Graduate School of Medicine, Chiba University, Pediatrics, Chiba, Japan; [2]RIKEN Research Center for Allergy and Immunology, Yokohama, Japan

Background: It is well known that skin colonization of *Staphylococcus aureus* is related with severity of atopic dermatitis in adults and children. However, it is not clear whether colonization of *Staphylococcus aureus* is related with development of atopic dermatitis as well as allergen sensitisation.
Method: In a birth cohort that recruited 306 mother-child pairs in Chiba city, Japan, we checked presence or absence of *Staphylococcus aureus* on the cheeks of babies at 1 and 6 months of age by means of stamp culture method. Atopic dermatitis was diagnosed at 1 year of age by examination by pediatric allergists based on Japanese guideline for atopic dermatitis. Also at 1 year of age serum specific IgEs (sIgEs) to house dust mite, Japanese cedar pollen, cat dander, cow's milk and egg white were measured by ImmunoCap.
Results: 13.2% of babies had atopic dermatitis and 39.1% were sensitised by at least one allergen (sIgE >0.7U/ml) at 1 year of age. Among five allergens examined egg white showed highest rate (32.2%). *Staphylococcus aureus* was detected in 43.8% at 1 month of age and 48.1% at 6 months of age, respectively. There was no difference in prevalence of atopic dermatitis at 1 year of age between *Staphylococcus aureus*-positive and negative groups at 1 month of age (11.7% and 14.4%, respectively). In contrast, prevalence of atopic dermatitis was significantly higher in *Staphylococcus aureus*-positive group (21.2%) than that in *Staphylococcus aureus*-negative group (5.7%) at 6 months of age ($P < 0.001$). Multivariate logistic regression analysis revealed colonization of *Staphylococcus aureus* at 6 months of age but not at 1 month of age is significantly associated with atopic dermatitis and egg

white sensitisation at 1 year of age ($P = 0.002$ and $P = 0.047$, respectively).

Conclusion: *Staphylococcus aureus* colonization at 6 months of age is an independent risk factor for atopic dermatitis and egg white sensitisation at 1 year of age. Whether *Staphylococcus aureus* colonization causes allergic diseases in infancy needs interventional study.

1206
Serum levels of sCD25 and sCD30 do not correlate with disease extent and severity in children and adults with atopic dermatitis

Rosińska-Więckowicz, AM[1]; Więckowicz, M[2]; Czarnecka-Operacz, M[1]
[1]Department of Dermatology, Poznań University of Medical Sciences, Poznań, Poland; [2]Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences, Poznań, Poland

Background: Previous studies suggested that evaluation of serum levels of the soluble form of CD25 (sCD25) and CD30 (sCD30) may be considered as a reliable marker of atopic dermatitis (AD) severity. Some authors proved, these markers correlate positively with disease extent and severity measured with SCORAD or EASI index. We investigated the relationship between evaluated SCORAD scores and serum levels of sCD25 and sCD30, as well as mutual correlations between aforementioned immune markers.

Method: One hundred and two patients with AD: 77 children (mean age 5.1 ± 4.1 years) and 25 adults (mean age 23.8 ± 4.4 years). Disease extent and severity in the group of adults (A-AD) and children with AD (CH-AD) was measured with SCORAD index. Serum levels of the soluble form of CD25 (sCD25) and soluble form of CD30 were determined in both investigated groups using ELISA. The statistical analysis was performed using STATISTICA version 8.0 (Stat Soft Inc. 2007).

Results: Statistical analysis of the data did not show any significant correlation between SCORAD and serum levels of sCD30 both in the A-AD group (Rs −0.129, $P = 0.538$), as well as in the CH-AD group (Rs −0.198, $P = 0.083$). Similarly, no correlation was found between serum levels of sCD25 and SCORAD in the two investigated groups of patients (A-AD: Rs −0.355, $P = 0.081$, CH-AD: Rs −0.111, $P = 0.334$). Moreover, a negative correlation was observed, between patients age and serum levels of sCD30 (A-AD: Rs −0.506, $P = 0.009$, CH-AD: Rs −0.712, $P = 0$). In the A-AD group no significant correlation between serum levels of sCD25 and patients age (Rs −0.333, $P = 0.103$) was observed, while in the CH-AD group significant negative correlation was observed (Rs −0.573, $P = 0$). Positive correlation was observed exclusively between serum levels of sCD25 and sCD30 both in adults (Rs 0.511, $P = 0.008$) and children with AD (Rs 0.667, $P = 0$).

Conclusion: In our opinion, serum levels of sCD25 and sCD30 are not unambiguously useful as markers of disease severity both in adult patients, as well as children with AD. Despite our expectations and data presented in our previous studies, sCD25 and sCD30 were not helpful either to monitor or predict disease severity with regard to patients age. Discrepancies between our data and previous studies may result from decreased number of patients in the previously investigated population, while in our presented study, as much as 102 patients with AD were included. Further studies are necessary in order to obtain explicit results.

1207
House dust mite sensitivity determines a distinct subtype in atopic dermatitis

Baris, S[1]; Ozen, A[1]; Akdeniz, T[2]; Aydin, O[3]; Karakoc-Aydiner, E[1]; Saricoban, HE[4]; Kıykım, A[1]; Cagan, HH[1]; Camcioglu, Y[5]; Cengizlier, R[6]; Demirkesen, C[3]; Yucelten, D[7]; Yanıkkya, GD[2]; Barlan, I[1]
[1]Pediatric Allergy-Immunology, Marmara University, Istanbul, Turkey; [2]Immunology, Yeditepe University, Istanbul, Turkey; [3]Pathology, Istanbul University, Istanbul, Turkey; [4]Pediatric Allergy-Immunology, Sureyyapasa Chest Diseases and Chest Surgery Training Hospital, Istanbul, Turkey; [5]Pediatric Allergy-Immunology, Istanbul University, Istanbul, Turkey; [6]Pediatric Allergy-Immunology, Yeditepe University, Istanbul, Turkey; [7]Dermatology, Marmara University, Istanbul, Turkey

Background: Atopic dermatitis (AD) is a heterogeneous disease with regard to clinical phenotype and natural history. We investigated T cell subtypes and cytokine responses in the peripheral blood of various AD subgroups and their skin biopsies.

Methods: A total of 50 subjects (30M, 20F) were studied. Immunologic studies were performed in 29 subjects: nine HDM-sensitised; six other sensitisations; seven non-allergic AD and five healthy controls. Skin biopsy samples were evaluated by immunohistochemistry in 16 subjects.

Results: The mean age of study participants was 8.93 ± 5.17 years (min = 1 years, max = 24 years). Th17 cell percentage was increased in all AD groups compared to healthy controls. HDM-allergic AD emerged as a distinct immunologic phenotype, with higher production of IL-2, IL-4, IL-5 both at rest and when stimulated by Der p-1 or SEB along with higher Th17 and CD4+ cell percentages, whereas, lower production of IL-17. AD with sensitisations other than HDM was mostly similar to non-allergic AD, with increased Th17 and CD4+CD69+IFN-g cell percentage. Overall, in the lesional skin expression of Foxp3+ and CD4+ was higher; and CD4+IFN-γ+ cells lower ($P = 0.002$, $P = 0.04$, $P = 0.024$, respectively). HDM-allergic AD had lower IFN-γ and IFN-γ co-expressing CD8+ cells compared to AD with other sensitisations ($P = 0.026$ and $P = 0.042$, respectively). Paired comparison of lesional vs non-lesional skin among HDM-allergic AD revealed higher CD4+ cells, Foxp3 and T-bet ($P = 0.018$, $P = 0.018$, $P = 0.018$, respectively).

Conclusion: HDM-allergic AD is a distinct subtype with predominant Th2 skewing and higher Th17 cell percentage along with a depressed Th1 response which may have therapeutic implications.

1208
The intrinsic type of atopic dermatitis differs from the extrinsic type in high frequencies of nickel/cobalt allergy with high levels of circulating Th1 cells

Tokura, Y[1]; Yamaguchi, H[1]; Kabashima-Kubo, R[2]; Bito, T[3]; Sakabe, J-I[1]; Tatsuno, K[1]; Hirasawa, N[4]
[1]Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan; [2]Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu, Japan; [3]Department of Dermatology, Kobe University Graduate School of Medicine, Kobe, Japan; [4]Department of Cellular Signaling, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan

Background: Atopic dermatitis (AD) can be divided into the extrinsic and intrinsic types. While extrinsic AD shows high serum IgE levels with specific IgE to some allergens, intrinsic AD has low serum IgE levels. The frequency of intrinsic AD is approximately 20%, and the female preponderance is clearly seen. We have reported that extrinsic AD patients have impaired barrier function, but intrinsic AD patients have normal barrier function. Intrinsic AD has much less filaggrin gene (*FLG*) mutations and increased frequency of circulating Th1 cells, implying that normal barrier-permeable, non-protein antigens, such as metals, may induce a Th1 response.

Method: To investigate metal allergy in intrinsic AD along with the background characteristics, enrolled in this study were 80 Japanese AD patients seen in three university hospitals, consisting of 51 extrinsic and 29 intrinsic AD patients. The patients' demographics were studied in various aspects. Patch testing was performed with three different patch test materials, focusing on nickle, cobalt, and chrome. In 17 AD patients (extrinsic, 12; intrinsic, 5), sweat was collected from the forearms by

exercise, and the amount of nickel in the sweat was measured.

Results: In the baseline features, seven of 21 extrinsic AD patients examined (33.3%) had one of the eight *FLG* mutations common to Japanese AD patients, whereas only one of 18 intrinsic AD patients had a mutation of *FLG* (5.6%). Prurigo-like lesions were more frequently observed in intrinsic AD. The percentage of interferon-gamma-producing Th1 cells was significantly higher in the peripheral blood of intrinsic than extrinsic AD. When analyzed collectively with the three materials, intrinsic AD showed significantly higher percentages of positive reactions than extrinsic AD to nickel (intrinsic 41.4% *vs* extrinsic 17.7%, $P = 0.033$) and cobalt (41.4% vs 11.8%, $P = 0.004$), but not chrome (24.1% vs 13.7%, $P = 0.359$). Taken together, the prevalence of metal allergy to one or more of the three metals was significantly higher in intrinsic AD than extrinsic AD ($P = 0.002$). The concentration of nickel was higher in the sweat of intrinsic AD than extrinsic AD patients (333.8 vs 89.4 ng/g, $P = 0.0005$).

Conclusion: Nickel and cobalt allergy may cause intrinsic AD. Given that the metals are excreted through sweat, the high amounts of nickel in the sweat might exaggerate intrinsic AD.

1209
Serum vitamin D concentration is associated with eczema risk and severity in Chinese children

Leung, TF[1]; Wang, SS[1]; Kong, APS[2]; Sy, HY[1]; Hon, KL[1]
[1]Department of Paediatrics, The Chinese University of Hong Kong, Hong Kong, Hong Kong; [2]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, Hong Kong

Background: Vitamin D was postulated to play crucial roles in many immunological diseases in recent years. Eczema is a chronic inflammatory disease of the skin. Vitamin D increased proliferation of type 2 T-helper cells that released interleukin-4 and interleukin-13 in eczema patients. An Italian study of 37 eczema children suggested an association between vitamin D deficiency and eczema severity. This study investigated the relationship between eczema diagnosis and severity and serum vitamin D levels in Hong Kong Chinese children.

Methods: Four hundred and ninety-nine Chinese children with eczema were recruited from both our hospital and local schools. Controls consisted of 328 non-allergic children. Their disease was diagnosed according to Hanifin and Rajka criteria. Eczema severity was evaluated by SCORAD (short-term) and NESS (long-term). Serum 25-hydroxyvitamin D concentrations were measured by enzyme-linked immunosorbent assay (IDS, Boldon, UK).

Results: Age of patients (10.5 ± 3.9 years) was significantly younger than that of controls (12.3 ± 4.1 years) ($P < 0.001$). There was no difference between percentage of males between cases (57.0%) and controls (57.5%) ($P = 0.943$). Vitamin D deficiency (<25 nM) was more common in eczema patients than controls (47.7% vs 26.8%; $P < 0.001$). The majority (61.0%) of controls had vitamin D level in the insufficiency group (20–50 nM). The rates for vitamin D sufficiency were similarly low in both eczema (11.4%) and control (12.2%) groups. Three hundred and six patients had data for SCORAD and 290 patients had NESS score. Inverse correlation was found between serum vitamin D levels and both short- and long-term eczema severity. The numbers of patients with SCORAD and the respective median levels of vitamin D were mild (104, 25.6), moderate (129, 20.3) and severe (73, 18.8) ($P < 0.001$). For NESS, the number of patients and the median levels of vitamin D were mild (71, 25.1), moderate (103, 22.7) and severe (116, 20.3) ($P = 0.004$).

Conclusions: Our study is novel in showing high rate of vitamin D deficiency in Chinese children. There is an inverse correlation between vitamin D level and eczema severity in our population. Considering the hypothesis that vitamin D is protective, oral supplementation may improve disease control in eczema patients. Molecular studies are needed to delineate how vitamin D as a hormone modulates eczema status.

Funding: Direct Grant for Research, CUHK.

1210
A study on causes of skin rashes in Iraqi patients

Al-Jebouri, MM
Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq

Background: A rash means some change has affected the skin. Rashes are generally caused by skin irritation which can have many causes. In some cases a rash does not go away or the skin may become so irritated that laboratory investigation and medical care are needed. The present study is a summary of our laboratory findings through 30 years on some causes of skin rashes among patients attended our laboratory.

Method: The following parameters were investigated for 500 patients complaining of skin rashes. These are fungi, bacteria, antinuclear factor, rheumatoid factor, cholestrol, complete blood counts including eosinophilia were carried out utilizing the conventional as well as the new manufactured kits internationally used. Hypertension was also recorded for the relevant patients.

Results: The causes of skin rashes tested were significantly different and the most common causes were bacteria and fungi. Bacterial infections were secondary to fungal incidences and vice versa was found. Autoimmune diseases were recorded particularly among females with low frequencies. Cholestrol and hypertention were also associated with skin rashes especially among peoples over 40 years old. Neutrophilia was mostly associated with bacterial infections and some allergens. Eosinophilia of unknown origin was seen.

Conclusion: A correlation between sex, age and rashes was reported. Fungal and bacterial infections were mostly interrelated with neutrophilia and eosinophilia. Lupus and rheumatoid factors were mostly associated with females of middle age. Cholestrol and hypertension were significantly associated and interrelated with skin rashes and few cases of neutropenia.

1211
Skin colonization with *Staphylococcus aureus* in patients with atopic dermatitis

Pezeshkpoor, F[1]; Miri, S[2]; Ghassemi, R[3]; Farid, R[4]; Ghenaat, J[5]
[1]Skin Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [2]Department of Laboratory Medicine, Harborview Medical Center, University of Washington Seattle, WA, United States; [3]Department of Allergy and Clinical Immunology, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran; [4]Allergy Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [5]Department of Microbiology, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran

Background: To investigate the presence of *S. aureus* in the skin of AD patients and compare with healthy control group.

Method: Forty patients with AD were recruited in to our study. *S. aureus* skin colonization was determined in AD patients and controls, also skin distribution of *S. aureus* colonization was compared in three age groups of AD patients.

Results: *S. aureus* was found on the skin of 42.5% and 7.5% of AD patients and control group, respectively ($P = 0.0003$). The most common involved skin areas with *S. aureus* colonization were face (in ≤2 years old), flexor surfaces (in >2 and ≤12 years old) and extremities (in >12 years old).

Conclusion: The incidence of *S. aureus* on the skin of AD patients was considerably higher rather than controls. Further studies are needed to investigate the clearance of *S. aureus* from the skin of AD patients using anti-staphylococcal treatment.

1212
Fullerene adducts attenuate delayed-type hypersensitivity reactions induced in mice

Bashkatova, E; Shershakova, N; Shilovski, I; Babakhin, A; Andreev, S; Khaitov, M
NRC Institute of Immunology FMBA, Moscow, Russia

Background: The purpose was to investigate the effects of water fullerene nanodispersion and fullerene amino derivatives (FDs) on delayed-type hypersensitivity (DTH) reaction and antigen-induced cytokine expression.

Method: A fullerene C_{60} nanodispersion (nC_{60}) and water-soluble FDs (nanoparticles) containing $C_{60}(Arg)_n$, $C_{60}(Lys)_n$ and $C_{60}(Piperazine)_n$ ($n = 3–5$) were prepared by the original method. The DTH reaction was induced in female BALB/c mice by subcutaneous injections (s.c.) with keyhole limpet hemocyanin (KLH) (100 μg/mouse) emulsified in complete Freund's adjuvant. On day 14 after s.c. injections mice were challenged with an intradermal (i.d.) injections of KLH in saline into one footpad while contra lateral footpad received i.d. of saline only. For comparison, FD samples (2 μg/mouse) were administered intravenously for 24 h prior to the KLH sensitisation and 24 h before the KLH challenge. The magnitude of the DTH reaction was determined by measuring of the footpad thickness prior and after KLH challenge using digital caliper. Cytokine expression was measured using ELISA in the supernatants of spleen cells stimulated with ConA and KLH collected on day 17 and 19 after KLH sensitisation, respectively.

Results: The treatment with $C_{60}(Piperazine)_n$ before KLH sensitisation significantly attenuated inflammatory reactions associated with DTH (44% of inhibition), while nC_{60}, $C_{60}(Arg)_n$ or $C_{60}(Lys)_n$ gave also statistically significant but somewhat smaller effect (25–30%). Assay of cytokine levels showed the significant increase of IL-4 and IL-5 after sensitisation and sharp decrease of these cytokines to the control level in 24 h after the challenge. In contrast, levels of IFNγ in mice treated with $C_{60}(Piperazine)_n$ were approximately the same as in control. It should be noted that the injection of FDs in 2 weeks after the sensitisation does not lead to the inhibitory effect.

Conclusion: These results demonstrate that some FDs may have a therapeutic potential for the treatment of DTH-mediated inflammatory diseases.

Poster Session 47

Mechanisms of allergic sensitisation

1213
Innate lymphoid cells type 2 expressing high GATA-3 levels are present in cord blood and in higher proportions in male than female neonates

Forsberg, A[1]; Bengtsson, M[1]; Eringfält, A[1]; Ernerudh, J[1]; Mjösberg, J[2]; Jenmalm, M[1]
[1]Linköping University, Linköping, Sweden; [2]Karolinska Institutet and University Hospital Huddinge, Stockholm, Sweden

Background: Innate lymphoid cells type 2 (ILC2), a recently discovered cell type associated with Th2 responses, may play a role in allergic reactions and can be detected in adult peripheral blood. An imbalance between Th1- and Th2-like immunity has been observed already at birth in children developing allergy. The aim of this study was to investigate if ILC2 cells are present in cord blood and if their proportions are associated with allergy development and gender.
Method: A population of ILC2-like lymphoid cells was detected by flow cytometry in cord blood and adult peripheral blood.
Results: These cells were tested negative for lineage markers including CD1a, CD3, CD4, CD11c, CD14, CD19, CD34, CD123, CD303, FCεR1α, TCRαβ, TCRγδ, but expressed CD161, CD127, CRTH2 and showed heterogeneous expression of CD117. Furthermore, cord blood ILC2 cells ($n = 8$) expressed higher levels of GATA-3 than adult peripheral blood ILC2 cells ($n = 7$, $P = 0.009$). GATA3 was recently demonstrated as a hallmark transcription factor of ILC2s and is important for IL-5 and IL-13 cytokine production. Cord blood ILC2 proportions were similar in children later developing allergic diseases ($n = 7$) compared with those who did not ($n = 7$), all with a clinical follow-up of 6 years. Newborn boys ($n = 14$) had significantly higher proportion of ILC2s than girls ($n = 13$, $P = 0.02$). In contrast, when comparing adult males ($n = 9$) and females ($n = 8$), no differences in ILC2 proportions were observed.
Conclusion: Interestingly, there is a male predominance early in life of asthma and other allergic diseases, as well as of high IgE levels and susceptibility to Th1-dependent infections. Since ILC2s may induce and enhance Th2 responses, the increased ILC2 proportions in male neonates could be associated with the heightened Th2 responses in boys during childhood.

1215
Predicted value of sensitisation to dust mites in the development of atopic diseases

Diaz Rodriguez, A[1]; Fabré Ortíz, DE[2]
[1]Allergy Department, Medical Center, Jose Manuel Segui, Guira de Melena, Artemisa, Cuba; [2]Allergy Department, Pediatric Hospital, Juan Manuel Márquez, Marianao, Havana, Cuba

Background: Sensitisation to dust mites has been described as a one of the most important risk factors for the development of atopic diseases. The purpose of this study was to determine the predicted value of sensitisation to dust mites in asymptomatic children in the development of symptomatic atopic diseases.
Method: A cross-sectional, prospective and analytical study was carried out in 100 school children aged 6–7 years in a primary school of San Antonio de los Baños, La Habana, Cuba in 2007. The ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire was applied to determine the presence of asthma, allergic rhinitis and atopic dermatitis. The sensitisation to dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides siboney and Blomia tropicalis*) was explored by skin prick tests (SPT). The children with positive SPT and no symptoms of asthma, allergic rhinitis and atopic dermatitis were reevaluated 5 years later (2012) with the same questionnaire to seek for the presence of new atopic symptoms. The possible statistical associations between new symptoms and family history of atopy, smoking habits in living together relatives and the presence of pets were determine by chi-square tests.
Results: The 60% presented asthma, allergic rhinitis or atopic dermatitis whereas the 40% of children had no symptoms of atopy. From the children with no symptoms, the 16% had (+) SPT to dust mites: 12.5% to *Dermatophagoides pteronyssinus*, 25% to *Dermatophagoides siboney* and 94% to *Blomia tropicalis*. When reevaluated 5 years later (in 2012) the 75% of children with positive SPT and no symptoms of atopy developed atopic diseases: 25% had asthma and 63% allergic rhinitis. There was a significant statistically relationship between the new-appearance symptoms of atopic diseases and the family history of atopy ($\chi^2 = 7.11$) and smoke habits in relatives ($\chi^2 = 5.33$).
Conclusion: From the children with no symptoms of atopy and a positive SPT to dust mites, the majority developed atopic diseases, been the allergic rhinitis the most common. There was a significant statistically relationship between the new symptoms of atopic diseases and the family history of atopy and smoke habits in living together relatives.

1217
Anaphylaxis after inhalation exposure to peanut

Fonseca Avendaño, JA; Burgos, A; Garcia, D; de Vicente, T; Montoro, A
Alergologia, Hospital Central de la Defensa, Madrid, Spain

Background: Peanut, a member of the legume family, is responsible for most food allergies in children over 0.6% of the population suffers. The common manifestations are cutaneous and gastrointestinal, and may infrequently respiratory symptoms after exposure by inhalation.
Method: This is a girl 28 months old with a history of atopic dermatitis and urticaria after eating fish Gallo (Lepidorhombus Wiffiaganis) comes to our clinic for symptoms of urticaria (rash generalised urticaria) after taking 1/2 peanut. Three months later after a peanut-free diet presents picture of erythema, facial, nasal congestion, rhinorrhea, conjunctival hyperemia and itching in the mouth in five times after smelling peanuts.
Results: Positive prick skin test to peanut. Doubtful positive for latex, pineapple and almond.

Negative for other nuts and legumes, My white and blue fish.

Total IgE 32.1. Tryptase 1.56 μg/l; 3.72 μg eosinophil cationic protein/l specific IgE: Peanut 8.82 kU/l; Pineapple <0.35 kU/l; Almond <0.35; Latex <0.35kU/l; Gallo <0.35; IgG 715 mg/dl; 67 mg/dl IgA, IgM 72.2 mg/dl.

Conclusion:

1 We present a rare case of food allergy with respiratory manifestations after sniffing.
2 Emphasize to patients the possibility of allergic reactions after exposure to air.
3 It is noteworthy that the demonstrations took place in a more severe type anaphylaxis.

1221
Disease severity is an outcome predictor in infants with atopic dermatitis

Tsabouri, S[1]; Valari, M[2]; Douros, K[3]; Magiakou, M-A[4]; Papadavid, E[5]; Theodoridou, M[6]; Priftis, K[3]
[1]Child Health Department, Medical School, University of Ioannina, Ioannina, Greece; [2]Department of Dermatology "Agia Sofia" Children's Hospital, Athens, Greece; [3]3rd Department of Paediatrics, University of Athens School of Medicine, "Attikon" General University Hospital, Athens, Greece; [4]Department of Endocrinology, Athens University Medical School, "Agia Sofia" Children's Hospital, Athens, Greece; [5]2nd Department of Dermatology, Athens University School of Medicine, "Attikon" General University Hospital, Athens, Greece; [6]1st Department of Pediatrics, 'Agia Sofia' Children's Hospital, University of Athens, Athens, Greece

Background: Atopic dermatitis (AD) is an inflammatory, chronically relapsing and intensely pruritic skin disease causing loss of skin structure. Although children showing more severe dermatitis have a higher risk of having more persistent AD, prognostic determinants of the long-term persistence of the disease are scarce.
Aim: To determine the clinical and laboratory parameters, at the initial assessment of the patients with AD and their relation to the subsequent natural course of the diseases up to school age.
Method: Eighty-nine infants with AD aged 3–24 months were recruited and followed up until the age of 8 year-old. At the time of the initial visit a number of parameters were determined: peripheral blood eosinophil count, serum ECP levels, total IgE levels and specific IgEs for a panel of allergens including food and inhaled allergens. The severity of AD was calculated by using the SCORAD index. Every second year, parents were interviewed about symptoms and diagnosis relevant to food allergy, allergic rhinoconjunctivitis (RC) and asthma using a standardised questionnaire. At the end-visit specific IgEs for the same panel of allergens used at the time of recruitment were measured. For the purpose of univariate, descriptive analysis Fisher's exact tests were used and for the multivariate analysis of data logistic regression models.
Results: Complete follow-up was achieved in 72 children. Food allergy at school-age was independent from IgE or ECP values, eosinophil count and SCORAD index. AD persistence was significantly related with SCORAD index ($P = 0.012$, OR: 1.10, CI: 1.02–1.20). For each unit increase in SCORAD index the mean likelihood of AD persistence at the age of 8 years was increased by 10%. Asthma development was significantly related only with ECP values ($P = 0.019$, OR: 1.10, CI: 1.01–1.20) and allergic RC diagnosis was significantly related only with IgE values ($P = 0.017$, OR: 1.01, CI: 1.00–1.01).
Conclusion: In this cohort of infants suffering from AD, the severity of the disease was the major outcome predictor for AD persistence in later childhood. In addition, ECP and IgE were significantly correlated with asthma and allergic RC development respectively.

1223
Environmental influences on hepatocyte growth factor in human colostrum

Munblit, D[1]; Treneva, M[2]; Dissanayeke, S[1]; Chow, L-Y[1]; Pampura, A[2]; Boyle, RJ[1]; Warner, JO[1]
[1]Paediatrics, Imperial College London, London, United Kingdom; [2]Allergy Department, Moscow Institute of Paediatrics and Child Surgery, Moscow, Russia

Background: Colostrum is the primary source of nutrition for a newborn, providing nutrients, growth factors and immunological components vital for an infant's health and development. Hepatocyte Growth Factor (HGF) is found in a very high concentration in colostrum. It is known to be an important mitogen and stimulator of hepatocyte proliferation, and acts as a repair factor. It has been shown that HGF levels can be influenced by various environmental factors, such as: different diseases, pregnancy or aging. High levels of this growth factor have been found at the late stages of pregnancy. HGF also shown its potential anti-allergic properties in the animal studies and may exert immunomodulatory effects in a neonate's developing immune system.
Methods: We recruited pregnant women at antenatal clinics in Moscow and London. Participants underwent allergy skin prick testing to a panel of inhalant and food allergens, and questionnaire interview. Colostrum samples (days 0–6) were analysed in duplicate using electrochemiluminescence (Meso Scale Discovery®) for level of HGF.
Results: We analysed HGF in colostrum samples from 196 women (120 Moscow, 76 London). In univariate analyses we found colostrum HGF level was lower in mothers who reported visible mould/mildew in their home [mean (SD) logHGF concentration mean 3.49 (0.52) pg/ml mould, 3.25 (0.46) no mould $P = 0.01$]. We also found an inverse correlation between time after birth and HGF level ($r = -0.37$; $P < 0.001$). Multivariate analysis adjusted to country of collection, maternal age, maternal allergy history, mode of delivery, previous pregnancies, smoking exposure and parental education level showed no significant difference between the levels of HGF in colostrum of mothers living in mouldy homes ($P = 0.15$) but the association with time after birth remained ($P < 0.001$).
Conclusion: HGF levels in colostrum drop rapidly after birth, so assessment of HGF levels in colostrum needs to be corrected for time after birth in the future studies. We found no association with positive maternal allergy history or a number of other maternal and environmental factors, suggesting that high expression of HGF in early breast milk is conserved in human populations. Future work will explore the relationship between colostrum and breast milk HGF levels and infant health outcomes.

1224
Body pletismography in pediatrics age

Viana, J[1,2]; Cunha, L[1]; Almeida, S[1]; Falcão, H[1]
[1]Serviço de Imunoalergologia, Centro Hospitalar do Porto, Porto, Portugal; [2]Serviço de Imunoalergologia, Hospitais da Universidade de Coimbra, Coimbra, Portugal

Background: Body Pletismography allows objective and thorough study of lung function. Its use in paediatrics age helps in earlier diagnosis of intrinsic or extrinsic lung pathology and its clinical follow-up. In spite of methodological requirements for this age group, lately there has been greater interest in its usefulness, especially if thoracic comorbidities are present.
Method: Retrospective review of records and clinical data of patients submitted to Body Pletismography at Hospital Maria Pia from January 2010 to July 2012. Each session results were assessed by criteria from ERS 1993 modified by Zapletal *et al.*
Results: Pletismography was performed in 109 children aged 7–20 years, with mean age 14 years and 64% of them male.

Most were able to complete the study successfully (80%). Results were found to be compatible with obstructive syndrome in 8%, in 18% with Restrictive Syndrome and in 10% with Mixed Syndrome. There were also 25% that showed Small and Medium Airway Obstruction and 11% with non-specific alterations. Twenty-seven percent of them were normal.

This study was useful for the diagnostic, differential diagnosis and functional assessment work-up of patients with neuromuscular disease (present in 22 cases, four of

them also with scoliosis), cystic fibrosis (in 13 cases), thoracic dimorphisms (*pectus excavatum and* scoliosis present each in five cases), and asthmatics (in 33 cases).

Conclusion: Plethismography is more selectively requested in Paediatrics due to its time and resources requirements. However it was used sucessfully to assess respiratory mechanisms, residual volume and total lung capacity in the majority of children (80%). Integrated in clinical evaluation, this allows better diagnosis and thorough follow-up of children with respiratory disease.

1225
Consumption of a follow-up formula containing docosahexaenoic acid, prebiotics, and beta-glucan reduced the incidence and duration of acute respiratory infections in 3–4 year old children

Jin, X[1]; Li, F[1]; Liu, B[2]; Zhuang, W[2]; Strong, P[2]; Scalabrin, D[2]
[1]Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine, Shanghai, China; [2]Mead Johnson Pediatric Nutrition Institute, Evansville, IN, United States

Background: Reduction in the incidence of respiratory infections has previously been reported in children receiving dietary docosahexaenoic acid (DHA), in infants receiving prebiotics, and in adults receiving yeast beta-glucan. The objective of this study was to determine if a follow-up formula containing the combination of DHA, the prebiotic blend of polydextrose (PDX) and galactooligosaccharides (GOS), and yeast beta-glucan reduced the incidence of respiratory infections in healthy children enrolled in daycare.

Method: A double-blind, randomised, controlled, prospective trial was completed in 3- to 4-yr old children fed three servings per day for 28 weeks of either an experimental follow-up formula ($n = 133$) containing DHA (25 mg/serving), a prebiotic blend of PDX and GOS (1.2 g/serving), and yeast beta-glucan (12.8 mg/serving), or a control powdered cow′s milk ($n = 131$). Fecal and blood samples were collected at the onset of the study and again at 28 weeks. The incidence and duration of acute respiratory infections (ARI) and antibiotic use were obtained from medical records. Incidence of ARI was analyzed with Cochran-Mantel-Haenszel row mean score test. Duration of ARI was analyzed with ANOVA. Antibiotic use was compared with Fisher's exact test. Fecal and serum immune markers and serum ferritin and zinc were analyzed with non-parametric tests.

Results: Children receiving the experimental follow-up formula had fewer episodes ($P = 0.04$) and shorter duration (3.4 ± 0.22 days vs 4.2 ± 0.19 days; $P = 0.01$) of ARI and less antibiotic use ($P = 0.01$) compared to the control group. Children consuming the experimental formula had a higher white blood cell (WBC) count at the end of the study ($P = 0.04$), a higher change from baseline of WBC ($P = 0.01$), and higher IL-10 at the end of the study ($P = 0.04$). There were no differences between groups in serum ferritin and zinc or fecal secretory IgA. There were no differences in formula consumption between groups.

Conclusion: In 3- to 4-year old children, daily intake of three servings of a follow-up formula containing DHA, a prebiotic blend of PDX and GOS, and yeast beta-glucan over a 28 week period resulted in fewer episodes and shorter duration of ARI, as well as reduced antibiotic use, compared with intake of three servings per day of powdered cow's milk. The children who consumed the experimental formula also increased IL-10 and WBC, suggesting an anti-inflammatory mechanism and/or an increase of effector immune cells.

Poster Session 48

Pediatric allergy and prevention and risk factors I

1226
Acetaminophen and antibiotic use in early life and the development of childhood allergic diseases

Liu, L-F[1]; Tsai, H-J[2]; Wang, J-Y[3]
[1]Gerontology, National Chen Kung University, Tainan, Taiwan; [2]Division of Biostatistics and Bioinformatics, National Health Research Institutes, Institute of Population Health Sciences, Zhunan, Taiwan; [3]Pediatrics, National Chen Kung University, Tainan, Taiwan

Background: Whether use of acetaminophen and/or antibiotics in early life can cause allergic diseases in later childhood remains inconclusive. The objective of the present study is to investigate the temporal relationship between exposure to acetaminophen and/or antibiotics in early life and developing allergic diseases in later childhood using two independent birth cohorts derived from the National Health Insurance Research Database (NHIRD) in Taiwan.
Method: The authors conducted a prospective birth cohort study using 263 620 children born in 1998 and 9910 children born in 2003, separately, from NHIRD. Exposure status of acetaminophen and/or antibiotics and potential confounding factors were included in the analyses. Cox proportional-hazard models were applied to determine the temporal effect of acetaminophen and/or antibiotic exposure on developing allergic diseases.
Results: We observed positive relationship between exposure to acetaminophen and/or antibiotics during the first year of life and the development of three examined atopic diseases in 1998 birth cohort [adjusted hazard ratio (aHR): 1.86 for both use of acetaminophen and antibiotics in atopic dermatitis (95%CI: 1.74–1.98); aHR: 1.60 for both use in asthma (95%CI: 1.53–1.67); aHR: 1.61 for both use in allergic rhinitis (95%CI: 1.55–1.67)]. Similar results were observed in 2003 birth cohort.
Conclusion: Our findings have provided suggestive evidence the temporal effect of exposure to antibiotics and/or acetaminophen influences the development of childhood common allergic diseases. Further functional studies and/or animal studies will be needed for better understanding underlying regulatory mechanisms on this important clinical/public issue.

1227
The association of water damage with the severity of atopic dermatitis in children, Korea

Seo, S[1,2]; Kim, J[3]; Han, Y[3]; Ahn, K[3]; Yoo, Y[2,4]; Choung, JT[2,4]; Lee, J-H[5]
[1]Korea University College of Medicine, Seoul, Korea; [2]The Environmental Health Center for Asthma at Korea University Medical Center, Seoul, Korea; [3]The Environmental Health Center for Atopic Dermatitis, Samsung Medical Center, Seoul, Korea; [4]Department of Pediatrics, College of Medicine, Korea University, Seoul, Korea; [5]The Environmental Health Center, Ulsan University Medical Center, Ulsan, Korea

Background: Damp environment could contribute to the exacerbation of asthma, but little is well-known for the association between dampness attributed to water damages on buildings and atopic dermatitis (AD). Moreover, the survey through the self-led questionnaire for the evaluation of water damage might not be enough for determining its health impact on AD due to chances for misclassification attributed to insufficient information. We compared the airborne concentrations among homes with and without water-damages determined by thermal images and explored the association of water-damages with the severity of AD in children.
Methods: We visited 56 homes with a patient(s) diagnosed with AD (SCORAD range: 5.7–53.1), and sampled airborne mold in both a living room and a child's bedroom(s) using an impactor sampler. Thermal assessments on the wall using the infrared camera and checking the presence of visible mold were performed for determining water-damages. We calculated the areas of water damage using the 'IMAGE J' program, and then classified the water-damaged homes with >0.2 m^2 or the combined area of water damage. The colony counting was used to get the mold level after 3-day incubation. We compared the concentrations of airborne mold among homes with/without water-damages. The association of water-damages with the severity of AD in children was analyzed.
Results: Of 56 homes, water-damages were observed in 33 homes (58.9%) and most of water-damages were found in children's rooms (24/33). The concentrations of mold were significantly higher in water-damaged homes (GM: 322.4 CFU/m^3) than those in non-damaged homes (85.3 CFU/m^3) ($P < 0.01$). Conversely, the mold levels were significantly lower in homes with any visible mold only (212.8 CFU/m^3) than homes without ones (271.1 CFU/m^3) ($P < 0.05$). The SCORAD indices of patients in water-damaged homes (GM: 24.0) were significantly higher than those in non-damaged homes (15.7) ($P < 0.05$). Children living in water-damaged homes had more than eightfold the risk of exacerbating AD than those in non-damaged homes (OR, 8.7; 95% C.I., 1.6–46.3) ($P < 0.001$).
Conclusions: These results indicate that a thermal camera-driven assessment could be a promising tool for determining the water-damage. Moreover, this method could be much practical for assessing exposure to mold in order to provide area information for intervention as well as savings for time and sampling/analysis costs before full assessment for dampness.

1228
Relationship between socioeconomic and sociodemographic risk factors and atopic dermatitis in Korean adolescents

Rha, YH[1]; Lee, KS[1]; Kim, MS[2]; Choi, SH[3]
[1]Pediatircs, Kyung Hee University Hospital, Seoul, Korea; [2]I Dream Children's Hospital, Daegu, Korea; [3]Pediatrics, Kyung Hee University Hospital at Gand Dong, Seoul, Korea

Background: There have been numerous studies in an attempt to prevent and treat atopic dermatitis (AD), and as a result, many medical, socioeconomic and sociodemographic risk factors have already been suggested. We conducted this study specifically focusing on the relationship between various socioeconomic and demographic variables, and AD in Korean children and adolescents.
Method: In this study, 79 202 Korean adolescents aged 12–18 years who participated in the 2010 Korea Youth Risk Behavior Web-Based Survey(KYRBWS, 2010) were used as sample. Dependent variable was atopic dermatitis; independent variables were gender, parents' education level, FAS, low subjective family economic status, and obesity. These were collected by the self-answering surveying method. Multivariate analysis was conducted in order to analyze

the relationship between socioeconomic and sciodemographic risk factors and AD.
Results: The prevalence of AD based on 2010 KYRBWS in Korean adolescents was 23.1%. In univariate analysis, female, urban, high parental education level, family affluence scale was strongly correlated with AD. In multivariate analysis, female, urban, college or higher parental education level, obesity, high family affluence scale, low subjective family economic status were correlated with AD.
Conclusion: This study proved that Korean adolescents' AD was strongly correlated with socioeconomic and sociodemographic risk factors. Thus, it is utmost important to modulate the socioeconomic and sociodemographic risk factors to control AD systematically in adolescent in Korea.

1229
Risk factors of extrinsic and intrinsic atopic dermatitis in Korean adolescents

Rha, YH[1]; Kim, MS[2]; Lee, KS[1]
[1]Pediatircs, Kyung Hee University Hospital, Seoul, Korea; [2]I Dream Children's Hospital, Daegu, Korea

Background: Atopic dermatitis (AD) can be classified into Extrinsic (EAD) and intrinsic AD (IAD) based on presence of specific IgE (sIgE). Even though it is known that EAD shows more severe clinical sign, no study with comparative analysis on EAD and IAD has been done in Korea. We analyzed EAD and IAD sample data to identify their risk factors among Korean adolescents.
Method: Based upon the data from Korea National Health & Nutrition Examination Survey (KNHNES V-1, 2010) on 12–18 year old adolescents' prevalence rate, the various variables presumably related to AD, including age, gender, income, habitat (rural/urban), obesity, smoking, drinking, vitamin D and total IgE (tIgE) concentration in blood, were used for statistical analysis.
Results: The group with positive sIgE, especially male, showed significantly higher level in vitamin D and tIgE concentration in blood than negative sIgE group in general survey population. Prevalence rate of EAD and IAD were 7.2% and 5.8% respectively. The tIgE concentration of EAD and IAD were respectively 356.5 mg/dl (quartile 194.5–708.0) and 109.6 mg/dl (quartile 53.2–243.3), showing a significant difference; however, there was no significant difference in other variables. The risk factors for EAD were the high tIgE concentration (OR, 8.4; 95% CI, 4.0–17.7; $P < 0.000$) and smoking (6.7;1.1–42.3; $P < 0.042$), and the risk factors for IAD was obesity (4.4; 1.1–17.4; $P < 0.037$).
Conclusion: In comparison between EAD and IAD, tIgE and smoking were found to be the risk factors for EAD and obesity for intrinsic AD. Therefore, the prevention of adolescent AD requires moderation in smoking for EAD and in body-weight control for IAD.

1230
Effect of breastfeeding on lung function of asthmatic children

Kim, HS[1]; Lee, HH[2]; Kim, KW[1]; Sohn, MH[1]; Kim, KE[1]
[1]Department of Pediatrics and Institute of Allergy, Severance Children's Hospital, Yonsei University College of Medicine, Seoul, Korea; [2]Department of Pediatrics, Myongji Hospital, Kwandong University College of Medicine, Goyang, Korea

Background: Evidence of beneficial effect of breast-feeding on asthma and lung function is conflicting. Methods

Feeding practices were evaluated based on questionnaires completed on visiting Severance Children's Hospital Allergy Clinic. We categorised the duration of breast feeding as not breastfed ($n = 52$), 0–6 months ($n = 77$), and >6 months ($n = 81$), in patients diagnosed with asthma ($n = 210$). Spirometry and methacholine challenge test were performed.
Results: The mean age was 7.6 ± 3.3 years and 130(61.9%) were male. Forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC, %) of not breastfed group was 100.1 ± 11.0, 100.9 ± 10.0 for 0–6 months group, 104.6 ± 7.9 for >6 months group ($P = 0.011$). However, FEV1 (% predicted) of not breastfed group was 100.4 ± 17.1, 102.9 ± 13.8 for 0–6 months group, 101.9 ± 16.5 for >6 months group ($P = 0.663$), and FVC (% predicted, $P = 0.51$), PEF (% predicted, $P = 0.358$) and FEF25-75 (% predicted, $P = 0.61$) did not show any differences between the groups. Considering history of parental allergic diseases ($n = 123$), longer duration of breast feeding resulted in better FEV1/FVC (%) ($P = 0.007$): 98.0 ± 12.7 of not breastfed group ($n = 26$), 98.8 ± 10.7 for 0–6 months group ($n = 49$) and 104.4 ± 7.4 for >6 months group ($n = 48$).
Conclusions: Longer duration of breastfeeding showed superiority in FEV1/FVC. However duration of breastfeeding has mixed influence on different parameters of spirometry and further study will be needed to clarify the effect of breastfeeding on pulmonary function in children with asthma.

1231
Reference values of total serum IgE and their significance in the diagnosis of allergy in Asian children: PATCH study

Tu, Y-L[1,2]; Tsai, H-J[3,4]; Chang, S-W[5]; Cheng, J-H[1]; Huang, J-L[1,2]; Yao, T-C[1,2]; PATCH Study Group
[1]Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan; [2]Department of Pediatrics, Chang Gung University College of Medicine, Taoyuan, Taiwan; [3]Division of Biostatistics and Bioinformatics, National Health Research Institutes, Institutes of Population Health Sciences, Miaoli, Taiwan; [4]Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, United States; [5]Clinical Informatics and Medical Statistics Research Center, Chang Gung University College of Medicine, Taoyuan, Taiwan

Background: Total serum immunoglobulin (IgE) test is usually performed to aid in the diagnosis of allergic diseases. Reference values of total IgE may vary among people of different ethnic backgrounds. This study aimed to establish reference values of total IgE levels in Asian children and to assess the significance of these values in the diagnosis of allergic sensitisation and allergic diseases.
Methods: A total of 1321 Asian children aged 5–18 years from the Prediction of Allergies in Taiwanese CHildren (PATCH) study, a population-based cohort, were evaluated for total and specific IgE levels by ImmunoCAP and ImmunoCAP Phadiatop Infant, respectively.
Results: The geometric mean total IgE was significantly higher for children with allergic sensitisation than those without (239.5 vs 27.5 kU/l, $P < 0.001$), and for boys than girls (112.8 vs 74.4 kU/l, $P < 0.001$). Multivariate regression analysis revealed that allergic sensitisation was the single most important independent variable explaining variability of total IgE levels in this population. The area under the receiver-operator characteristic (ROC) curve of total IgE for diagnosing allergic sensitisation, asthma, allergic rhinitis and atopic dermatitis were 0.92, 0.72, 0.70 and 0.70, respectively. The sensitivity, specificity, and positive (PPV) and negative predictive values (NPV) of total IgE at the opitmal cutoff of 77.7 kU/l on the ROC curve for diagnosing allergic sensitisation were 82.3%, 87.1%, 89.5%, and 78.6%, respectively; whereas the corresponding values using the upper 95% CI of total IgE levels (164.3 kU/l) in children without allergic sensitisation were 61.2%, 95.0%, 94.3%, and 64.6%, respectively.
Conclusion: Total serum IgE test discriminates Asian children with and without allergic sensitisation independent of allergic symptoms, with an optimal cutoff of 77.7 kU/l. This study confirms the insufficient diagnostic accuracy of total IgE levels alone to detect allergic diseases.

1232
Impact of environmental factors on the population of allergic children

Adamia, N[1]; Katamadze, N[2]; Chkhaidze, I[3]; Jorjoliani, L[4]; Khachapuridze, D[5]; Ubiria, I[3]; Kherkheulidze, M[3]; Zerekidze, V[6]; Saginadze, L[6]; Gotsadze, K[7]; Barabadze, K[7]; Kherkheulidze, V[8]; Ghughunishvili, M[3]; Chkhaidze, N[3]
[1]Department of General Pediatrics, Tbilisi State Medical University, Tbilisi, Georgia; [2]Batumi Medical Center, Tbilisi, Georgia; [3]Tbilisi State Medical University, Tbilisi, Georgia; [4]Javakhishvili Tbilisi State University, Tbilisi, Georgia; [5]Tskaltubo Center of Clinical Immunologists and Allergologists, Tbilisi, Georgia; [6]Pediatric Clinic, Tbilisi, Georgia; [7]Tatishvili Medical Center, Tbilisi, Georgia; [8]AIETI Medical Institute, Tbilisi, Georgia

Most allergic diseases are related with worsening of environmental condition, pollution of atmosphere, water and soil, unsound food products.

Goal of our work is study of impact of environmental factors and worsening of life quality of population of children with allergies.

Materials and methods: Research was conducted through questioning of random and representative groups of patients of Tbilisi, Kutaisi and Batumi including 7989 children from 3 to 16 years. Outdoor risk factors were studied through questioning. Case group included cases of allergic rhinitis (19.5%), bronchial asthma (9.8%), atopic dermatitis (5.7%) and food allergies (3.7%), control group included healthy population of respective age and sex.

Data processing was provided by SPSS/V12.5 software.

Results and consideration: Questionnaire allowed identification of significant differences in almost all parameters, in healthy individuals and patients with allergic diseases. Frequency of the identified variables was reliably higher ($P < 0.05$) in families of children with allergic rhinitis and bronchial asthma, compared with healthy population. For the patients with allergic rhinitis, bronchial asthma, atopic dermatitis, with environmental problems, inherited allergic load was confirmed in 76.8% of cases, while in the healthy population this figure was 8.9% ($P < 0.001$). 1/3 of children with allergic rhinitis and bronchial asthma allergic load was identified from the side of both parents. Environmental factors were considered as risk factors provoking worsening of condition in certain conditions or increasing probability of disease. Various negative ecological factors were represented almost equally.

Outdoor undesirable environmental factors were reflected in skin allergic response. Skin prick-test was positive for outdoor allergens. Skin prick-test to fungi was positive in 23.9% of cases.

Conclusions: Obtained results show that the problem of environment pollution is quite significant in Georgia. Environmental factors, together with genetic predisposition significantly impacts frequency of sensibilization to the allergens in children and development of allergic diseases. Management of increased allergic load is possible through early targeted preventive program, taking into consideration the peculiarities of the region.

1233
How far do German parents conform to international recommendations of primary allergy prevention concerning breastfeeding and timing of introducing solid foods?

Bellach, J; Trendelenburg, V; Ahrens, B; Schwarz, V; Beyer, K
Pediatric Pneumology & Immunology, Charité Universitätsmedizin Berlin, Berlin, Germany

Background: Exclusive breastfeeding for at least 4 months is recommended for primary prevention of allergic diseases for children at risk. If breastfeeding is not possible or desired, hypoallergenic formulas should be used.

The aim of our study was to investigate to what extent parents that participated in the 'Hen's Allergy Prevention Study' (HEAP) followed these recommendations.

Method: So far 242 children were recruited at birth into the HEAP Study. Based on the questionnaires developed in EuroPrevall a standardised baseline-questionnaire was employed that included questions addressing the allergy history of the family. Upon weaning between 4 and 6 months of age the families were invited to the study centre where another questionnaire was answered that contained questions to the dietary history of the child including breast feeding, formula feeding and the introduction of solid foods. The study participants in the HEAP Study are observed until the age of three.

Results: Seventy-two percent ($n = 172$) of the children had atopic heredity and were thus at risk to develop allergic diseases. Of all children, 78% ($n = 189$) were breastfed, of those 46% ($n = 111$) were exclusively breastfed for at least 4 months and 32% ($n = 78$) were partially breastfed. Twenty-two percent ($n = 53$) were not breastfed. Children with atopic heredity were more often exclusively breastfed than children without atopic heredity (51% vs 32%, $P < 0.050$).

Among children with atopic family background, only 37% of those partially breastfed and 40% of those not breastfed received hypoallergenic formula as recommended in the national guidelines. One child with atopic family background received soy protein based formula.

Ninety-seven percent (167/172) of atopic families started introduction of solids only after the child was 4 months old, according to the recommendations.

Conclusion: Despite clear national and international guideline for allergy preventions only 40% of the children at risk to develop an allergic disease received hypoallergenic formula if not or only partially breast-fed. This highlights that recommendations are so far not well implemented and actions are required to do so.

1234
Emollients are relevant sources of exposure to contact sensitisers

Kordus, K; Spiewak, R
Department of Experimental Dermatology and Cosmetology, Jagiellonian University Medical College, Krakow, Poland

Background: Atopic diathesis and dry skin are major causes for the use of emollients. However, the majority of emollients, just as any other cosmetics, contain numerous sensitisers – preservatives and fragrances in first instance. The objective of this study was to analyse compositions of emollients available at pharmacies, with the focus on the occurrence of active substances, as well as ingredients whose use in cosmetics is subject to restrictions imposed by the EU 'Cosmetic Directive' due to known risk of sensitisation.

Method: Emollients offered by Internet pharmacies were analyzed with special attention paid to occurrence of 'problematic' ingredients as listed in the annexes to the by the European 'Cosmetic Directive'.

Results: We identified 177 cosmetics marketed in pharmacies as emollients that contained in total 522 different ingredients, including 181 active and 49 'problematic' ingredients (preservatives and fragrances). Methylparaben (occurrence in 38% of analysed products), phenoxyethanol (36%) and propylparaben (33%) were the 'problematic' ingredients most commonly used in the emollients. Out of 177 analysed cosmetics marketed in pharmacies as emollients, only 37 (21%) were completely free of potential sensitisers listed in the European Cosmetics Directive's annexes III and VI.

Conclusion: The vast majority of emollients available from pharmacies contain ingredients with sensitising potential that are subject to restrictions of use in cosmetics on account of the risk of sensitisation. Lack of correlation between the numbers of active and 'problematic' ingredients

occurring in the analysed products indicates on the possibility of creating safer emollients while maintaining effectiveness.

1235
Probiotic microorganism lactobacillus reuteri impact on the prevalence of allergic diseases in 1 and 3 years old Slovene children

Besednjak-Kocijancic, L
Zdravstveni dom Nova Gorica, Primary Pediatric Health Centre, Nova Gorica, Slovenia

Background: Human microflora and her changes in newborn are very important for the maturation of the immune system. Nowadays our environment is free from germs to the point that the immune system is not stimulated sufficiently. Probiotics, viable non-pathogenic bacteria beneficially affect the host intestinal microbial balance and may improve immunity and prevent development of allergic diseases. The aim of presented study was to evaluate the efficacy of probiotic microorganism Lactobacillus reuteri (LR) in the prevention of asthma, atopic dermatitis (AD) and food allergy in children with positive family history for an allergic disease.
Method: This prospective study included 316 maturely born infants with a positive history of parental allergy confirmed by allergy testing. According to the duration of exclusive breastfeeding and to the addition of LR in to the child diet, they were divided in two groups:

Group A- 201 children exclusively breastfed for 4–6 months and
Group B- 115 children breastfed with addition of LR from fourth week of life for 12 weeks.

The prevalence of asthma, AD and food allergy at the age of 1 and 3 years was observed. The study results were in part compared to the results of the study performed before availability of LR in Slovenia. Statistical analysis was performed with SPSS 11.0 using chi-square analysis with Yates' correction. P-values < 0.05 were considered significant.
Results: In the total population significantly higher prevalence of allergic diseases was observed at the age of 3 years (1 year: 12.3%; 3 years: 24.4%), ($P < 0.01$). The percentage of allergic children at the age of 1 year (group A: 20.1%; group B: 7.9%) and at the age of 3 years (group A: 28.3%; group B: 17.4%) was significantly lower in group B ($P < 0.05$). No significant between group difference in asthma, AD and food allergy prevalence was confirmed at the age of 3 years ($P > 0.05$). But comparison of results with those obtained in 3 years old children before availability of LR in Slovenia showed decrease of the asthma and AD prevalence ($P < 0.01$).
Conclusion: The study confirms that addition of LR to early child's diet is beneficial. It decreases the percentage of allergic children and the likelihood of the development of asthma and AD.

Table 1

	Odds ratio	*P*-value	IC 95% minimum value	IC 95% maximum value
Age 5–14	1.06	0.938	0.24	4.59
Age 15–34	0.06	0.001	0.01	0.29
Age 35–69	0.19	0.003	0.06	0.57
Age 70 and more	0.41	0.242	0.09	1.8
Severe anaphylaxis	7.65	0.001	3.02	19.4
Sex male	1.12	0.784	0.48	2.62
Presence of previus anaphylaxis	2.27	0.061	0.96	5.37

1236
Factors for the use of one or more doses of adrenaline in anaphylaxis episodes attended in a general hospital

Farias Aquino, EG; Tejedor Alonso, MA; Mugica Garica, MV; Moro Moro, M; Vila Albelda, C; Rosado Ingelmo, A; Gomez Traseira, C; Tobón Franco, JD
Allergy Unit, Hospital Universitario Fundación Alcorcón, Alcorcón, Spain

Background: Some patients need one or more doses of adrenaline to attain full control of anaphylaxis episodes. Our aim was to establish the factors associated with the use of one or more doses of adrenaline in patients treated in our hospital by episodes of anaphylaxis.
Method: We selected 203 episodes of anaphylaxis who were treated in emergency of our hospital (183) or in the hospitalisation wards (20). 52.7% were women and the median was 35.8 years (IQR 19.2–55.4). The University Hospital Alcorcon Foundation is the referral hospital for the city of Alcorcon whose population is 165 000 inhabitants.

The definition of anaphylaxis is established by NIAIF-FAAN Symposium. We use alpha-numeric strings to find symptoms, signs or causes of anaphylaxis in the digital records of our hospital.
Result: Seventeen percent of the episodes of anaphylaxis were treated with adrenaline (1 dose in 12.8% and 2 or more in 4.9%). 22.2% of anaphylaxis were severe and 77.8% were moderate. In an exploratory univariate analysis the type of food (milk, egg, nuts…) or gender did not influence the use of adrenaline. In an ordinal logistic regression model, whose dependent variable was the use of adrenaline (0 doses, 1 dose and 2 or more doses), adrenaline was less used in the 5 and 14 years and 15 and 34 years, compared to the group of 0–4 years. Adrenaline was used higher in severe anaphylaxis than in moderate anaphylaxis (OR 7.7). Patients who had had previous episodes of anaphylaxis were more likely to be given adrenaline, although in borderline the statistical significance (Table 1).
Conclusion: The use of adrenaline is mostly influenced by the severity of anaphylaxis, and the use of two or more doses of adrenaline to treat episodes of anaphylaxis is unlikely, including the severe anaphylaxis.

1237
Dog and cat ownership affect the risk of allergic diseases in different ways

Lee, S-Y[1]; Kim, B-J[2]; Park, K-S[3]; Yu, J[4]; Hong, S-J[4]; Lee, H-R[1]
[1]Pediatrics, Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Anyang, Korea; [2]Inje University Heundae Paik Hospital, Pusan, Korea; [3]Presyterian Medical Center, Junju, Korea; [4]Childhood Asthma Atopy Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Background: Studies on pet ownership as a risk or protective factor for atopy and allergic diseases show inconsistent results. The objective of this study was to describe the relationship between cat and dog ownership and allergic outcomes in childhood.
Method: A cross-sectional study of 1556 children aged 9–12 years was conducted. Demographic and disease-related information was obtained via a detailed questionnaire, and skin prick tests were performed.
Results: Dog ownership showed a reduction in risk of current allergic rhinitis (aOR 0.660; 95% CI 0.457–0.955). Dog ownership was not associated with the risk of current asthma, current atopic dermatitis, atopy, and dog sensitisation. Cat ownership was associated with a increased risk of cat sensitisation (aOR 2.627; 95% CI 1.219–5.660). Cat ownership was not related to the risk of allergic diseases and atopy.

Conclusion: Dog ownership decreased the risk of allergic rhinitis, but cat ownership increased sensitisation to cat. Dog and cat ownership may affect the risk of allergic diseases in different ways.

1238
Risk factors for recurrent wheezing following acute bronchiolitis: a 24-month study

Vasconcelos Pinela, A[1]; Cruz, C[1]; Machado, V[2]; Didenko, I[1]; Tomaz, E[1]; Viseu, R[1]; Peres, MJ[1]; Inacio, F[1]; Caturra, L[2]
[1]Imunoalergology Department, Centro Hospitalar Setubal, EPE, Setubal, Portugal; [2] Pediatric Department, Centro Hospitalar Setubal, EPE, Setubal, Portugal

Background: One of the earliest and most common infectious respiratory condition of childhood is bronchiolitis. Different authors agree that a child who has severe bronchiolitis is at increased risk for recurrent wheezing. The aim of this study was to identify risk factors (type of respiratory virus, type of delivery, age and number of admissions days) for recurrent wheezing (two or more episodes of wheezing in the next 24 months) following acute bronchiolitis.
Method: In December of 2012 we analyzed the clinical reports of 80 children with the diagnosis of bronchiolitis admitted to our hospital (Paediatric inpatient and emergency) between October 2010 and March 2011. Children mean age was 9 months, minimum 1 month and maximum 48 months at the admission time, 34 females and 46 males. The viral infection was confirmed by PCR/RT-PCR multiplex and subsequent visualisation on low density micro arrays detection in nasopharyngeal exsudate. A statistical model of linear regression was used in the analysis.
Results: Respiratory sincicial virus (A or B) were identified in 50% ($n = 40$) of patients, Bocavirus in 7.5% ($n = 6$), Influenza in 5% ($n = 4$), Adenovirus in 3.8% ($n = 3$), others respiratory virus in 6.3% ($n = 5$) and in 27.5% ($n = 22$) were infected with multiple virus. One third of the children ($n = 27$, 33.8%) had recurrent wheezing (two or more episodes of wheezing in the next 24 months of age). Analyzed factors did not show to increase the risk of recurrent wheezing, considering the whole population. Nevertheless when we analyzed only children under the age of 12 months we found that Infants born by cesarean section had a diagnosis of recurrent wheezing in 54.5% vs 25% of those born by vaginal delivery. Delivery by cesarean was a risk factor for recurrent wheezing, the OR was 3.91 (95% CI, 1.256–12.21).
Conclusion: In our study recurrent wheezing was frequent after bronchiolitis (up to one third of our sample). Children under 1 year with a medical diagnosis of bronchiolitis who were delivered by cesarian section had a greater risk of developing recurrent wheezing in the next 24 months.

1239
Identifying suitable indicators for assessing the impact of allergic rhinitis on school achievement in France

Ebersold, S[1]; Robin, B[2]; Robineau, S[3]; Sheikh, A[4]; Pham Thi, N[5]
[1]INS HEA, Suresnes, France; [2]Stallergenes, Antony, France; [3]Alcimed SA, Paris, France; [4]Centre for Population Health Sciences, University of Edimburgh, Edimburgh, United Kingdom; [5]Hôpital Necker-Enfants-Malades, AP-HP, Service de Pneumologie et d'Allergologie Pédiatrique, Paris, France

Background: Poorly controlled allergic rhinitis can impair quality of life and impact adversely on school performance and achievement.
Objectives: We sought to indentify the most relevant indicators to assess the impact of allergic rhinitis on school achievement in France.
Methods: We conducted a review of international publications (1993–2011) focusing on the impact of various conditions on school performance to identify the range of indicators of educational performance that have been studied. The relevance of these types of indicators was then discussed, based on medical and educational experts' interviews.
Results: We identified 11 types of indicators that we ranked as priority ones when cited in publications focusing directly on allergic rhinitis or secondary ones when cited in publications focusing indirectly on this pathology (symptoms, co-morbidities). Priority types of indicators were school grades and cognitive performance. In the French educational context, grades are used at all ages of school attendance. As for cognitive tests, although several are appropriate for use in school-aged children, they require specialist psychologists input and are therefore costly to undertake. Secondary types of indicators were the level of acquired school knowledge, the level of schooling advancement (school grade according to age) and school support. They are relevant in France but only for the younger students.
Conclusion: Examination grades and cognitive tests seem to be the best indicators to assess the impact of allergic rhinitis on school performance, although use of cognitive tests is more prohibitive because of the costs of undertaking these assessments.

1240
Hygroscopicity and particle mass analysis of intra nasal cellulose used in the treatment of seasonal allergic rhinitis

Josling, PD[1]; Sewell, B[2]; Emberlin, J[3]
[1]Nasaleze International Limited, Research and Development, Ramsey, Isle of Man; [2]College of Human Health Sciences, Swansea University, Singleton Park, United Kingdom; [3]Allergy UK, Sidcup, United Kingdom

Background: An important factor influencing particle deposition of Hydroxy propyl methyl cellulose (HPMC IOM915K) is the hygroscopic nature of the powder which causes it to absorb moisture from the ambient air. Hygroscopicity is the intrinsic tendency of a material to take up moisture from its surroundings which can lead to particle size increase through aggregation due to local dissolution and recrystallisation.

HPMC powders are polydisperse which means they contain molecules of a range of degree of polymerisation and molecular weight and thus a large quantity of different particle sizes. For maximum efficacy an optimal deposition of the powder within the nasal cavity is desirable since sufficient gel formation is thought to be crucial for its effect in alleviating symptoms of SAR. The important factor for efficacy is not the number of particles deposited but the mass of these particles.
Method: The measurements were conducted at an ambient temperature between 23 and 28°C and a relative humidity between 30% and 50%. The conditions in the nose are different with the inhaled air being humidified to about 80% relative humidity by evaporation of moisture from the watery mucus and warmed to 30–36°C.

Particle size analyses were all performed using a Beckman Coulter LS13 320 machine. Robust analysis by laser diffraction depends on several instrument design parameters and an approved ISO standard is in force. Batch analyses are presented for powders ranging from newly produced to several years after storage at optimum conditions.
Results: Our experiment shows that the mass median diameter (MMD) of HPMC lies between 10.0 and 12.5 μm. More than 50% of the powder mass is therefore contributed by particles larger than 10.0 μm. Data indicates HPMC particulate increases in size by approximately 14% during storage.
Conclusion: It is postulated that HPMC particles absorb water from nasal air and grow in diameter causing them to deposit in a higher position within the respiratory tract. This could lead to augmented deposition within the nose which increases in efficiency with increasing particle size.

1241
Association between childhood asthma and autism spectrum disorders

Ohya, Y; Narita, M; Futamura, M; Hamaguchi, M; Yamamoto, K; Tsumura, Y; Nomura, I; Kitazawa, H; Morita, K; Kawaguchi, T; Yomase, M; Saito, H; T-Child Study Group
National Center for Child Health and Development, Division of Allergy, Tokyo, Japan

Background: Recent few decades, we experienced rapid increase in prevalence of childhood allergic diseases such as asthma and eczema all over the world especially in highly civilized lesions. At the same time, pervasive developmental disorders such as autism spectrum disorders (ASD) have also attracted attention in pediatrics since their high prevalence was reported. The aim of this study is to investigate whether allergic diseases and ASD might be associated based on the date from our birth cohort study.

Method: Tokyo Children's Health Illness and Development (T-Child) Study is a birth cohort study in which 1550 children and their mothers participated from their pregnancy. When the children became just 5 years old, physical and psychological assessment were carried out by using ISAAC questionnaire, UK working party diagnostic criteria of atopic dermatitis, PARS interview and blood examination to detect allergen components.

Results: One thousand and three children participated the examination at their 5 years of age. One hundred children had been diagnosed as having asthma at that point and 67 children were classified as having ASD defined by high score of PARS. Children with asthma had higher prevalence of ASD than non-asthmatics (13.0% vs 6.1%, $P < 0.05$). Children with atopic dermatitis had also higher prevalence of ASD than those without atopic dermatitis (11.8% vs 6.4%, $P = 0.12$) had, however, there was no significant difference between the prevalence of the two groups. Children who showed positive to the allergen component such as Derf1, Derf2, Derp1, Derp2, Canf1, Feld1 had higher prevalence of ASD than those without ASD (8.3% vs 5.4%, 8.7% vs 5.4%, 8.1% vs 5.6%, 8.9% vs 5.4%, 10.3% vs 6.3%, 9.0% vs 6.3%, respectively).

Conclusion: Childhood asthma and ASD showed positive association in their prevalence and some allergen sensitisation may be correlated with a part of pathophysiological mechanisms of ASD.

Poster Session 49

Understanding the links between environmental exposure and allergy

1242
Indoor volatile organic compounds exposure within first year of life and atopic dermatitis at the age of 3

Kwon, JH[1]; Chang, M-H[2]; Park, E[1]; Hong, Y-C[3]; Ha, M[4]; Park, H[2]; Kim, Y[5]; Ha, E-H[2]; Lim, Y-H[2]; Kim, E[2]; Kim, B-N[6]; Chang, N[7]; Oh, S-Y[8]; Kim, Y-J[9]; MOCHE Study
[1]Pediatrics, School of Medicine, Ewha Womans University, Seoul, Korea; [2]Preventive Medicine and Ewha Medical Research Institute, School of Medicine, Ewha Womans University, Seoul, Korea; [3]Preventive Medicine, Seoul National University College of Medicine, Seoul, Korea; [4]Preventive Medicine, College of Medicine, Dankook University, Cheonan, Korea; [5]Occupational and Environmental Medicine, Ulsan University Hospital, Ulsan, Korea; [6]Division of Child and Adolescent Psychiatry, Department of Psychiatry and Institute of Human Behavioral Medicine, Seoul National University College of Medicine, Seoul, Korea; [7]Department of Nutritional Science and Food Management, Ewha Womans University, Seoul, Korea; [8]Department of Food and Nutrition, Kyung Hee University, Seoul, Korea; [9]Department of Obstetrics, School of Medicine, Ewha Womans University, Seoul, Korea

Background: Allergic disease related with genetic background and environmental conditions. Exposure of indoor air pollutants causes allergic inflammation. Among the environmental factors, indoor volatile organic compounds (VOCs) are one of the aggravating factors of atopic dermatitis. But the influence of VOCs on the development of atopic dermatitis is ambiguous in clinical field. So that, we investigate the relationships of indoor VOCs and atopic dermatitis development in Korean young children.
Method: From a birth cohort of a Mothers and Children's Environmental Health (MOCHE) study, 257 infant whose parents agreed with the environmental measurement were enrolled. Total VOCs (TVOCs) were measured using passive sampling systems (3M 3500 OVM badge) over 24 h, in the infant's bedrooms between 6 and 12 month. The parents answered a questionnaire at 36 month. Concentrations of TVOCs divided two groups by third quartile (75th). Correlations between high TVOCs expose group and atopic dermatitis development at the age of three were calculated by univariate logistic regression.
Results: The average concentration of TVOCs was 174.7 ± 115.1 /m^3. We determined high TVOCs expose group more than 152.1 /m^3 (75 percentile). High TVOC expose group more used carpets and pesticides compared than low TVOC expose group. There was no relationship between atopic dermatitis and smoking at home, papering of wall, new furniture, moving within 1 year. Increase risk of atopic dermatitis was observed to high TVOCs expose group (OR = 2.447; 95% Cl: 1.040–5.767). When using the calibration parameters as smoking and household income, the risk was still increased (OR = 2.577; 95% Cl: 1.080–6.145). When using the calibration parameters as parental history of allergy, the risk was not increased (OR = 2.148; 95% Cl: 0.873–5.281).
Conclusion: Our results show that exposure to high concentrations of TVOCs, the indoor pollutant increase the risk of atopic dermatitis. The data give concept that exposure of TVOCs during infancy influence to early childhood atopic dermatitis. But genetic background of allergic disease is also important to the development of atopic dermatitis.

1243
Comparative association of secondhand tobacco smoke with allergic rhinitis and asthma in a population based study

Lin, S[1]; Reh, D[1]; Navas-Acien, A[2]
[1]Department of Otolaryngology-Head & Neck Surgery, Johns Hopkins University School of Medicine, Baltimore, MD, United States; [2]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Background: Allergic rhinitis (AR) is estimated to affect up to 40% of the population in the U.S., and asthma 8%. Secondhand tobacco smoke (SHS) has been associated with both asthma and allergic rhinitis. In this study, we evaluate the association of SHS in a comparative manner to AR and asthma in a community-based study of adult nonsmokers. As a secondary objective we compared physician recommendations to avoid exposure to SHS among participants with AR, asthma and controls.
Methods: In a single large metropolitan community, 200 participants were recruited and interviewed. Subjects were assessed for allergic rhinitis (physician diagnosed or skin test positive), asthma (self-reported physician diagnosis) and compared to controls. A validated questionnaire was used to assess past and present SHS exposure as well as disease-specific quality of life, and administered by trained personnel in one-on-one interviews.
Results: AR was significantly associated with current SHS exposure ($P = 0.01$) and SHS exposure 20 years ago ($P = 0.03$); asthma was significantly associated with SHS exposure 20 years ago ($P = 0.05$) but not with current SHS exposure. Physicians were more likely to recommend avoidance of SHS to those with asthma (45.8%) than AR (28.6%) or controls ($P < 0.05$). Among controls, only 19.5% of non allergic participants and 15.8% of non-asthmatic participants reported that a physician had ever recommended to avoid SHS exposure.
Conclusion: While both past and current SHS were significantly associated with AR, only past SHS was associated with asthma. More participants with asthma self-reported that a physician had recommend to avoid SHS compared to AR participants, potentially contributing to past but not current SHS exposure with asthma. In view of the increasing evidence on the role of SHS in asthma and AR, physicians need to improve their medical recommendations regarding avoiding exposure.

1244
Relationship between the indoor residential environment and atopic dermatitis symptoms

Lee, S[1]; Kim, E[2]; Kim, J[1,3]; Han, Y[1]; Jeong, H[2]; Ahn, K[1,3]
[1]Samsung Medical Center, Environmental Health Center for Atopic Diseases, Seoul, Korea; [2]Department of Social and Preventive Medicine, Sungkyunkwan University School of Medicine, Seoul, Korea; [3]Samsung Medical Center, Department of Pediatrics, Sungkyunkwan University School of Medicine, Seoul, Korea

Background: We aimed to investigate the relationship between indoor air pollutant level at home and atopic dermatitis (AD) symptoms.
Method: In this panel study, 72 children with AD were recruited, and their demo-

graphic information was collected. We regularly measured indoor air pollutant levels more than two times during the study period from August 2008 to July 2012, and assessed AD symptom severity on the same day. The average concentration of indoor air pollutants such as particulate matter ($PM_{2.5}$, PM_{10}), formaldehyde (HCHO), Volatile Organic Compounds (VOCs), Bacterial aerosols and Airborne fungi was obtained according to the Korean Indoor Air Quality Standard Method. Linear Mixed Model (LMM) was used to assess the impact of the indoor air pollutants on the atopic dermatitis.

Results: PM, TVOC and bioaerosols did not show correlation with AD symptoms. In contrast, our results showed that AD symptom score was positively correlated with formaldehyde, while humidity was negatively correlated with pruritus. After adjustment with temperature, humidity and season, an increased concentration of formaldehyde by 10 ppb was associated with a 15.7% (95% confidence interval: −0.21–3.39) increase of AD symptoms.

Conclusion: Our data suggest that concentrations of indoor air pollutants such as formaldehyde may aggravate skin symptoms in AD.

1245
The exposure to tobacco smoke in children with wheezing

Aberle, N[1,2]; Kljaic Bukvic, B[1]; Vuckovic, M[1]; Blekic, M[1]
[1]Paediatric Department, General Hospital 'Dr Josip Bencevic', Slavonski Brod, Croatia; [2]Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

Background: Bronchoconstriction is frequent clinical sign in childhood. Epidemiological studies showed at least one bronchoconstriction in up to 50% of children while only 10% of children develop asthma. Many factors, mostly viruses and allergens but also excessive environmental tobacco smoke exposure (ETS) have influence on recurrent wheezing. Increased bronchial hyperresponsiveness (BHR) is a universally recognised phenomenon of asthma, but it is also present in children with recurrent non asthmatic wheezing. ETS exposure induces airway inflammation, mucociliary system injury, increase epithelial penetration, IgE sensitisation, and eosinophil count. The exposure to tobacco smoke is therefore one of the most important causes for the occurrence of wheezing.

Method: We examined 248 with allergic asthma (165% or 66.5% boys and 83% or 33.5% girls) and 129 children with nonallergic recurrent bronchoconstriction (75% or 58.1% boys and 54% or 41.9% girls) in relation to environmental tobacco smoke exposure.

Results: In the group of children with asthma 199 (80.2%) children were exposed to tobacco smoke (131% or 52.8% boys and 68% or 27.4% girls). In the group of children with recurrent bronhoconstriction of nonallergic aetiology, 102 (79.1%) children (52% or 40.3% boys and 50% or 38.8% girls) were exposed to tobacco smoke. It is clear that children in both examined groups were equally and very often exposed to tobacco smoke and the comparison showed that there is no statistically significant difference between children with allergic asthma and children with nonallergic asthma in relation to environmental tobacco smoke exposure ($\chi^2 = 0.08$, df = 1, $P > 0.05$).

Conclusion: The children in both groups were very often exposed to the environmental tobacco smoke and ETS exposure is probably a very important factor for wheezing evolution.

1246
Indoor air quality at day care centers is a risk factor for wheezing

Marques, JG[1,2]; Martins, P[1,2,3]; Papoila, AL[4,5]; Alves, M[5]; Caires, I[2,3]; Pedro, C[2,3]; Martins, JA[2,3]; Manilha, MC[6]; Cano, MM[6]; Mendes, AS[6]; Teixeira, JP[6]; Virella, D[5]; Viegas, J[7]; Aelenei, D[8]; Leiria-Pinto, P[1,2]; Rosado-Pinto, J[9]; Neuparth, N[1,2,3]
[1]Immunoallergy, Hospital de Dona Estefânia, Lisbon, Portugal; [2]Faculdade de Ciências Médicas da Universidade Nova de Lisboa, CEDOC, Lisbon, Portugal; [3]Pathophysiology, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Lisbon, Portugal; [4]Informatics and Biostatistics, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Lisbon, Portugal; [5]Centro Hospitalar de Lisboa Central, EPE, Gabinete de Apoio Estatístico e Epidemiológico, Lisbon, Portugal; [6]Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal; [7]Laboratório Nacional de Engenharia Civil, Lisbon, Portugal; [8]Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Lisbon, Portugal; [9]Hospital da Luz, Lisbon, Portugal

Background: Almost half of the children have at least one episode of wheezing before 6 years of age. Poor indoor air quality (IAQ) at day care centers (DCC) has been proposed has a risk factor for wheezing. Our aim was to study the wheezing incidence in two clusters of DCC, different in what concerns to IAQ.

Methods: In the scope of ENVIRH study (Environment and Health in children day care centers – PTDC/SAU-ESA/100275/2008) 19 DCC from Lisboa and Porto were selected. Stratified sampling followed by cluster analysis, to guarantee the heterogeneity of DCC concerning IAQ, was used. The selection was performed after an IAQ assessment at the end of 2010 in a larger number of DCC. A new IAQ assessment in those 19 DCC was performed at the end of 2011, with measurements of CO_2, CO, volatile organic compounds (VOCs), PM_{10}, indoor air temperature (IAT) and relative humidity (IRH). A questionnaire addressing wheezing symptoms was distributed ($n = 1229$). Hierarchical and logistic models were used for data analysis.

Results: From the 583 questionnaires considered in the analysis, 54% were boys and the mean age was 38 months (SD ± 19 months). At the end of 2011, one of the clusters had higher values of CO_2 ($P < 0.001$) and VOCs ($P = 0.021$), lower IAT ($P = 0.037$) and higher IRH ($P = 0.008$). No differences were found between clusters for PM_{10} ($P = 0.585$). In the multivariable analysis, only age (OR: 0.97, CI 95%: 0.96–0.98, $P < 0.001$) and belonging to the worst IAQ cluster (OR: 1.45, CI 95%: 1.01–2.04, $P = 0.042$) were associated with wheezing (45.6% in the worst IAQ cluster vs 36.6% in the other cluster).

Conclusion: Attending DCC with poor IAQ was a risk factor for wheezing. More attention should be dedicated to improve IAQ standards in DCC.

1247
Aspirin-intolerance and smoking history in Japanese patients with adult asthma

Hayashi, H; Taniguchi, M; Mitsui, C; Fukutomi, Y; Watai, K; Minami, T; Tanimoto, H; Oshikata, C; Ito, J; Sekiya, K; Tsuburai, T; Tsurikisawa, N; Otomo, M; Maeda, Y; Mori, A; Hasegawa, M; Akiyama, K
Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, Sagamihara, Kanagawa, Japan

Background: Chang et al. reported that there is a higher percentage of smokers among patients with aspirin-intolerant asthma (AIA) than in the general population (AAAI, 2012). However, there are few studies on aspirin sensitivity and smoking history in asthmatics.

Method: A case–control study was performed to compare smoking history (never smokers vs past or current smokers) among Japanese patients with AIA, those with aspirin-tolerant asthma (ATA), and healthy controls (HCs). The AIA patients ($n = 127$) were diagnosed by a systemic aspirin provocation test. The ATA-1 patients ($n = 100$) were definitely diagnosed by the systemic aspirin provocation test. The ATA-2 patients ($n = 1270$) were recruited from the Sagamihara National Hospital Database of Asthma. The HCs ($n = 1270$), who were living in Sagamihara City, were extracted using an epidemiological questionnaire. We compared smoking history among these groups with gender and age matching.

Results: The percentages of smokers (past or current) were 38.6% in the AIA group,

43.0% in the ATA-1 group, 39.2% in the ATA-2 group, and 33.1% in the HC group. The percentage of smokers (past or current) in the ATA-2 group was significantly higher than that in the HC group. However, there were no significant differences between the AIA group and the ATA-1, ATA-2, or HC group.
Conclusion: Aspirin sensitivity in Japanese asthmatics may not be affected by smoking history.

1248
Urban design for increasing quality of life of allergic asthma patients in immunotherapy process

Dincer, E[1]; Gungor, OC[1]; Yalcin, AD[2]
[1]Architecture Faculty, Istanbul Technical University, Istanbul, Turkey; [2]Internal Medicine, Allergy and Clinical Immunology, Antalya Education and Research Hospital, Antalya, Turkey

Background: Antalya district has been chosen for the case study. Because of rapid increase in population, illegal and unsanitary housing can be seen in Antalya. Besides these, agricultural and industrial structuring also affect allergic reactions in Antalya. Antalya is also a leading market for both cultural and coastal tourism.
Method: Three hundred Controlled allergic asthma patients studied. Fifty-one percent of the controlled patients are having allergic symptoms <5 years. Seventeen percent of the patients complained about highway, 6% of the patients complain about agricultural areas, 5% of the patients complained about industrial areas and 1% of the patients complained about tourism areas as urban facilities. Forty-two percent of the studied patients live in high-income (wealthy) residential areas though 28% of the patients live in low-income residential areas. Forty-four percent of the patients live in houses which have 100–150 m² living area, 32% of the patients live in houses which have 80–100 m² living area. Fifty-nine percent of the buildings built before <15 years and 95% of the buildings are concrete. Forty-five percent of the patients work in tourism areas, 7% of them work in industrial areas and 5% of the patients work in agricultural areas. Twenty-eight percent of the studied patients are either not working or housewives.
Results: Fifty-nine percent of the buildings built before <15 years and 95% of the buildings are concrete. As the building age increases allergic symptoms increases too. Patients who lives buildings which are older than 20 years, are patients who have allergic sypmtoms for medium (*P*:0.016) and long (*P*: 0.049) term. Patients who live in buildings which are 0–10 years old have allergic symptoms on Fall (*P*:0.009) and Winter (*P*: 0.000, 0.020), and patients who live in buildings which are 0–15 years old have allergic symptoms on Summer (*P*:0.009, 0.009, 0.016). Patients who live in wood homes have allergic symptoms mostly in Winter (*P*: 0.000). Also household concerns affect having allergic symptom period. While patients who are disturbed by Noise have allergi symptoms for short (*P*: 0.031) and medium (*P*:0.026) term, long term patients are commonly disturbed by cockroaches (*P*: 0.023) and wall cracks (*P*: 0.025) in their living areas. Moreover smoking have effect on allergic symptoms for short (*P*: 0.027) and long (*P*:0.002) term patients.
Conclusion: District, smoking, profession, building age, building type and building material have effects on allergens which patients are sensible to.

1249
Cigarette smoke as environmental factor induce inhibition of immune functions and DNA damage in alveolar macrophages

Takeuchi, M[1]; Hirono, Y[1]; Nose, M[1]; Inoue, S[1]; Sasaki, K[1]; Sakura, M[1]; Pinkerton, KE[2]
[1]Life Science, Kyoto Sangyo University, Kyoto, Japan; [2]UCD, Center for Health and the Environment, Davis, CA, United States

Background: Cigarette tobacco smoke is consisted from main-stream tobacco smoke (MTS) and side-stream tobacco smoke (STS). STS is released into the atmosphere, and may impact lung health and development of allergic lung diseases in non-smoker. STS is inhaled into the lung by respiration and affect to alveolar macrophage (AM). AM is playing an important role of immune system in the lung. However, the effect of STS on AM is not yet fully demonstrated compared with MTS. In this study, we investigated the effect of STS on DNA damage and immune functions in AM.
Method: Mice were exposed to STS of 20 cigarettes/day during 10 days by using STS exposure auto-machine. Extract of water soluble side-stream cigarette smoke (WSTS) was obtained by which STS was bubbled in sterile distilled water and freeze-dried. After STS exposure, AM were obtained by brochoalveolar lavage (BAL). TLRs and phagocytic activity, reactive oxygen species(ROS) generation of AM were determined by FACS. Expressions of cytokines mRNA of AM were measured by RT-PCR. DNA damage of AM was evaluated by comet assay.
Results: The number of AM was significantly increased in STS exposed mice. The cell size and intra-cellar structure of AM were changed by STS. Phagocytic activity of AM was significantly inhibited by STS. Expressions of CD11b, TLR-2, TLR-4 and CD14 on AM were significantly inhibited by STS. ROS generations of AM were significantly increased by STS exposure. Expression of TNF-α mRNA in AM was significantly inhibited by STS. Tail moment and length of AM as indicator of DNA damage were significantly increased by STS. DNA damage in AM was also induced by WSTS at dose dependent.
Conclusion: STS exposure caused the change of cell size and intracellular structure in AM. STS and WSTS induced significantly increase of DNA damage in AM. The phagocytic activity, expressions of CD11b, TLR-2, TLR-4, CD14 and TNF-α mRNA in AM were decreased by STS. STS was a risk factor for DNA damage of AM and inhibited the immunological functions in AM mediated by DNA damage. These results suggest that inhibition of phagocytosis, TLR expression and STS induced-DNA damage of AM may associated with infection and development of allergy in the lung.

1250
Use of kotinin levels in creating awareness for environmental tobacco smoke exposure

Nacaroglu, HT[1]; Can, D[1]; Gunay, I[1]; Unsal Karkıner, CS[1]; Gunay, T[2]; Cimrin, D[3]; Nalcabasmaz, T[1]
[1]Department of Pediatric Allergy, Dr Behcet Uz Children's Hospital, Izmir, Turkey; [2]Department of Public Health, Faculty of Medicine, Dokuz Eylul University Izmir, Turkey; [3]Department of Biochemistry, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey

Background: Environmental tobacco smoke (ETS) exposure is a known cause of chronic respiratory problems. This is important for wheezy child as number and severity of attacks may increase. Our aim in this study is to find if parents would take necessary steps and decrease ETS exposure when they know the level of exposure by means of urinary kotinin levels.
Method: Our study group was consisted of families whose children were under age 3 and had episodic wheezing and at least one of the parents was smoking but not in the same environment with the child. They were given leaflets about ETS exposure after enrolling the study. Demographic information and data on smoking features were recorded. Urinary kotinin levels were determined at the first visit. Children with positive kotinin levels randomly assigned to two groups. Study groups were informed about kotinin levels by phone call. Control group was not informed

about kotinin levels. Children from both groups were called for a follow-up visit. At the follow-up visit, urinary kotinin were repeated.

Results: Kotinin levels of 237 children with episodic wheezing were evaluated. Kotinin levels were negative in 44 children. A total of 59 (30.5%) children from both groups did not come to follow-up visit and excluded from the study. In the study group there were 65 children (46 boys-70.8% and 19 girls-29.2%) and in the control group 69 children (52 boys-75.4% and 17 girls-24.6%). Mean age on admittance were 24.4 ± 8.9 months for study group and 25.3 ± 9.8 months for control group. There were no significant difference between sex and age of the groups. Kotinin levels for both study and control groups were decreased in follow-up but there were no significant difference ($P > 0.05$). Number of cigarettes smoked by father was decreased significantly in the study group. Changes in smoking habits (Number of cigarettes smoked in the house by mother, smoking in the car, etc) were mostly same between two visits of the both groups except number of. Also the percent of kotinin negative children in the follow-up visit were identical in both groups ($P > 0.05$).

Conclusion: Decreasing environmental smoke exposure is crucial prophylaxis of chronic respiratory illnesses including episodic wheezing. Mentioning the ETS exposure as a risk factor would increase the awareness of the family. Measuring kotinin levels is a practical, efficient and noninvasive procedure. Families with risk, would benefit from getting informed on kotinin levels.

1251
The prevalence of allergic rhinitis with a remarkable association with passive smoking

Souzanchi, G; Fazlolahi, MR; Pourpak, Z; Tazesh, B; Entezari, A; Moin, M
Immunology Asthma and Allergy Research Institute, University of Medical Sciences, Tehran, Iran

Background: Allergic rhinitis (AR) is a global health problem and one of the most common respiratory disorders that reduces quality of life. The prevalence of Allergic rhinitis in Iranian adults is not precisely determined. The aim of this study was to evaluate the prevalence of AR and it's relation with smoking behavior in Iranian adults.

Method: A telephone interview survey was performed using 16 500 randomly selected telephone numbers in Tehran, Iran from 2009 to 2010. A modified European Community Respiratory Health Survey (ECRHS) questionnaire was completed for adults aged between 20 and 44 years old.

Results: There were 5500 responses to 7150 eligible telephone numbers dialed (76.92%). The study populations included 3412 female (62%) and 2088 men (38%) with the mean age of 31.15 ± 7.33. Among all participants, 26/7% suffered from allergic rhinitis.

In this study, there was no significant relation between smoking and AR, however we found a remarkable association between passive smoking and AR (*P*-value = 0.02).

Conclusion: The prevalence of allergic rhinitis in our study was higher than the mean prevalence of allergic rhinitis in the word. We found an interesting result in our study that indicates the significant association between passive smoking and AR.

In addition to increase the diesel exhaust fumes in Tehran, the other considerable reasons for the higher prevalence of allergic rhinitis, may be because of people's unawareness from the main cause of allergic rhinitis and their avoidance to allergen and irritant (such as tobacco smoking) exposure.

1252
Environmental risk factors contributing to allergic symptoms among Georgian schoolchildren

Abramidze, T; Gotua, M; Rukhadze, M; Dolidze, N; Gamkrelidze, A
Center for Allergy and Immunology Research, Tbilisi, Georgia

Background: The epidemiological investigations have revealed significant differences in allergy prevalence even amongst those of the same ethnic background, advocating that, notwithstanding the importance of genetic factors, environmental factors are likely to be responsible for the observed prevalence gradients and time trends. This study examined the relationship between environmental risk factors and presence of allergic symptoms among Georgian schoolchildren.

Method: We used ISAAC phase III study methodology and core questionnaire for design and data collection. Questions about environmental factors were included in an additional questionnaire. Associations between environmental factors and presence of allergic symptoms were investigated using logistic regression.

Results: The prevalence of asthma, rhinoconjunctivitis and atopic dermatitis symptoms in the western part of Georgia were 11.2% (95%CI, 10.3–12.1), 7.1% (95%CI, 6.4–7.8) and 4.2% (95%CI, 3.7–4.7) correspondently. The risk factors associated with asthma symptoms: family history of allergy [OR 2.3; (95% CI, 1.9–2.8)], food allergy [OR 3.0; (95% CI, 2.5–3.7)], drug allergy [OR 1.8; (95% CI, 1.4–2.4)], mother smoking at present [OR 2.2; (95% CI, 1.3–3.6)], cat exposure at present [OR 1.4; (95% CI, 1.1–1.7)] and consumption of cereal three times or more per week [OR 1.3; (95% CI, 1.1–1.6)]. The risk factors associated with rhinoconjunctivitis: family history of allergy [OR 2.8; (95% CI, 2.3–3.6)], and food allergy [OR 2.9; (95% CI, 2.2–3.6)], drug allergy [OR 2.8; (95% CI, 2.1–3.6)], cat exposure at present [OR 1.5; (95% CI, 1.1–2.0)], cooking with open fire [OR 1.75; (95% CI, 1.1–2.9)] and consumption of fast-food three times or more per week [OR 1.4; (95% CI, 1.1–1.7)]. The risk factors associated with atopic dermatitis symptoms: family history of allergy [OR 3.3; (95% CI, 2.5–4.4)], and food allergy [OR 5.42; (95% CI, 4.1–7.2)], drug allergy [OR 3.04; (95% CI, 2.1–4.4)], mother smoking at present [OR 2.7; (95% CI, 1.4–5.5)], father smoking at present [OR 1.4; (95% CI, 1.0–1.8)], cat exposure at present [OR 1.8; (95% CI, 1.2–2.5)] and dog exposure at present [OR 1.5; (95% CI, 1.1–2.0)].

Conclusion: Family history of allergy, presence of other allergy disorders, pet's exposure; tobacco smoke exposure and diet are among the risk factors associated with atopic disease in Georgian schoolchildren.

1253
Nasal inflammatory response against a tobacco leaves extract

De Mateo, B[1]; Chivato, T[2]; Nuñez, A[1]; Bartolomé, B[3]; Gómez de Terreros, FJ[4]; Gutierez, C[4]
[1]Alergología, Hospital Central de la Defensa Gómez Ulla', Madrid, Spain; [2]School of Medicine, Boadilla, Madrid, Spain; [3]Laboratorio Bial Aristegui, Bilbao, Spain; [4]Hospital Central de la Defensa Gómez Ulla', Madrid, Spain

Background: The association between exposure to environmental smoked tobacco and development of allergic diseases has been studied previously without definitive conclusions. Thus, the relationship between tobacco, inflammation and allergic rhinitis precises studies to clarify this possible relationship.

Objectives: To test whether the extract of tobacco leaves causes inflammation of the nasal mucosa.

Methods: Forty-three patients diagnosed with idiopathic chronic rhinitis (ICR) and 39 healthy subjects (control group) were evaluated. Skin tests were performed with common pneumo allergens and tobacco leaves extract. Nasal challenge test was performed with tobacco leaves extract.

Total IgE, tobacco specific IgE, Tryptase, Myeloperoxidase and ECP were determined in serum and nasal lavage. Nasal lavage cells were quantified before and after nasal challenge test. Results were statistically analysed.

Results: Group ICR had 17 men and 26 women with a mean age of 46.6 years. Control group had 11 men and 28 women with a mean age of 39.4 years. Thirteen patient from the ICR group and 14 from the control group were active smokers. Hay fever was diagnosed in 17 patients in ICR group and seven in the control group. Significant differences were observed between concentrations of total IgE in patients with and without hay fever (97.9 vs 50 KU/l) and between serum ECP of smokers vs nonsmokers (16.7 vs 11.9 μg/l). Positive skin tests were obtained with tobacco leaves extract in five cases. Thirty-two subjects showed positive nasal challenge tests. There were no significant serum levels of specific IgE to tobacco leaves extract (<0.35 kU/l). No differences in cell counts in nasal washes were observed.

Conclusion: The extract of tobacco leaves is capable of generating inflammation of the nasal mucosa. The inflammatory response to extract tobacco leaves is most common in individuals with chronic rhinitis. Inflammation generated is not dependent of IgE.

1254
The effect of PM10 on the symptoms of allergic rhinitis patients in spring season

Kim, S-T[1]; Ju, Y-H[1]; Jung, J-H[1]; Kang, I-G[1]; Ko, K-P[2]; Oh, D-K[2]; Kim, Y-H[3]; Jang, T-Y[3]
[1]Gil Medical Center, Otorhinolaryngology, School of Medicine, Gachon University, Incheon, Korea; [2]Department of Preventive Medicine, School of Medicine, Gachon University, Incheon, Korea; [3]Department of Otorhinolaryngology-Head and Neck Surgery, Inha University College of Medicine, Incheon, Korea

Background: PM10 (particulate matter <10 μm) is known as a major components of air pollutant which affect the allergic symptoms. We have studied the effects of PM10 on the symptoms of allergic rhinitis patients in spring season.

Method: We have investigated the allergic symptom change (rhinorrhea, sneezing, nasal obstruction and sleep disturbance), nasal obstruction level which was measured by portable peak nasal inspiratory flowmeter (PNIF) in 108 allergic patients. Fourty-seven control people were also included. The spring pollen counts and PM10 concentration of Incheon city were also evaluated. We have done the long term period observation of PM10 effect on allergic symptoms for 120 days from February to May and also compared the symptom score change before and after 2 days when the PM10 concentration was peaked over 100 $\mu g/m^3$ (short term).

Results: The highest concentration of PM10 was <150 $\mu g/m^3$ during the study period. The statistics showed that there was no significant correlation between the PM10 and the nasal obstruction ($P = 0.0670$), rhinorrhea ($P = 0.1485$), sneezing ($P = 0.3991$), itching ($P = 0.2044$), sleep disturbance ($P = 0.4413$) and the sum of all symptoms ($P = 0.0816$). However, the variability of nasal obstruction, which was checked by PNIF, was significantly correlated with the PM10 ($P = 0.0410$). There was also a significant correlation between the out-door activity time and the sum of clinical symptoms ($P < 0.001$). The PNIF in the afternoon has shown a significant correlation with the out-door activity, however no correaltion with the pollen concentration. The frequency of drug use and PNIF were not affected by the short term change of PM10. In the long term.

Conclusion: This study has shown that PM10 concentration in the spring season might influence the nasal obstruction, which was checked by PNIF and this nasal obstruction was also influenced by the out-door activity period.

1255
Location of smoking at home, asthma control and lung function in school children

Suzuki, S; Watanabe, H; Nezu, Y; Sato, K; Nishimuta, T
Department of Pediatrics, Shimoshizu National Hospital, Yotsukaido, Japan

Background: Tobacco cessation in family members is essential in management of pediatric asthma but is often difficult to achieve. It is unclear whether stopping indoor smoking in family members can be recommended for patients with pediatric asthma.

Method: We evaluated school children with asthma who visited our hospital between December 2011 and January 2012 and collected data on presence of smokers in family members and location of smoking (indoor or only outdoor). Urinary cotinine was measured by ELISA. The primary outcome was acute exacerbations during the last 1 year and lung function after inhalation of bronchodilator.

Results: A total of 93 patients were evaluated. The proportion of patients with a low urinary cotinine level (below 4 ng/mg creatinine: 20th percentile in participants) was 100% in patients without smoking family members ($n = 35$), 90% in patients with outdoor-smoking family members ($n = 22$), and 44% in patients with indoor-smoking family members ($n = 36$). The rates in regular use of inhaled corticosteroids and leukotriene receptor antagonists were similar among three groups. The proportion of patients who had no acute exacerbation during the last year were 82%, 85%, and 58%, and the average peak expiratory flow rate after inhalation of bronchodilator were 102%, 100%, and 92%, respectively. Only the values in patients with indoor-smoking family members were significantly lower than other groups of patients. Similar results were found on maximal midexpiratory flow and flow at 50% remaining vital capacity after inhalation of bronchodilator.

Conclusions: Outdoor smoking in family members, unlike indoor smoking, seems not to be associated with poor asthma control and decreased lung function in school children with asthma. Our results may substantiate the recommendation that smoking family members of patients with pediatric asthma should smoke outdoor for better outcomes.

1256
Effect of second hand smoking on athma severity in children

Irani, C[1,2]; Saliba, J[1]
[1]Internal Medicine/Allergy&Immunology, Hotel Dieu de France Hospital, Beirut, Lebanon; [2]Pulmonary, University of Alberta, Edmonton, AB, Canada

Background: Asthma is a common chronic disease involving airflow obstruction, bronchial hyperresponsiveness and an underlying inflammation. Many immunologic and non immunologic environmental factors are important triggers of asthma including cigarette smoking and second hand smoke (SHS). Cigarette smoking and SHS in asthmatics lead to detrimental effects in patient outcomes and effectiveness of steroid therapy.

It is associated with worse lung function, higher acuity of exacerbations, more health care utilisation, and greater bronchial hyperreactivity. Passive smoking is also related to poor quality of life among asthmatic adolescents and increased respiratory-related school absenteeism.

Method: A cross sectional study was conducted from February 2012 to December 2012. The study included 100 children between 2 and 18 years old with asthma. The information concerning the patients was collected from their medical records filled out by the physician in a clinical setting in direct communication with the patients, or their parents when it is a little child.

Children were divided into two groups: the first was experimental (E) and included 28 children from smoking families, and the second consisted of controls (C) which contained 73 children from non-smoking families. We explored the effect of second-hand smoking on the number of respiratory infections, asthma exacerbations per year, nocturnal symptoms and hospital admissions.

Results: Using multiple correspondence analysis, we found that Second hand smoking is positively correlated with nocturnal asthma symptoms, at least one exacerbation per year, at least one respiratory infection per year and at least one hospital admission per year for asthma exacerbation with a test value (>2). The absence of second hand smoking is negatively correlated with nocturnal symptoms, presence of exacerbation/year, respiratory infection and hospital admission.

Conclusion: Second Hand Smoking causes respiratory illness, asthma, poor growth, neurological disorders in children. Counseling parents for smoking cessation may reduce asthma severity and the rate of asthma exacerbation in children. To avoid the risk of respiratory and allergic disease by environmental tobacco smoke, absolute smoking cessation by parents is strongly recommended.

1257
Correlation of tropomyosin sensitisation of mite and shrimp and the severity of asthma in children

Lin, Y-T; Cheng, J-H; Huang, J-L; Yeh, K-W
Chang Gung Memorial Hospital, Taoyuan, Taiwan

Background: Food allergen and aeroallergen sensitisation are risk factors of asthma. Muscle tropomyosin is one of the major allergen responsible for ingestion-related allergic reaction of crustacean (shrimp or crab) and this allergen is also a cross-reacting allergen of aeroallergens, such as dust mites or cockroach. This study investigated the relationship between sensitisation to allergen of tropomyosin and the severity of asthma in children.

Method: A total 88 children (0.7–12 years) with physician-diagnosed mild to severe asthma were recruited in the Pediatric Allergy and Asthma Center of Chang Gung Memorial Hospital during September to December 2012. Their serum were collected and evaluated specific IgE of shrimp, crab, mite, cockroach, Pen a1 (shrimp tropomyosin) and Der p10 (mite tropomyoisin) by ImmunoCAP (Phadia, Sweden). An allergen-specific IgE level ≥0.35 KU/l was defined as positive. Classification of asthma severity was based on GINA guideline by a pediatric allergist. The subjects were divided by three groups by age to investigate different distribution of sensitisation.

Results: It is significant that 95% of asthmatic children in group of age of 4–6 years sensitised to mite than the other groups (Chi-square, $P < 0.001$). Otherwise, shrimp, cockroach, crab, Pen a1 and Der p10 sensitisation didn't show significant difference by age. Regarding to asthma severity presentation, children with age above 6 years had 5.8-fold risk presenting moderate severity than the group below age of 4. Furthermore, children sensitised to shrimp, cockroach or mite also increased risk of severity of asthma. After adjusting for age and shrimp, cockroach and mite sensitisation, sensitisation to cockroach and age were stronger associated with the severity of asthma (>6 years, OR = 5.19, $P = 0.012$; cockroach, OR = 3.67, $P = 0.033$). However, children who sensitised to Pen a1 and Der p10 had no risk for severity of asthma.

Conclusion: Children at age group of 4–6 years had higher sensitisation rate to mite. Children with cockroach sensitisation or older age had higher risk for the severity of asthma. Tropomyosin sensitisation, whatever of mite or shrimp, was not related to severity of asthma in children.

1258
Cat sensitisation increases risk of asthma and allergic rhinitis among mite sensitive children

Panichwattana, W; Piboonpocanun, O; Veskitkul, J; Visitsunthorn, N; Pacharn, P; Vichyanond, P
Division of Pediatric Allergy and Immunology, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Background: Sensitisation to cat allergen is an important risk factor for the development of allergic diseases in children. In Thailand, it was estimated that 6–10% of allergic children were sensitised to cat. Recently, pet keeping has become a common practice in Thailand.

Objective: We aim to study prevalence of sensitisation to common aeroallergens including to cat and dog among children attending pediatric allergy clinic at Siriraj Hospital from January 1st to December 31st, 2011.

Method: A retrospective chart review of 501 children seen at pediatric allergy clinic was conducted. Nine charts were excluded because of incompleteness of data. Demographic data including age, sex, type of allergic diseases and presence of pets in household were reviewed. Positive skin prick test was defined as wheal size ≥3 mm.

Result: Mean age of 492 patients was 6.9 ± 3.9 years. There were 302 males (61.4%) and 190 females (38.6%). Three hundred and eighty-four patients had allergic rhinitis (78%) whereas 156 had asthma (31.7%) and 72 had atopic dermatitis (14.6%). Overall, 329 patients had positive skin testing (66.9%). Prevalence of sensitisation to house dust mites (HDM), cockroach, grass, cat and dog were 53.5%, 46.9%, 18.9%, 12.8% and 10.4%, respectively. The increase in prevalence of cat sensitisation compared to our earlier data (1997) was not statistically significant (OR 0.76, CI 0.37–1.53, $P > 0.05$). Among 63 patients who were allergic to cats, 57 were also sensitised to HDM (90.4%). Asthma as well as allergic rhinitis was more commonly observed among dual sensitised (cat and mite) individuals than among those sensitised to mite only (OR = 2.84, CI 1.54–5.24; OR 2.88, CI 1.3–6.3, $P < 0.01$).

Conclusion: Prevalence of cat sensitisation among Thai children appeared to be increasing. Dual sensitisation to cat and mite appeared to increase risk of asthma as well as allergic rhinitis compared to sensitisation to mite only.

1259
The high prevalence of sensitisation to pollen allergens in adult patients with respiratory allergy in Kuwait

Alenizi, AA[1]; Arifhodzic, N[1]; Alenezi, AA[2]; Alanezi, AT[3]
[1]Al Rashed Allergy Centre, Allergy Department, Kuwait, Kuwait; [2]Sabah Al Nasser Polyclinic, Kuwait, Kuwait; [3]Jahra Hospital, Kuwait, Kuwait

Background: Sensitisation to inhalant allergens in patient with Allergic rhinitis and/or Asthma is rather high in Kuwait. Because of that respiratory allergies are one of the most common chronic diseases affecting all age groups.

Aim: To assess the most common sensitising allergens in adult patients with clinical symptoms of respiratory allergy.

Methods: One thousand four hundred and fifteen randomly selected adult patients, mean age 42.9 ± 12.9 years, both gender, who were referred to the tertiary Allergy Centre for further evaluation have been skin tested with a battery of inhalant allergens (Stallergenes), which includes main indoor allergens, pollens typical for desert climate and molds. Both local people and expatriates were included. All patients were divided into three age groups (15–40, 41–60, and >60 years). Skin prick test (SPT) was done using prick lancets, and was considered as a positive if the longest wheal diameter is 3 mm or bigger than negative control.

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

Results: From total of 1415 patients, 1044 (73.8%) had positive SPT either to indoor allergens (DP, DF, Cat dander), pollens (Salsola kali, and Bermuda grass) and molds (Cladosporium, Alternaria, and Aspergillus). Rate of sensitisation was similar in both Kuwaitis and expatriates (74.3% vs 72.6%; $P < 0.50$). There was no statistically significant difference between genders (71.8% males vs 75.4% females; $P < 0.13$). Sensitisation to outdoor allergens highly prevailed in all patients. 51.5% of all skin positive patients have been sensitised to pollens only, 19.2% to both indoor and outdoor allergens and 7.3% to molds ($P < 0.02$). Sensitisation to pollen allergens was significantly more frequent in younger and middle age group (58.9%, and 45.8%), and also remained the highest sensitising factor in elderly patients (43.2%).

Conclusion: Regardless of desert climate in Kuwait, sensitisation to local pollen allergen is the leading cause of sensitisation in adult asthmatic and/or allergic rhinitis patients. Pollens in desert environment, known as having high allergen potency, together with other factors (typical desert climate, high pollution) play significant role in development of respiratory allergy in our region.

Poster Session 50

Airborne allergens I

1260
The trend of airborne pollen in Parma, Northern Italy from 1994 till 2011

Ugolotti, M[1]; Vitali, P[1]; Albertini, R[2,3]
[1]Hospital Hygiene Unit, University Hospital of Parma, Parma, Italy; [2]Department of Clinical and Experimental Medicine, University of Parma, Parma, Italy; [3]Medical Immunology Unit, University Hospital of Parma, Parma, Italy

Background: Pollen grains, their allergens and environment play an important role in the pathogenesis of respiratory allergies increasing worldwide following anthropogenic and non-anthropogenic factors. Further, climate change influences the production, dispersion and allergenicity of anemophilous pollen and the growth and distribution of weeds, grasses and trees that produce it. The present study was carried out to ascertain the variations of the pollen season parameters in Parma (Northern Italy) from 1994 till 2011.
Method: The monitoring of pollen was performed from our survey center in accordance with standard methods of the Monitoring Italian Network in Aerobiology (R.I.M.A.®) of Italian Aerobiology Association (AIA). Airborne pollen grains were collected using a type Hirst 7-day recording volumetric spore-trap, standard equipment used for aerobiological sampling worldwide. Ten taxa were taken into account (*Alnus, Betula*, *Corylus*, Cupressaceae, Poaceae, *Artemisia, Plantago, Platanus, Ambrosia*, Urticaceae), considering: season start, end, duration, peak day, peak value and seasonal pollen index (SPI).
Results: The data shown:

1 a significant negative trend of duration (*Platanus*, Urticaceae) and SPI (*Corylus, Betula*, Poaceae);
2 a positive trend of start (*Artemisia*), peak day (*Platanus*); SPI and peak value (*Ambrosia*);
3 significant correlations between end and duration (*Alnus* and Poaceae); start and duration (*Betula; Plantago*); SPI and peak value (Corylus; Cupressaceae; *Betula; Plantago; Platanus*; Poaceae; *Artemisia; Ambrosia)*. The shortest pollen season was for *Platanus*, while the longest was for Urticaceae. The highest SPI was for Urticaceae.

Conclusion: The aim of our aerobiological observation was to define our regional' pollen spectrum. Although we didn't found significant trend of temperature and rainfall we have observed significant changes of some season pollen parameters. However, not all the changes were in the same direction. Our results can supplying information about the release of airborne pollen grains related to some climate parameters, that apparently not confirm a climate change in our area of observation, at least considering rainfall and temperature data of last 17 years. We believe that our data can be useful in managing the symptoms of patients suffering from pollinosis.

1261
Pollen forecasts to reduce hay fever symptoms may impact psychological conditions of allergic patients

Gelman, E[1]; Rodinkova, V[2]; Krivonos, T[3]; Kondratuk, A[3]; DuBuske, L[4]; Slobodianiuk, LV[5]
[1]Medical Division ■1, Vinnitsya National Pirogov Memorial Medical University, Vinnitsa, Ukraine; [2]Pharmacy, Vinnitsa National Pirogov Memorial Medical University, Vinnitsa, Ukraine; [3]Medical Psychology and Psychiatry, Vinnitsa National Pirogov Memorial Medical University, Vinnitsa, Ukraine; [4]Immunology Research Institute of New England, Gardner, United States; [5]Pharmaceutical Chemistry, Vinnytsia National Pirogov Memorial Medical University, Vinnytsia, Ukraine

Background: Allergy including hay fever may induce psychological conditions, especially at the beginning of the pollen season including increased anxiety level related to the expectation of hay fever symptoms.
Method: To determine the volunteers' psychologic status, tests including 'Assessment of degree of asthenic conditions' (Malkova), 'Assessment of degree of low mood sub-depression' (Zung), and the 'Mini-Mult Questionnaire' (Spielberger) were used. The study occurred at the beginning of the grass pollen season in Vinnitsa in 2012 including 30 men and 20 women age 18–50 years old with hay fever symptoms. The control group included 25 males and females 18–50 years old without seasonal allergy symptoms. The utility of pollen forecast were determined by testing the patients who use the forecast during the pollen season. Testing was done with the assistance of the Psychology Department of the Vinnitsa National Pirogov Memorial University. The aim of the test was to establish the level of anxiety of users while waiting for the pollen peak days. The test was placed in the '20 Minutes' local daily newspaper web-site and in the web-site of radio company 'Misto nad Bughom' in connection with the pollen forecast from July to October, 2012. The questionnaire was answered by 124 persons, 73 females (58.9%) and 51 males (41.1%).
Results: Most basic psychological tests were similar in the control group and allergy patients. The Mini-Mult test showed more depression (49 vs 43 points) and low mood for females in comparison with males of the control group (54 vs 47 points). However, for patients a reverse low mood pattern was observed: 40 low mood points for allergic women vs 53 low mood points for allergic men. The internet questionnaire showed the largest number of answers (34.7%) in a variant determining the forecast as a means to reduce the personal anxiety of patients associated with sub-depression during the pollen season. The second position (24.2%) was held by the utility of the pollen forecast associated with the symptoms which appeared.
Conclusion: Women both suffering from allergy symptoms and interested in the pollen forecast were a more sensitive group in relation to hay fever anticipation before the pollen season and anxiety during the pollen season. Information about pollen counts may impact allergy patient's mood and anxiety level.

1262
Back-trajectories analysis of olive pollen in Eastern Spain

Soriano, V[1]; Crespo, J[2]; Varea, M[2]; Cueva, B[3]; Gonzalez, P[3]; Ramon, A[4]; Fernández, J[5]
[1]UMH, Allergy, Clincal Medicine, Alicante University Hospital, Alicante, Spain; [2]Department of Physics and Computer Architecture, Universidad Miguel Hernandez, Elche, Spain; [3]UMH, Allergy, Alicante University Hospital, Alicante, Spain; [4]Allergy, Alicante University Hospital, Alicante, Spain; [5]UMH, Allergy, Clinical Medicine, Alicante University Hospital, Alicante, Spain

Background: Olive pollen is highly allergenic, but not all olive varieties have the same content on the molecular allergens. In our area of Alicante in eastern Spain, olive varieties are grown different from

southern Spain. Our aim was determined the origin of pollen in highest concentration peaks by back-trajectories from 2009 to 2012.

Method: We analyzed the data pollen counts of Elche city since 2009 until 2012. Elche (Alicante) is located in eastern Spain, with mild temperatures and early spring. The pollen counts were obtained with Burkard type collector and peaks of high concentration were selected each year. In order to identify the pollen origin area, for the selected sampling days, 3-day back-trajectories of the air masses arriving to Elche were calculated. We used the HYSPLIT 4 transport model (NOAA Air Resource Laboratory) at three different levels above the ground (100, 500 and 1000 m).

Results: We found 5–6 peaks of high concentrations of olive pollen on average per year, with variations in the onset of the first peak from March to May. The pollen concentration of the highest peaks not exceeded 700 pollen grains per cubic meter of air (g/m^3), far away from the 2500 g/m^3 of Jaen and Seville in southern SpainBack-trajectory analysis showed that, the late and high peaks come from southern Spain, especially Jaen and Seville. The earliest peaks coincide with flowering of the local community and the Murcia nearby.

Conclusion: The study of the movements of air masses allows us to know the place of origin. Knowing the olive variety grown in the places of origin of our olive spring pollen, we can improve the knowledge of the different molecular allergens present in our area.

1263
Aerobiological and biochemical studies on airborne fungal allergens: a major contributory factor to respiratory allergy related hospitalisation

Chakrabarti, HS; Gupta-Bhattacharya, S
Division of Plant Biology, Bose Institute, Kolkata, India

Background: In India more than 70% of the total allergic patients are suffering from respiratory allergy, of which 35% are due to fungal aeroallergen. The aims of this study were (i) to study prevalent fungal aero-allergenic fungal species in Kolkata (ii) to analyze their clinical contribution to manifestation of respiratory allergy and related hospitalisation, and (iii) to identify and characterise the major allergens in *Trochoderma harzeanum*.

Method: Five years aeromycological sampling was done in Kolkata. Allergenic activities of prevalent fungi were studied by SPT, PFT & ELISA. Detailed medical history of allergic patients and hospitalisation data were collected from hospitals. Major allergens were identified and characterised using different proteomic tools.

Results: Twenty-one prevalent airborne fungal spores were recorded in Kolkata. Their concentration peaked during monsoon (June–August) and lowered during winter (January–February). Local meteorological factors showed significant effects on aerospora load. A predictive model to forecast aerospora load was developed by multiple regression analysis with 58–82% accuracy level, considering the common meteorological parameters as predictors. 68.7% of the studied respiratory allergic patients (2154 in no.) were noticed sensitive to 14 different fungal species in SPT; 37% of them showed 2+/3+ level of reaction. ELISA revealed that all the sensitive patients exhibited high concentration of total IgE, as well as respective allergen specific IgE in their sera. When Pulmonary Function Test was performed, 84% of patients were noticed to have mild type of lung function impairment. Emergency hospitalisation due asthma and rhinitis showed positive correlation ($r = 0.745$; $P = 0.011$) with the aerospora concentration. 1D & 2D immunoblotting with *T. harzeanum* sensitive patients' sera showed 3 and 7 IgE reactive bands & spots respectively, among which a 34.5 kDa spot was detected as the major allergen, which was identified by MASCOT score, MALDI-TOF-TOF, followed by Uniprot and Kognitor database analysis. This 34.5 kDa protein was found to be highly allergenic when assessed by Allermatch™ web tool.

Conclusion: The studied area is heavily contaminated with fungal aeroallergens, which are significant causative agents for severe respiratory allergy. The spore calendar and prediction model can be useful for the right diagnostics and treatment of inhalant allergy. *T. harzeanum* carries a 34.5 kDa protein which is highly allergenic in nature.

1264
Fungal contamination of households as a risk factor for respiratory allergy

Maslova, L[1]; Novikova, T[1]; Titov, LP[2]; DuBuske, LM[3]
[1]Belarusian Medical Academy of Postgraduate Education, Minsk, Belarus; [2]Republican Scientific and Practical Center for Epidemiology and Microbiology, Minsk, Belarus; [3]Immunology Research Institute of New England, Gardner, United States

Background: Indoor mycobiota impact development and occurrence of symptoms of respiratory allergy including allergic rhinosinusitis and asthma.

Method: A questionnaire was used to assess the living conditions of patients including evidence of fungal contamination. Mycological examination of premises including sampling locations of mold damage and selection of fungi for culture was done.

Evaluation of 174 patients living in the fungal contamination included a medical history, allergic history, physical examination, skin testing, and blood samples for mold specific IgE.

Results: Mycological analysis of the isolates showed microscopic fungi spores including genera Penicillium, Cladosporium, Aspergillus and Ulocladium. A total of 174 people were living in areas affected by fungi. Fifty-nine people (33.9%) had sensitisation to fungal allergens, including presence of allergen specific IgE. Almost half of the sensitised patients (16.7%) were sensitised to several species of fungi. Thirty-three people (19%) had asthma. Asthma with fungal sensitisation was seen in 21 patients, six of whom had isolated fungal sensitisation, while 15 had fungal sensitisation combined with sensitisation to house dust mite allergens.

Conclusion: Among patients living in residential areas having significant fungal growth, there is increased sensitivity to fungal allergens in isolation or in combination with other types of sensitisation.

1265
Relevance of ash pollen exposure and sensitivity in school children assumed by microarray

Gassner, M[1,2]; Gehrig, R[3]; Pietragalla, B[3]; Schmid-Grendelmeier, P[4]
[1]Allergology, Grabs, Switzerland; [2]Institut of Primary Care, University Hospital of Zurich, Zurich, Switzerland; [3]Bio-Environmental Applications, Federal Office of Meteorology and Climatology MeteoSwiss, Zurich, Switzerland; [4]Allergy Unit, Department of Dermatology, University Hospital, Zurich, Switzerland

Background: Patterns of allergic sensitisation to pollens have changed. Allergies to tree pollen with here antibody cross reactivities to food allergens becomes more important industrialized countries. Ash is a common indigenous tree of the oleacea, growing in middle and nordish European countries, where other *Oleacea* allergens such as olive pollen are not growing.

Methods: We gathered information on allergic symptoms, serologic findings among 15 year-olds unselected school children in Grabs from 1983 through 2007 and pollen exposition. Atmospheric pollen levels were measured in Buchs since 1984. IgE antibodies against ash trees (*Fraxinus americana*, CAP t15) birch and grass pollen are compared with the pollen count. Sera from 46 students in 2006 were compared to 103 molecular allergens by using ImmunoISAC test.

Results: IgE antibodies against ash tree pollen (Fraxinus americana, CAP t15) were found in as often as the main tree pollen allergen birch, but not in a strong correlation, most probably due to very limited crossreactivity. Sensitisation to Ole e1 (15%) was even more frequent than to Bet v 1 (11%). The exposition to ash pollen in this region was the highest in Switzerland, but also comparing to the pollen counts of France, Germany and Austria. The exposition shows a very high annual variation (total pollen count 1996: 1240 to 2003: 15190) The comparison of IgE t15 (CAP) and IgE a nOle e 1 and nOle e 2 (assessed by ImmunoCAP ISAC) shows different sensitisation patterns.

Conclusions: Sensitisation to ash pollen is very common also in central Europe, but often ignored. The allergological diffenences between the olive and ash pollen are still unclear. Actually the ash trees are suffering by an epidemic of a detrimental mould (chalara fraxinea). The effect of this environmental interactions will be important also for the further changing of sensitisation pattern to three pollen.

Sometime ash trees are also plantend in towns with an unknown consequence.

Plant trees in towns, but consider also the allergenic potential of their pollen.

1266
Atopy and functional gastrointestinal disorders – what is in the air?

Santosa, A[1]; Siah, KTH[2]; Bigliardi, PL[1]
[1]Department of Medicine, National University Health System, Singapore, Singapore; [2]Department of Gastroenterology & Hepatology, National University Health System, Singapore, Singapore

Background: It has been documented that up to 80% of patients with functional gastrointestinal disorders (FGIDs) report atopic symptoms. In addition to this epidemiological observation, the relation between allergy and FGID symptoms has been further strengthened through histological and serological evidence pointing to a central mast cell role in the pathogenesis of FGID. Food allergens have been the main suspect. However, results of food sensitisation and food elimination diets have been inconsistent. In a study of patients with FGID, sensitisation to inhaled allergens was found to be in excess of sensitisation to food allergens. We aim to further define the relationship between aeroallergens and FGID.

Method: Sixty-three consecutive volunteers attending various clinics in one institution were subjected to questionnaires and blood draws. We collected data on demographics, atopic and gastrointestinal symptoms, family history and exposures to aeroallergens. Asthma, allergic rhinitis (AR), eczema and FGID were defined based on internationally validated diagnostic criteria (European Community Respiratory Health Survey II, ARIA, GA2LEN network and ROME III, respectively). We conducted skin prick tests to 18 common allergens; total and specific serum IgE levels to 120 allergens were measured by Phadia ImmunoCAP and ImmunoCAP ISAC.

Results: We analyzed 63 patients: 50% were female and the mean age was 36.6 years (95% CI 33.1–40.2). In subjects with FGID the prevalence of AR was 80.6%, while those of eczema and asthma were 52.8% and 25%, respectively. In non-FGID patients, the proportions were 66.7%, 48% and 7.4%. House dust mites (HDM) were the major aeroallergen, with sensitisation in 78% of subjects. Thirty percent of subjects were sensitised to animal dander; this did not correlate with pet ownership in general. However, pet ownership after the age of 18 years was associated with the irritable bowel syndrome (IBS) subset of FGID (OR 4.19, $P = 0.017$). Cat sensitisation was significantly higher in IBS subjects (72.7% vs 27.3%, $P = 0.017$). Additionally, we observed a trend toward IBS in dog sensitised subjects. Sensitisation to HDM and food allergens was not found to be associated with FGID. Mean IgE levels were similar across all FGID and atopy groups.

Conclusion: Atopy is common in patients with FGID. While aeroallergens have been studied widely in atopy, they deserve further attention as a potential target for intervention in patients with FGID as well.

1267
Dust mites culture for allergenic vaccines manufacturing

Fernandez Duro, BI; Labrada Rosado, A; Luis Rodriguez, B; Torralba Averoff, D; Oliva Diaz, Y; Cruz Jimenez, R
Biocen, Alergenos, Habana, Cuba

Introduction: Dust mites are the most important allergens in the home, causing respiratory allergies. In Cuba we have identified three allergological important species: *Dermatophagoides pteronyssinus* (Trouessart, 1897), the most abundant worldwide; *Blomia tropicalis* (van Bronswijk, 1973), typical of tropical and *Dermatophagoides siboney* (Dusbabek, 1982) reported in Cuba, in some Caribbean countries and more recently in Mexico (Cuervo, 2011). About 80% of Cuban asthmatics are sensitive to any of these species.

Objective: To develop a method of growing mites, for these three species, as raw material allergenic (MPA) for the development of standardised allergen vaccines.

Materials and methods: The cultivation of mites was performed with a new diet of synthetic amino acids and animal protein. Their standardisation was based on the development and application of analytical essays as ELISAs, Lowrys and SDS-PAGE.

Results: Achieved an original culture method, which is obtained with high yields and rates of MPA consistency between batches.

Conclusions: Set acceptance limits of MPA as an intermediate in the production process of allergen vaccines and demonstrated the stability of these products preserved at −20°C for more than 2 years. These MPA were licensed for production in Cuba, being the first of its kind in the country, and the first in the world for the species Dermatophagoides siboney. The availability of these materials ensures standardised production of high quality vaccines.

1268
Prevalence of sensitisation to aeroallergens in adult patients attended in allergy clinic in Monterrey, México

Hernandez-Sanchez, H; Gonzalez-Díaz, S; Arias-Cruz, A; Gallego-Corella, C; Macias-Weinmann, A; Canseco-Villarreal, JI; Dominguez-Sansores, L; Yáñez-Perez, V; Rangel-Garza, L
Centro Regional de Alergia e Inmunologia Clinica, Universidad Autonoma de Nuevo Leon, Monterrey, Mexico

Background: Aeroallergens have an important role in the pathogenesis of allergic disease. Prevalence of the different aeroallergens varies worldwide and might depend on the geographical region and weather. The aim of this study is to determine the prevalence of sensitisation to aeroallergens in adult patients evaluated at the Allergy Clinic at a tertiary-care academic referral center in Monterrey, México.

Method: We performed an observational and descriptive study to determine the prevalence and pattern of sensitisation in adult patients attended at the Allergy Clinic from January 2009 to December 2012. All patients were evaluated by medical history, physical examination, and underwent skin prick tests to 36 common aeroallergens in the region (See Table 1).

We determined the pattern of sensitisation (indoor, outdoor or mixed aeroallergen sensitisation) and the presence of mono vs polisensitisation in the study population. The variables were analyzed with statistical program SPSS 20, and was used Fisher's exact test for relationship.

Results: Of the total of 2170 patients tested, 81% ($n = 1777$) had at least one

Table 1

Indoor aeroallergens	Mites Dermatophagoides farinae, Dermatophagoides pteronyssinus, Periplaneta americana, Blatella germanica	Animal dander and others Felis domesticus, Canis familiaris Snuff Cotton Wool	Fungi Alternaria alternata, Aspergillus fumigatus, Penicillium chrysogenum, Helminthosporium cladosporioides, Hormodendrum cladosporioides, Rhizopuz nigricans
Outdoor allergens	Grasses *Bromus* spp., Cynodon dactylon, Holcus lanatus, Lolium perenne Timothy grass, Sorghum halepense	Weeds Amaranthus palmeri, Ambrosia elatior, Artemisia ludoviciana, Atriplex canescens, Chenopodium ambrosioides, Helianthus annuus, Salsola kali),	Trees Fraxinus americana, Junglans regia, Juniperus sabinoides, Ligustrum vulgare, *Populus alba*, *Prosopis* spp., *Quercus* spp.

positive skin test. With a sex distribution of 42.6% males ($n = 758$) and 57.3% female ($n = 1019$), mean age was 35.1 years, following diagnoses were records: allergic rhinitis (82.7%), asthma (8%), atopic dermatitis (2.4%) and urticaria (6.9%). Age group distribution was: <20 years with 12.3% ($n = 220$), 21–40 years with 56.2% ($n = 999$), 41–60 years with 26.3% ($n = 469$), and 61–80 years with 5% ($n = 89$). Sensitisation to outdoor aeroallergens was 71.5% and indoor aeroallergens were 67%, monosensitised 8%, polysensitezed 92%. The most common aeroallergens were *Dermatophagoides pteronyssinus* in 57.9%, *Dermatophagoides farinae* in 52.4%, *Blatella germanica* in 25.7%, *Prosopis spp* in 32%, *Cynodon dactylon* in 31%, and *Amaranthus palmeri* in 28.9%. The higher prevalence of aeroallergens was found in the age group of 21–40 years.

Conclusion: In our clinic allergy in our allergy clinic in adult patients found higher prevalence in patients polysensitised relacion to monosensitised. Aeroallergen sensitisation varies with age and geographical location of the subjects. *Dermatophagoides* spp. were found to be the most common aeroallergen.

1269
Sensitisation rates to allergens, which may dispread due to climate change: first results of a survey conducted in two German Federal States

Balakirski, G[1]; Hajdu, Z[2]; Hoeflich, C[3]; Baron, JM[1]; Kaiser, L[3]; Merk, HF[1]; Gerdsen, S[1]; Strassen, U[2]; Bas, M[2]; Bier, H[2]; Dott, W[4]; Muecke, H-G[3]; Chaker, A[2]; Roeseler, S[1]; Straff, W[3]
[1]Department of Dermatology, University Hospital of Aachen, Aachen, Germany; [2]ENT-Department, University Hospital of Technical University of Munich, Munich, Germany; [3]Federal Environmental Agency, Section II 1.5 Environmental Medicine and Health Effects Assessment, Berlin, Germany; [4]Department for Environmental Medicine, University Hospital of Aachen, Aachen, Germany

Background: It is widely accepted that industrial activities within the last century increasingly lead to global climate changes. For Germany, this may imply an increase in mean annual temperatures of 1.5–3.7°C until 2100. These changes may influence vegetation periods and the geographical spread of allergenic plants, and this may cause new allergen challenges in public health. Therefore, we started to analyse allergy patients from two German Federal States with different climatic conditions, specifically from North Rhine-Westphalia and Bavaria, for their status quo sensitisations to (i) common allergens, (ii) recently established allergens and (iii) allergens expected to dispread by climate change.

Methods: From 2011 to 2013, 1000 patients suffering from allergic symptoms of the upper respiratory tract, 500 living for at least 20 years in North Rhine-Westphalia, 500 living for at least 20 years in Bavaria, are analysed for specific IgE levels to 112 allergen components by ImmunoCAP ISAC technology. In parallel, patients complete epidemiological and medical questionnaires, and GA²LEN prick testing, prick testing for latex, ash, l. destructor and b. tropicalis, spirometry, nasal curettage and nasal lavage are performed.

Results and conclusions: Analysis of ImmunoCAP ISAC data from the first 493 patients (250 from North Rhine-Westphalia, 243 from Bavaria) revealed sensitisation rates similar to the main allergy trend in Germany: 57% (North Rhine-Westphalia 56%, Bavaria 58%) showed sensitisation to birch pollen, 55% (North Rhine-Westphalia 51.6%, Bavaria 58.8%) had sensitisation to grass pollen. In addition, 8.5% (North Rhine-Westphalia 8.4%, Bavaria 8.6%) showed sensitisation to cypress pollen, and 8.7% (North Rhine-Westphalia 7.2%, Bavaria 10.3%) had sensitisation to plane tree pollen. Sensitisation to pr–10 protein, which is associated with oral allergy syndrome, was seen in 56% of patients (North Rhine-Westphalia 55.2%, Bavaria 57.6%).

Significant differences between North Rhine-Westphalia and Bavaria were found for sensitisation to pollen of the olive tree, one of those allergenic plants which may dispread by climate change: North Rhine-Westphalia 18.4%, Bavaria 27.6% ($P < 0.05$, Chi-squared test).

This study aims to identify the status quo sensitisations to established allergens and to allergens expected to dispread by climate change in two German Federal States with different climatic conditions. In the long run, this study is part of an idea to establish an early detection system for new allergen challenges due to climate change.

1270
Sensitisation to gymnosperms in the centre of Spain

Alonso, MD[1]; Rosado, A[1]; Moro-Moro, M[1]; Feliu, A[2]; Trujillo, MJ[2]; Ruíz-Hornillos, J[3]; Henríquez-Santana, A[3]; Blanco, S[3]; Domínguez, J[4]; Rodríguez, B[4]; González, E[5]; Losada, A[5]; López-Matas, MA[6]; Carnés, J[6]
[1]Allergy Unit, Hospital Universitario Fundación Alcorcón, Alcorcón, Spain; [2]Allergology Area, Hospital del Tajo, Aranjuez, Spain; [3]Allergology Area, Hospital Infanta Elena, Valdemoro, Spain; [4]Allergology Service, Hospital de Getafe, Getafe, Spain; [5]Allergology Section, Hospital Infanta Cristina, Parla, Spain; [6]Laboratorios LETI, R & D, Tres Cantos, Spain

Background: Sensitisation and allergic symptoms to Gymnosperm trees (Cupressaceae and Pinaceae family) has increased dramatically in the last years because they are massively planted in gardens and hedges. The objectives of the study were to investigate the prevalence of sensitisation to different Gymnosperms species, including three Cupressacea and three Pinaceae members; and to evaluate the characteristics of a population residing in the centre of Spain.

Method: Extracts from *C. arizonica*, *C. sempervirens*, *P. pinea*, *J. communis*, *C. atlantica* and *L. recurrens* were prepared. Skin prick tests were manufactured (5 and 10 mg/ml for Cupressaceae and 2 mg/ml for Pinaceae). Patients were skin prick tested with a standard battery of aeroallergens and with Gymnosperms prick extracts. Serum samples were obtained from *C. arizonica* positive individuals and

sIgE to *C. arizonica* and Cup a 1 were measured by ImmunoCAP.

Results: The prevalence of sensitisation to *C. arizonica* and *P. pinea* was 17.8% and 2.4% respectively. Sensitised population consisted in 102 individuals (46.5% men; 36.6 ± 11.6 years old). All of them had respiratory symptoms, 72.5% during the *C. arizonica* pollination period (January to April). 86.3% were also sensitised to other pollens, being the most prevalent grasses (73.5%) and olive tree (61.8%). A high percentage of the population was also sensitised to other Gymnosperms: 91.2% to *C. sempervirens*; 80.4% to *J. communis* or 70.6% to *L. recurrens*. Greatest wheal sizes were obtained with *C. arizonica* (41.7 ± 28) and *P. pinea* (35.4 ± 40.4). 86.6% had positive specific IgE to *C. arizonica* (8.3 ± 11.6 kUA/l) and 90.7% to Cup a 1 (16.4 ± 21.1 kUA/l).

Conclusion: The prevalence of sensitisation to Gymnosperms in the centre of Spain is similar than those observed in other areas. Most patients were sensitised to more than one species. All patients with positive sIgE to *C. arizonica* were also positive to Cup a 1.

1271
Sensitisation to *Tetranychus urticae*: prevalence and clinical relevance

Santos, N; Placido, JL
Immunoallergology, Centro Hospitalar Sao Joao, E.P.E., Porto, Portugal

Background: *Tetranychus urticae* (*Tu*) is a phytophagus mite that has been associated with rhinitis, asthma, contact dermatitis and urticarial symptoms.

Aim: To determine the frequency of sensitisation to *Tu* in an unselected population followed in the Allergy Department of a Tertiary Hospital in Portugal, to assess it's clinical relevance with conjunctival provocation test (CPT) and to evaluate possible environmental risk factors.

Methods: Skin prick test (SPT) with a commercial extract of *Tu* 2 mg/ml (Leti) was performed in a consecutive population of patients ≥6 years old referred by their allergist to be skin prick tested to a panel of airborne allergens ($n = 196$); a mean wheal diameter ≥3 mm at 15 min was considered positive. Patients ≥18 years old sensitised to *Tu* and a non-sensitised control group were invited to perform CPT with *Tu* and answer a questionnaire on environmental exposure to *Tu* and allergy symptoms/diagnosis ($n = 24$). A single-blinded placebo-controlled CPT with *Tu* was performed with increasing concentrations (0.002-0.02-0.2-2 mg/ml); it was considered positive if objective signs of conjunctival hyperemia, palpebral edema or lacrimation were observed in the tested eye after 10 min. Statistical analysis was performed with SPSS 18.0 ($P > 0.05$).

Results: Seventy (38.3%) patients (62.7%♀, mean age 29.2 ± 4.7 years) were sensitised to Tu. Among atopic patients, it was the fifth more frequent sensitisation (46.0%), after house dust and storage mites; eight patients were monossensitised. Of the 24 patients who performed CPT, 12 were sensitised to *Tu* (10♀, mean age 28.8 ± 9.9 years, three monosensitised, nine sensitised to mites, nine with rhinitis, mean wheal size 4.4 ± 1.5 mm) and 12 controls (10♀, mean age 36.8 ± 10.8 years, five atopic, two sensitised to mites, six with rhinitis). Of *Tu* sensitised patients, 9 (75%) had a positive CPT, including the three monossensitised. The SPT presented a good diagnostic acuity: AUC = 0.952, sensitivity = 100%, specificity = 80%, positive likelihood ratio = 5, negative likelihood ratio = 0. The correlation between wheal size and CPT eliciting dose was not significant ($r = -0.112$. $P = 0.774$). No differences were found regarding atopy, allergic disease, housing type, region, distance to farming fields or farming activities.

Conclusion: A high prevalence of allergy to *Tetranychus urticae* was found. Future studies with larger number of patients are needed to evaluate the relation of sensitisation with clinical symptoms and the impact of environmental factors.

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

Poster Session 51

New perspectives in occupational allergy

1273
Quantification of bovine allergens in different dust and food samples – comparison of polyclonal and monoclonal antibody-based ELISAs

Zahradnik, E[1]; Sander, I[1]; Brüning, T[1]; Chapman, M[2]; Raulf-Heimsoth, M[1]
[1]Institute for Prevention and Occupational Medicine, German Social Accident Insurance, Ruhr University Bochum (IPA), Bochum, Germany; [2]Indoor Biotechnologies Inc., Charlottesville, VA, United States

Background: Cow hair and dander are important inducers of occupational allergies in cattle-exposed farmers. The lipocalin Bos d 2 is a major allergen in cow antigen extracts. The aim of study was to compare a sensitive sandwich ELISA based on polyclonal antibodies against a mixture of hair extracts from different cattle breeds with a monoclonal antibody based commercial ELISA for the single major allergen, Bos d 2.
Method: For cow hair allergens, a polyclonal antibody based ELISA had been developed with a lower detection limit (LOD) of 0.1 ng/ml of cow dander protein standard (Allergon, Angelholm, Sweden). The Bos d 2 ELISA (Indoor Biotechnologies, Charlottesville, USA) was amplified using a poly-peroxidase streptavidin conjugate (Fitzgerald, Concord, USA). In total, 122 dust samples were analysed using both assays, including airborne dust from cow stables ($n = 36$) and homes of cattle farmers ($n = 24$) as well as floor settled dust from homes of cattle farmers ($n = 31$) and urban dwellers ($n = 31$). Several extracts from foods (e. g. milk, cheese, beef) were tested for the presence of bovine allergens. Results from both assays were compared by calculation of Pearson correlation coefficients.
Results: The use of the poly-peroxidase streptavidin- conjugate resulted in nearly 8-fold improved sensitivity of the Bos d 2 ELISA. The average LOD of the amplified Bos d 2 assay was 0.028 ng/ml in comparison to the original assay (LOD = 0.22 ng/ml). All 122 dust samples were positive in the cow hair allergen ELISA, only two samples were negative for Bos d 2 (both samples from urban homes). All tested food extracts were positive in both assays. Cow hair allergen and Bos d 2 concentrations measured by both assays were highly and significantly correlated for airborne dust samples ($r = 0.970$, $P < 0.0001$, $n = 60$) and for floor dust samples ($r = 0.988$, $P < 0.0001$, $n = 62$). Cow hair allergen concentrations were on average 4-fold higher than Bos d 2 values.
Conclusion: Amplification of the Bos d 2 ELISA enhanced the sensitivity of the assay by 8-fold. Both assays to quantify bovine allergens were highly correlated and could be used to measure environmental exposure to cattle in dust or air samples. Apparent exposure to cows in urban homes most likely represents the detection of bovine allergens derived from foods.

1278
Occupational anaphylaxis. Four case reports

Folletti, I[1]; Crivellaro, MA[2]; Murgia, N[3]; Paolocci, G[3]; Giuliani, A[3]; Gambelunghe, A[3]; Dell'Omo, M[3]; Siracusa, A[4]
[1]Clinical and Experimental Medicine, Occupational Allergy Unit, University of Perugia, Terni, Italy; [2]Occupational Allergy Unit, University of Padova, Padova, Italy; [3]Section of Occupational Medicine, Respiratory Disease and Toxicology, University of Perugia, Perugia, Italy; [4]Formerly University of Perugia, Perugia, Italy

Background: Main triggers of anaphylaxis are food, medications, insect stings/bites and natural rubber latex and similar triggers have been reported to cause occupational anaphylaxis.
Method: We describe four cases of anaphylaxis that occurred during work in a truck driver, a worker employed in fish cleaning in a restaurant, a worker employed in an herbal medicine factory and in a worker employed in a plant processing hazelnuts.
Results: A 56-year-old truck driver affected by honey-bee allergy (laryngeal edema), while opening the cloth covering his truck noticed an infestation of numerous wood ants (*Formica rufa*) and many of those contaminated his clothes. Forty minutes after first contact with woods ants, skin itching, light-headness, difficulty talking and hypotension developed requiring pharmacologic treatment in an emergency unit. A 33-year-old female allergic to house dust mites (HDM) and affected by conjunctivitis and atopic dermatitis, after 2 weeks working in a restaurant preparing fish, experienced one generalised urticaria episode. She returned to work and continued cleaning fish with gloves without development of any symptoms until during work she ate bread and anchovies and developed lip angioedema. She left work and underwent allergy examination and skin prick tests showed and Anisakis allergy. A 33-year-old male employed for 7 years in an herbal medicine factory suddenly had face angioedema, dyspnoea, generalised itching and rhino-conjunctivitis handling dry senna leaves; treatment in an emergency unit was necessary. Specific inhalation challenge (SIC) test with dry senna was performed and he immediately developed the same symptoms described above. SPT were positive for HDM and dry senna leaves and nasal cytology showed an increase of neutrophils after SIC. A 54-year-old male, warehouseman, employed for 17 years in packaging of hazelnuts in shell experienced laryngeal edema after eating toasted hazelnuts. Before this event he referred some episode of oral allergy syndrome eating hazelnuts. Allergologic examination showed positive SPT for hazelnuts and specific recombinant allergens Cor a8 was positive (LTP protein family).
Conclusion: These four cases confirm that work could be associated with anaphylactic reactions. Atopy and cross-reactivity may have a role in sensitisation and occurrence of symptoms. Anaphylactic reactions at work have socio-economic consequences. Safety measures and advice on specific work tasks may be effective.

1279
Sensitisation to acrylates caused by artificial acrylic nails in beauticians

Zaragoza, V[1]; Sierra, C[1]; Alamar, R[2]; Palomar, F[1]; Fornés, B[1]; Ferraro, V[3]; Alegre, V[1]
[1]Dermatology, Consorcio Hospital General Universitario, Valencia, Spain; [2]Allergology, Hospital General Castellón, Castellón, Spain; [3]Union de Mutuas, Valencia, Spain

Background: Sensitisations to acrylates, in beauticians, have increased considerably in the past few years, due sculptured artificial acrylic nails have gained popularity among the population. There are three distinct types of sculptured acrylic nails: nail tips that use adhesive cyanoacrylate prepara-

tions, acrylate nails that polymerize at room temperature in the presence of an organic peroxide and accelerator, and photo-bonded sculptured nails in which polymerization requires exposure to UV radiation.

Objective: To study the clinical characteristics and the different allergens involved.

Method: A retrospective study (2001–2012) of 29 beauticians diagnosed allergic contact dermatitis to acrylates used in sculpting artificial nails. Patch tests were performed with an acrylics contact allergens series (14 allergens).

Results:

The area commonly affected, in all the beauticians, was the fleshy parts of the fingers and the external edge of hands. The most habitual symptoms included itching, redness, swelling, flaking and fissures.

13% showed affectation of the forearms.

24% presented rash on their eyelids, face, or neck and in this group two beauticians had asthma.

The most relevant sensitisers were the groups of: methacrylate (82, 7%), dimethacrylate (62%) and acrylate (41.3%). We observed 75% beauticians presented polysensitisation and the most frequent (44, 8%) were methacrylate and dimethacrylate.

Conclusion:

Acrylate monomers used for sculpting artificial nails are considerably irritant, strong allergens and important occupational sensitisers.

Clinical characteristics evolve with the employed type acrylic nails.

The most important consideration is prevention. Conventional protective gloves are not appropriate to prevent sensitisation to acrylate, workers need impenetrable gloves like 4H (GLOVE).

1280
Identification of a new allergen showing cross-reactivity between *Artemia* spp. and *Mysis* spp.

Ceballos Uribe-Etxebarria, M[1]; De Castro, C[2]; Gabriel, MDF[1]; Postigo, I[1]; Martínez, J[1]
[1]Microbiology, Immunology and Parasitology, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, Spain; [2]Clinica Adeslas, Córdoba, Spain

Background: *Artemia* is a genus of branchiopod crustaceans inhabiting brackish inland. There are several species of *Artemia*, the most known are *A. salina* and *A. franciscana*, being the second one the most worldwide commercialized for its employment in aquaculture. As *Artemia*, *Mysis* is used in aquaculture too, considered as a nutritionally complete fish food. There have been reported some cases as occupational allergy to these crustaceans. In this study we present the case of a 49 years-old man referring allergic symptoms such as rhino conjunctivitis, asthma and ocular angioedema after feeding his aquarium fishes with various species of crustaceans and several constituents of zooplankton (mollusks and insects).

After demonstrate that the species implicated in the allergenic process were the crustaceans *Artemia* and *Mysis*, the aim of this study was to determine which allergens of both species were the cause of sensitisation and if there was any cross-reactivity between them.

Methods: The allergens were revealed by 2D-immunoblotting. SDS-PAGE-IgE-immunoblotting-inhibition was used to study the cross-reactivity between both species. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was used to identify the nature of the allergens.

Results: The analysis by 2D-immunoblotting demonstrated the presence of several IgE binding proteins in both extracts. The SDS-PAGE-IgE-immunoblotting-inhibition showed that there was cross-reactivity between both species. It was shown by MALDI-TOF analysis that *Artemia* had a 37 kDa IgE binding protein identified as fructose 1,6-biphosphate aldolase. *Mysis* also demonstrated the presence of a homologous protein belonging to the same aldolase family.

As far as we know there is only another one allergen belonging to the aldolase family which has been identified in *Candida albicans*.

Conclusions: Both, *Artemia* and *Mysis* have a clinical relevant allergen identified as fructose 1,6-biphosphate aldolase.

1281
A new variant of occupational asthma in a Spanish cook

Sénent Sanchez, C[1]; Piraino Sosa, P[1]; Siraj, A[1]; Perez Sencion, JO[1]; Miguel Polo, L[1]; De la Osa Puebla, V[1]; Hernandez Agujetas, R[1]; Bartolome, B[2]
[1]Allergy Department, Virgen Del Valle Hospital, Toledo, Spain; [2]Bial Aristegui Laboratories, Bilbao, Spain

Introduction: Occupational asthma is classified into two types, occupational asthma due to irritants and occupational asthma due to allergens (immunological asthma). We describe a peculiar case of a cook who presented symptoms when exposed to fumes from frying dough made of wheat flour and water to make a Spanish pastry called 'churros' in which we observed what seemed to be the debut of occupational asthma due to irritants which later on behaved as IgE mediated/immunological asthma.

Methods: Full blood count, total IgE, specific IgE to cereals as well as lung function tests (spirometry, provocation test with metacholine, peek expiratory flow test during working and vacation periods and specific bronchial and nasal provocation tests with wheat flour) were performed. We placed an AIR SENTINEL collector to filter airborne particles in the kitchen and elaborated an extract from the material isolated with which we performed skin prick tests, SDS-PAGE and Immunoblotting.

Results: Full blood count: eosinophilia, elevated total IgE and elevate specific IgE to cereals. Skin prick tests: positive to cereals (wheat, rye, barley, oat, corn and rice). Basal spirometry: normal. Bronchial dilatation test: negative. PC20 metacholine: 1.33 mg/ml. Fractionated exhaled nitric oxide: 68 ppb. Peak expiritory flow rate in working and vacation periods: 45% reduction exclusively in exposure periods. Specific bronchial and nasal provocation tests (acustic rhinometry) with wheat flour: Negative. Skin prick tests with elaborated extract: positive in our patient, negative in two control patients. SDS-PAGE showed varios protein bands especially of 14 kDa. Immunoblotting showed fixation of IgE at 66, 45, 18 kDa.

Conclusion: We describe a case of occupational asthma showing mixed characteristics which began as asthma due to irritants and later on behaved as asthma of an IgE mediated/immunologic variant. One has to consider the possibility of diagnosing a mixed variant of occupational asthma: 'IgE mediated asthma associated with irritants'. Variants of asthma which share characteristics of both types of occupational asthma due to low molecular weight proteins/allergens (isocyanates) have been described in the past. To our knowledge our patient is the first case to be described of a new mixed variant of occupational asthma due to a high molecular weight protein.

1282
Basophil activation test as a useful tool in occupational asthma due to iroko wood

de las Marinas, MD[1]; Martorell, C[2]; Martorell, A[1]; Cerda, JC[1]; Felix, R[1]; Sanz, ML[3]
[1]University General Hospital, Valencia, Spain; [2]University Clinic Hospital, Valencia, Spain; [3]Clínica Universidad de Navarra, Pamplona, Spain

Background: Iroko wood has been described as an occupational allergen in exposed workers. Although it has been suggested that immune mechanisms are

involved, to date, no tests have been able to confirm this assessment unequivocally, because percutaneous tests using wood extract are usually negative and no specific IgE measurement is available. We present the case of a male patient, aged 37, who reported a 3-year history of episodic cough and wheezing due to contact with iroko wood in his workplace, with difficulty breathing that improved with inhalation of bronchodilators and deteriorated at night or after physical exertion. The patient is bronchially asymptomatic when not in contact with iroko wood, and reports mild nasal symptoms during the spring.

Method and Results: Skin tests and specific IgE for common aeroallergens were slightly positive for mites and several pollens. Total IgE was 221 kU/l. Prick test with commercial iroko wood extract (Diater) and prick-prick with iroko sawdust extract (10% wt/vol) were both negative. Baseline spirometry was within normal limits. Methacholine challenge test was positive (PC_{20}: 1.16 mg/ml). Bronchial challenge test with iroko sawdust (handling the dust for 15 min) showed a 13.58% reduction in baseline FEV1 once exposure was over, and the serial measurement of peak flow values (PEF) declined by 8.44% and 16.7% after 8 and 11 h respectively. An exposure test conducted using the same methodology with sapele wood dust showed no immediate or late changes. Serial PEF measurement on a workday without handling iroko wood remain unchanged. Conversely, on a day involving work with iroko wood, there was a progressive decrease in serial PEF values after 4 h of exposure (15.71%), reaching a nadir 8 h after onset (28.57%), with wheezing and breathlessness 2 h after completion of the work.

Basophil activation test (BAT) with iroko extract at two final concentrations (0.03 and 0.01 mg/ml) showed a positive response in the patient (52.2% and 47.6% respectively), but negative in three tested controls. BAT was also carried out with sapele wood (0.03 and 0.01 mg/ml) with negative response of both the patient and the healthy controls.

Conclusion: We report a new case of occupational bronchial asthma induced by inhalation of a tropical wood in an atopic patient who is a carpenter. We describe BAT as an effective mechanism for attributing the immunoallergic involvement of iroko wood in his illness.

1283
Occupational allergy by inhalation of allergenic proteins of *Anisakis simplex*

Garcia Navarro, DJ[1]; Montoro de Francisco, A[2]; Chivato Perez, T[2]; Burgos Pimentel, A[2]; Fonseca Avendaño, J[2]; De Vicente Jimenez, TM[2]; Membrillo de Novales, FJ[2]; De Mateo Hernandez, B[2]; Nuñez Hernández, MÁ[2]; Mateos Galvan, JM[2]; Fernández López, M[2]
[1]Allergy, Hospital Central de la Defensa "Gómez Ulla", Madrid, Spain; [2]Allergology, Hospital Central de la Defensa "Gómez Ulla", Madrid, Spain

Background: Anaphylaxis can affect 20% of sensitised to Anisakis simplex. There have been reports of occupational asthma due to inhalation of allergens in patients who handle raw fish. Anisakis has several proteins which are capable of producing sensitations, primarily by ingestion, but may also occur through contact or inhalation.

Method: We report two cases:

1. Case 1: 50 year old woman, who works as a cook. A year ago she presented two episodes of anaphylaxis after eating undercooked fresh fish. Thereafter she removed raw fish from her diet. Ten months later after handling fish (eviscerating it), an hour after hand injuring, she presented itching hands and head, epigastric pain, nausea and severe generalised urticaria. That forward with adrenaline, corticosteroids, and antihistamines. She manipulates fish daily with gloves because of her profession.
2. Case 2: Woman 50, who works as a kitchen assistant. Refers nasal congestion without rhinorrhea. She doesn't improvement with oral antihistamines or nasal steroids. She ate fresh fish until October 2012, when she presented urticaria and epigastric pain after ingestion of anchovies. Works cleaning and preparing fresh white and blue fish, then freezing. She prepare pickled anchovies 1 time per week.

Prick test (PT) with allergenic extracts of fish, Anisakis, food (Alk Abelló). Total and specific IgE to Anisakis, white and blue fish (Thermofisher). Gastroscopy. Rhinomanometry.

Results: Cases 1 and 2: Negative PT both raw and cooked whiting. Anisakis simplex PT positive both immediate and 48 h later reading. Case 1: Allergic response test against aeroallergens negative. Total IgE 1821KU/l. Specific IgE levels were always kept high Anisakis: >100KU/l. Normal Gastroscopy: Anisakis larvae not observed. Not taking biopsy

Case 2: positive PT Anisakis simplex, olive, cypress, mugwort, Chenopodium and dog. Total IgE 72.4 kU/l IgE Specific: Anisakis * 13.1 kU/l. Rhinomanometry: Normal.

Conclusion: We report two cases of allergy occupational following ingestion, contact and inhalation of various Anisakis simplex proteins, after handling fresh fish continuously, as part of their normal work, producing in each of the patients, respiratory, skin and digestive symptoms.

1284
Specific IgE to African penguin serum and mucus proteins

Potter, PC[1,2]; Fenemore, B[1]; Foot, L[1]
[1]Allergy Diagnostic & Clinical Research Unit, University of Cape Town Lung Institute, Cape Town, South Africa; [2]Department of Medicine, Observatory, University of Cape Town, South Africa

Background: Animal behaviourists working in aquariums may become sensitised to fish feeds and also to crustaceans in fish feeds, by direct contact, or via the airborne route in poorly ventilated work areas. Sensitisation to penguins has not previously been reported.

Method: A 42-year old female animal behaviourist who cared for a small African Penguin colony at an Aquarium in Cape Town developed progressive asthma in the work environment, worse when cleaning the penguin enclosure and feeding the penguins. Her asthma symptoms abated when she was out of the penguin enclosure. In addition she had a severe hand dermatitis and latex allergy.

Results: Skin prick tests confirmed positive sensitivity to sardine, tuna and white fish mix. Immunocap tests were positive to duck feathers (67.2 Ku/l), mould mix (47.4 Ku/l), latex (5.5 Ku/l), pilchards (100 Ku/l) and squid (100 Ku/l). ISAC tests were positive to cod par albumin, egg serum albumin and anisakis tropomycins. Western blots of the nesting material, penguin serum and penguin oral mucus confirmed strong specific IgE binding to penguin serum proteins of 25 kd and 70 kd, as well as 12 kd proteins in the faeces from the penguin nests. The 12 kd proteins are considered to be par albumin from the fish eaten by the penguins.

Conclusion: This is the first reported case of specific sensitisation to penguin serum proteins in an atopic animal behaviourist, in the occupational environment.

1285
Development and validation of an electronic patient data acquistion tablet for allergy symptom collection in an environmental exposure chamber and at-home

Wilson, D[1]; Nandkeshore, H[2]; Patel, P[2]
[1]Logos Technologies Inc., London, United Kingdom; [2]Inflamax Research Inc., Mississauga, ON, Canada

Background: Patient Reported Outcomes (PRO) such as allergy symptom scoring are

recognised by FDA and EMA as important and acceptable endpoints in allergy trials. PRO have historically been recorded on paper diaries requiring subsequent transcription and which can introduce human error, data queries and time delays. Its electronic format (ePRO) offers many inherent benefits in terms of speed and validity, but it has often been challenging to implement in practice due to both cost and logistics. We have developed the electronic patient data acquistion tablet (ePDAT) system to address these issues while ensuring compliance with FDA and EMA requirements for validated ePRO data collection tools.

Method: ePDAT was developed through the following stages. First, the definition stage where user requirements and specifications are determined. Next, the planning stage where a validation plan is developed and regulatory and risk assessment performed. In the design stage, there is a system design and testing plan. Utilizing the ePDAT there is the installation and configuration stage which takes place during the installation qualification (IQ). Finally user acceptance testing is performed to examine that the developed tool is indeed performing as originally intended and involves test script development and test execution.

Results: The ePDAT system has a touch screen graphical user interface via a marketed tablet. Patient questionnaires are presented on-screen with multiple choice options as well as visual analog scales (VAS). Patient data are recorded in real-time and are directly transferred into the database once the tablet has an active wireless or cell phone connection to the internet. The data are also stored locally on the tablet as back-up. Other features include time logging, data entry time interval windows, detailed audit trails, compliance incentives and follow-on questions for the electronic diary card. IQ and User Acceptance Testing demonstrated 21 CFR Part 11 compliance for computerized system.

Conclusion: The ePDAT system is a validated tool suitable for use for allergy symptom input and collection in allergy trials. The same ePDAT can be used seamlessly across EEC and at-home symptom recording making multicenter EEC trials feasible and practical.

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

Poster Session 52

Molecular biomarkers for allergen-specific immunotherapy

1286
Mitogen-activated protein kinase serum levels and allergen immunotherapy response

Gawlik, R[1]; Jawor, B[1]; DuBuske, L[2]
[1]Silesian University School of Medicine, Katowice, Poland; [2]Immunology Research Institute of New England, Gardner, United States

Background: MEK1 mitogen-activated protein kinase kinase is a 393 amino acid protein involved in tyrosine kinase signaling cascades which are involved in the pathogenesis of allergic airway inflammation. This study assesses plasma concentration of MEK-1 in allergic rhinitis patients during allergen immunotherapy (AIT).
Method: Patients who had both positive skin prick tests and increased allergen specific IgE to house dust mites were studied including 26 patients (14 females and 12 males) mean age 32.2 ± 7.6 years. No other significant disease was present. AIT was given to 16 patients using a weekly course D.pt – 50% and D.far – 50% allergen extract (Novo Helisen Depot, Allergopharma, Germany) injections given over 1 year. The concentration of MEK1 in serum was determined by Titer Zyme EIA (Assay Designs, Ann Arbor, USA). The control group consisted of 10 subjects with similar allergy (mean age 24.7 ± 4.5 year) on pharmacotherapy.
Results: One year of AIT gave a statistically significant reduction of MEK-1 concentration in serum of AIT patients to 462.0 ± 144.9 pg/ml. In the control group patients there were no significant changes of MEK-1 serum levels (598.4 ± 166.6 pg/l). Serum MEK1 concentrations were not significantly different between groups at the beginning of the study (584.3 ± 142.6 pg/ml; controls 566.5 ± 144.4 pg/ml). AIT also reduced rhinitis symptoms. The reduction of MEK-1 serum concentration correlated with decreased severity of rhinitis symptoms (r = 0.42; $P < 0.05$) Successful AIT impacted both symptoms and MEK-1 concentrations in serum.
Conclusion: MEK1 concentrations in serum decrease with AIT suggesting an influence of allergen immunotherapy on kinase activity or expression perhaps related to reduction in allergic nasal inflammation and nasal symptoms. Serial MEK1 serum concentrations may serve as a marker for successful allergen immunotherapy.

1287
The effect of subcutaneous immunotherapy for specific allergens on clinical symptoms and T regulatory and T helper cells cytokines in patients with allergic rhinitis

Jabbariazad, F; Faridhosseini, R; Shirkani, A; Tehrani, H; Zamani, MA; Jafari, M; Yousefzadeh, H
Mashhad University of Medical Sciences, Mashhad, Iran

Background: Allergic rhinitis is one of the most common allergic disease and characterised by sneezing, rhinorea, nasal congestion and nasopharyngeal itching. Subcutaneous immunotherapy (SCIT) for specific allergens is an effective treatment and decreases clinical symptoms in allergic rhinitis. In this study effect of subcutaneous immunotherapy with specific allergens on clinical symptoms and T regulatory and T Helper cells cytokines, in patients with allergic rhinitis were evaluated.
Method: In this randomised clinical trial study, 30 patients with moderate to severe allergic rhinitis according to clinical criteria and positive skin prick test for common aeroallergens were selected and treated by SCIT. Clinical symptoms (according to TNSS and TSS) and T cells cytokines IL4, IL17, IFN gamma, TGF beta, GITR, FOXP3 and IL10 (by RT-PCR) were evaluated before and 1 year after initiation of treatment.
Results: Thirty patients with allergic rhinitis at age range 15–45 years old were treated by SCIT that 23 (14 female, nine male) patients continued the study and seven patients were excluded. After 1 year immunotherapy, clinical symptoms decreased significantly. The specific cytokines TGF beta and IL10 levels increased and changes were statistically significant (P = 0.013 and P = 0.05 respectively). IL17 level was also increased, but not statistically significant (P = 0.8). IFN gamma, IL4, GITR, FOXP3, all decreased, but the changes were not statistically significant ($P > 0.05$).
Conclusion: Subcutaneous Immunotherapy for specific allergens decreases clinical symptoms in patients with allergic rhinitis and induces tolerance in T lymphocytes, especially by increasing T regulatory cells cytokines, TGF beta and IL10.

1288
In vitro immunomonitoring of birch pollen-allergic patients during allergen-specific immunotherapy

Cifuentes, L[1]; Ploetz, S[1]; Chaker, A[2]; Pennino, D[3]; Durham, S[4]; Shamji, M[4]; Ollert, M[1]; Ring, J[1]; Schmidt-Weber, C[3]; Darsow, U[1]
[1]Department of Dermatology and Allergy, Technical University Munich, Biederstein, Munich, Germany; [2]Hals-Nasen-Ohrenklinik und Poliklinik, Technische Universität München, Munich, Germany; [3]ZAUM – Center of Allergy and Environment, Technische Universität and Helmholtz Center Munich,Munich, Germany; [4]Molecular Immunology, Section of Allergy and Clinical Immunology, National Heart and Lung Institute, Imperial College London, London, United Kingdom

Background: Identification of predictive early markers in a successful allergen-specific immunotherapy (ASIT) provides the basis for a personalized treatment, stratifying the therapy according to individual needs, reducing the time and costs of a standard immunotherapy and improving the effectiveness.
Aim: To characterise early surface markers for successful ASIT using proliferation assays and multicolour flow cytometry technics.
Method: In this prospective study, PBMCs of n = 14 patients undergoing ASIT with birch pollen were analyzed before ASIT, before high allergen doses, 6, 18, 30, 48 h and 1 week after high doses. Each patient within this group had a history of rhinoconjunctival symptoms during birch season and a positive skin test with birch pollen extracts. Further characterisation included allergen-specific IgE against birch pollen extracts (i1, i3 ≥ 0.10 kU/l) measured by the Immulite system (Siemens). PBMCs characterisation and monitoring of the specific T cell proliferation in ASIT patients was performed by multicolour flow cytometric staining. Humoral sIgE and sIgG4 reactivity against Bet v 1 during the mentioned time-points were analyzed by ELISA. In addition the number of specific IL-4, IL-10 and double positive T cells has been measured by means of ELISPOT.

Results: CD137 and CD40L expression remained stable at all time points investigated in the totality of patients. CD27 became progressively dominant after the high dose in all patients. The regulation of CD69 divides the birch-allergic patients into two distinct populations, one group displayed a significant up-regulation 6 h after high dose and the other group underwent a down-regulation. The same tendency was observed with the CRTH2, a specific Th2 marker.
Conclusion: Surface marker repertoire differs during different time-points in the early phase of immunotherapy. A correlation with the outcome of the nasal challenge will establish the clinical relevance of the results. Surface marker repertoire differs during different time-points in the early phase of immunotherapy. A correlation with the outcome of the nasal challenge will establish the clinical relevance of the results.

1289
Flagellin suppresses allergen-stimulated Th1 and Th2 responses by the induction of regulatory T cells in asthmatics

Koh, Y-I[1]; Shim, J-U[1]; Rhee, J-H[2]; Kim, D[1]
[1]Allergy, Asthma and Clinical Immunology, Chonnam National University Medical School, Gwangju, Korea; [2]Microbiology, Chonnam National University Medical School, Gwangju, Korea

Background: Bacterial flagellin, which activates TLR5, has a strong immunomodulatory activity. We previously reported that intranasal co-administration of flagellin, *Vibrio vulnificus* FlaB, with ovalbumin inhibited asthma in a mouse model of asthma. We determine whether the effect of flagellin on asthma in mice experiments can be reproduced in human blood system.
Method: Peripheral blood mononuclear cells were obtained from patients with house dust mite (HDM)-sensitive asthma. To test the effect of FlaB-treated autologous dendritic cells (DCs) on HDM-stimulated blood CD4+ T cells, FlaB-treated or untreated DCs were co-cultured with CD4+ T cells in the presence of HDM extracts.
Results: FlaB treatment increased the expression of CD80, CD83 and CD86 molecules on DCs, in which the degree of the expression was similar to that as treated with LPS, and increased the production of IL-10, but not IL-12, from DCs. FlaB-treated DCs decreased the production of IL-13 and IFN-gamma but increased the production of IL-10 and TGF-beta from HDM-stimulated CD4+ T cells. Intracellular cytokine staining showed that FlaB-treated DCs reduced the production of IL-4 and IFN-gamma from HDM-stimulated CD4+ T cells but increased the production of IL-10. Furthermore, FlaB-treated DCs increased the number of Foxp3+ cells from HDM-stimulated CD4+ T cells.
Conclusion: Flagellin may suppress allergen-stimulated Th1 and Th2 responses via the induction of IL10-producing DCs and regulatory T (Treg) cells in asthmatics. Flagellin may be an effective adjuvant to enhance Treg cells and thus promote the efficacy of allergen-specific immunotherapy in allergic asthma.

1290
High-dose allergen immunotherapy induces a strong down-regulation of genes involved in Th2 and Th1 activation and function in adenoid tissues

Masieri, S[1]; Trabattoni, D[2]; Nebuloni, M[3]; De Luca, M[2]; Dell'Albani, I[4]; Cavaliere, C[1]; Incorvaia, C[5]; Marcucci, F[6]; Moingeon, P[7]; Frati, F[4]
[1]Department of Otolaryngology, La Sapienza University, Rome, Italy; [2]Department of Biomedical and Clinical Sciences, L.Sacco Hospital, Milan, Italy; [3]Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy; [4]Medical and Scientific Department, Stallergenes Italy srl, Milan, Italy; [5]Allergy/Pulmonary Rehabilitation Unit, ICP Hospital, Milan, Italy; [6]Spin-Off ATRP Srl, Allergic Tests Research and Production, Perugia, Italy; [7]Stallergenes France, Research and Development, Antony, France

Background: Tonsils and adenoids are part of the Waldeyer ring, the basic function of which is antibody formation to antigens.

Allergen immunotherapy (AIT) is an effective and safe treatment for allergic rhinitis (AR). As demonstrated by trials performed with grass tablets, during sublingual immunotherapy (SLIT) it is observed an increase of specific IgEs and a dose-dependent rise of specific IgGs but these immunological modifications do not correlate with efficacy. By the model of the Allergen Challenge Chamber (ACC) the 5-grass pollen SLIT tablets were found to be rapidly effective (after 30 days). Moreover, previous studies showed that only high-dose SLIT is able to reduce the nasal production of IgE in pediatric patients with grass-induced AR. This response suggested a rapid loco-regional and immunological effect of SLIT where Waldeyer ring could have an important role. The aim of this study was to investigate the immunologic changes in adenoid tissues in children with AR undergoing AIT (SLIT).
Method: Adenoid samples from 16 children (mean age (SD): 7.12 (1.15) years, range 5–9 years, seven males, nine females) with adenoids hypertrophy of 4th level and clinical indication to adenoidectomy were collected. Of them, five children were not allergic and 11 suffered from house dust mite (HDM) and grass-induced AR. In four children AR was treated by antihistamines, while in seven patients by high-dose HDM and grass SLIT (Stallergenes, Antony, France). The immunologic evaluation addressed the Th1, Th2 and Th3 cells by performing a PCR Array on mRNA extracted from adenoid samples.
Results: SLIT induced a strong down-regulation of genes involved in Th2 and Th1 activation and function. In particular, in SLIT-treated allergic children IL-4, CCR2, CCR3 and PTGDR2 (Th2 related genes) and CD28, IL-2 and INHA (Th1 related genes) expression was reduced compared with anti-histamines-treated allergic children.
Conclusion: These preliminary findings suggest that AIT (SLIT) is able to influence the immunological environment in the adenoids, especially by affecting the Th2 and Th1 patterns of response. These results could explain the efficacy of SLIT yet after the first month of treatment and its strong immunological effect, both humoral and cellular.

1291
Induction of antigen-specific B cells using novel tetramer-based approach occurs early during peanut oral immunotherapy

Patil, SU[1]; Ogunniyi, AO[2]; Kubala, S[3]; Brennan, A[1]; Ma, A[1]; Moon, J[1]; Love, C[2]; Shreffler, WG[1]
[1]Allergy and Immunology, Massachusetts General Hospital, Harvard University, Boston, United States; [2]Chemical Engineering, Massachusetts Institute of Technology, Boston, United States; [3]Temple University Medical School, Philadelphia, PA, United States

Background: Peanut oral immunotherapy (PNOIT) variably increases tolerance in patients with an IgE-mediated peanut allergy and has been inconsistently correlated with increased specific IgG4 levels. To investigate whether specific antibody induction is an epiphenomenon or a potentially important B cell mechanism of immunotherapy, we used a novel tetramer-based approach to identify and track Arah2-specific B cells in patients undergoing peanut oral immunotherapy.
Method: Patients aged 7–21 ($n = 20$) with IgE-mediated peanut hypersensitivity (positive specific IgE, skin prick testing, and history of reaction) underwent PNOIT in a single-center, randomised trial (3:1, active: placebo). Peripheral blood mononuclear cells from before and during dose-escalation of PNOIT in active patients were stained with fluorescent Arah2 tetramers and analyzed using flow cytometry. Tetramer+ cells are reported as per million $CD19^+$ cells. Non-food allergic patients (control, $n = 5$) were similarly analyzed. Paired immunoglobulin heavy and light chains were amplified and sequenced from

sorted, tetramer+ B cells using nested single-cell RT-PCR for subsequent expression and characterisation.

Results: The frequency of Arah2$^+$ B cells is similar in non-allergic control and peanut-allergic patients before PNOIT (142.6 vs 377.5, $P = 0.2$). In allergic patients during PNOIT, there is a significant increase in the CD19$^+$CD27$^+$IgM$^-$ memory subset of Arah2$^+$ B cells from baseline (28.3–118.9, $P = 0.01$). Induction also occurred in Arah2$^+$ CD19$^+$CD27^{++}IgM$^-$ plasmablasts (2.5–14.4, $P = 0.03$), but not significantly in CD19$^+$CD27$^-$IgM$^+$ naïve B cells (90.4–161.5, $P = 0.2$). This induction of Arah2$^+$ memory cells occurs early, during the first 2 months of PNOIT. Comparative analysis of 20 heavy chain immunoglobulin genes from Arah2$^+$ cells demonstrated varied heavy chain usage (20% V1, 50% V3, 30% V4), and three clones demonstrated homology greater than 85%, suggesting related clonality.

Conclusion: Using this novel affinity selection and single cell cloning approach, we can demonstrate the expansion of putative antigen-specific B cells in patients undergoing PNOIT. More importantly, by characterising the specificity and functional capacity of these antibodies, we can now begin to more directly test the hypothesis that specific immunoglobulin induction is an important mechanism for long-lived post-treatment tolerance in food allergy immunotherapy.

1292
Establishment of an Alt a 1-specific FAB-assay as a method to evaluate the outcome of *Alternaria*-immunotherapy

Mertens, M[1]; Arlt, B[1]; Kupczyk, M[2]; Klysner, S[1]; Kahlert, H[1]

[1]Allergopharma GmbH & Co. KG, Research & Development, Reinbek, Germany; [2]Department of Internal Medicine, Asthma and Allergy, Barlicki University Hospital, Medical University of Lodz, Lodz, Poland

Background: The only causal treatment of allergic disease is specific immunotherapy (SIT). During SIT regulatory mechanisms, like the generation of inhibitory factors in patient serum, are induced. However, at present the primary parameter for documenting successful therapy is a decrease in symptom medication score (SMS). To avoid the dilemma of subjective evaluation, the facilitated antigen binding (FAB)-assay was presented recently as potential solution. The assay measures binding of allergen-IgE-complexes to B-cells. When sera from patients undergoing SIT are applied, binding to B-cells is often blocked, which is proposed to be associated with treatment success. The aim of the present work was to establish the FAB-assay for *Alternaria*-specific immunotherapy.

Method: Indicator serum, alone or with serum before, within or after SIT, was incubated with allergen and B-cells. After addition of fluorescence-labelled antibodies against IgE and CD23, IgE-binding to B-cells was analysed by flow cytometry. The following issues were addressed:

1 Identification of a suitable indicator serum with high *Alternaria*-specific IgE-titer.
2 Identification of a suitable allergen by applying *Alternaria*-extract and recombinant (r)Alt a 1.
3 Investigation of sera from patients either successfully treated with verum (*A. alternata* adsorbate) or placebo.
4 Specificity and reproducibility of the assay.

Results: Application of *Alternaria*-extract results in maximally 30% IgE-binding to B-cells, whereas binding of up to 70% was observed with two of four potential indicator sera using rAlt a 1. Thus, a suitable indicator serum and allergen for following experiments was identified. To investigate the correlation between inhibitory capacity and successful SIT, sera from three verum and three placebo patients from four different time points, were analysed. All verum patients showed complete IgE-binding inhibition already after the first treatment year in contrast to no inhibition before therapy, while sera from placebo patients showed only minor IgE-inhibitions. This effect is allergen-specific and highly reproducible.

Conclusion: An *Alternaria*-specific FAB-assay was established with a selected indicator serum and recombinant Alt a 1. Here, all investigated, successfully treated patients showed complete IgE-inhibition. However, investigation of more patients, including SIT non-responders, is needed in order to proof the concept of IgE blocking capacity as a surrogate marker for successful SIT.

1293
Identification and characterisation of IL-4 and IL-4 receptor as novel intervention targets for allergy treatment

Ahmed, N; Ramsbottom, K; Carr, S; Suphioglu, C
School of Life and Environmental Sciences, Deakin University, Geelong, Vic., Australia

Background: Allergen-reactive Type 2 helper T cells (Th2) are responsible for the activation and/or recruitment of IgE antibody-producing B cells in allergic cascades. Briefly, cytokine IL-4 binds to IL-4 receptor (IL-4R), which activates the STAT6 phosphorylation pathway that leads to gene activation of allergen-specific IgE antibodies by B cells. Consequently, IgE production leads to clinical symptoms of allergy. Allergies are associated with high morbidity and economic cost; hence, deeming it critical to establish novel therapies. The overall aim of the study was to characterise novel synthetic peptide inhibitors of IL-4 and its receptor, which will inhibit IgE production and therefore pave way for the future development of novel allergy therapeutics.

Method: Phage display technology was used to screen a random peptide library with a biologically active purified human IL-4 and IL-4R to identify peptide antagonists. Once identified, the peptides were synthesised and used for *in vitro* immunoassays and a HEK-Blue IL-4/IL-13 reporter cell line model, transfected with a gene producing secreted embryonic alkaline phosphatase (SEAP). SEAP acts as a substitute to IgE when cells are stimulated with cytokine IL-4. QUANTI-Blue was added as a substrate that breaks down in the presence of SEAP, producing blue coloration. The blue color confirmed the activation of STAT6 pathway using a spectrophotometer.

Results: We have successfully used phage display to identify M13 phage clones that demonstrated specific binding to IL-4 and IL-4R. All of the 10 individual phage clones characterised had identical peptide sequence motifs, and only one peptide was synthesised for use in ELISA, demonstrating significant binding to IL-4 and IL-4R. We have successfully used peptides K1 to IL-4 and N1 to IL-4R that demonstrated inhibition of SEAP production in HEK-Blue cells. A colorimetric analysis showed a >50% inhibition, resulting in less blue color development. A Student's *t*-test revealed the significance of the results.

Conclusion: We have successfully identified and characterised synthetic peptide antagonists against IL-4 and IL-4R, which effectively inhibits IL-4 interaction with IL-4R and vice-versa. Since IL-4 interaction with IL-4R is a common pathway for many allergies, a prophylactic treatment can be devised by reducing this interaction for future treatment of allergies.

1294
Alum adjuvant in birch pollen immunotherapy decreased body drop temperature but did not contribute to the adaptive immune response in mice

van Rijt, LS[1]; Logiantara, A[1]; van Ree, R[1,2]; Opstelten, D-J[3]; van der Kleij, H[3]
[1]Academic Medical Center, Experimental Immunology, Amsterdam, The Netherlands; [2]Academic Medical Center, Otorhinolaryngology, Amsterdam, The Netherlands; [3]HAL Allergy BV, Leiden, The Netherlands

Background: Aluminum hydroxide (alum) is widely used in subcutaneous immunotherapy (SCIT) for more than 50 years. While the prevailing theory has been that adsorption of allergens to alum induces slow release of allergens, reduces adverse effects and increases effectiveness of treatment, this was not proven formally.
Method: In a mouse model for birch pollen-mediated allergic airway inflammation, mice were de-sensitised by six subsequent subcutaneous injections with birch pollen extract alone or adsorbed to alum. As an objective parameter of a shock response, body temperature was measured after each subcutaneous injection. After the de-sensitisation phase, mice were challenged intranasally with birch pollen extract. Subsequently, the antigen-specific T cell response was determined and antigen-specific antibody responses were analysed.
Results: The adsorption of the birch pollen extract to alum resulted in a significant reduction of the shock response as measured by a decreased drop in body temperature. The drop in body temperature became gradually less with subsequent SCIT injections in both groups. SCIT decreased the production of Th2 cytokines IL-5 and IL-13 and increased birch pollen specific IgG1. These effects were independent of the presence of alum in the preparation.
Conclusions: This study showed that alum did not enhance the suppression of the antigen specific T cell response nor was it required for the induction of the humoral response. Alum did increase the safety profile of the preparation during the immunotherapy phase. This study underscores the need for more detailed research concerning the use of alum in immunotherapeutic preparations. Furthermore, alternative adjuvants to optimise SCIT need to be explored.

1295
Long term maintenance of regulatory T cells induced by specific epicutaneous vs sublingual immunotherapy in mice sensitised to peanut

Dioszeghy, V[1]; Mondoulet, L[1]; Dhelft, V[1]; Ligouis, M[1]; Puteaux, E[1]; Dupont, C[2]; Benhamou, P-H[1]
[1]DBV Technologies, Bagneux, France; [2]Hôpital Necker, Université Paris Descartes, Paris, France

Background: Epicutaneous immunotherapy (EPIT) decreases both clinical responses to allergen exposure and allergen specific TH2 responses, and increase local and peripheral Foxp3+ regulatory T cells (Tregs) in sensitised mice. The aim of this study was to evaluate the long term maintenance of suppressive Tregs after end of treatment in peanut sensitised mice treated by EPIT or sublingual immunotherapy (SLIT).
Method: Thirty mice were sensitised orally to peanut protein extract (PPE) with cholera toxin and then treated for 8 weeks by either EPIT or SLIT ($n = 15$ each). After treatment, mice were left untouched for eight more weeks and CD25+CD4+ Tregs were then isolated from spleen and transferred into two groups of new PPE-sensitised mice ($n = 8$ per group). The allergen-specific cytokine responses of splenocytes and the recruitment of eosinophils in esophagus after intensive oral exposure to PPE were measured in receiver mice and compared to sensitised EPIT, SLIT or Sham treated mice and naïve mice.
Results: As expected, EPIT and SLIT decreased PPE-specific Th2 cytokines compared to Sham (IL-5: 45.9 ± 24.3 and 37.9 ± 29.2 vs 207.5 ± 60.4 pg/ml; IL-13: 56.9 ± 34.9 and 69.1 ± 49.2 vs 452.4 ± 221.3 pg/ml respectively; $P < 0.05$), and SLIT, but not EPIT, increased IL-10 (114.1 ± 53.0 vs 25.4 ± 9.1 pg/ml in Sham, $P < 0.05$). When transferred, Tregs isolated 8 weeks after stopping EPIT did suppress allergen specific Th2 cytokine production as efficiently as EPIT (IL-5: 58.3 ± 34.5 and IL13: 67.4 ± 40.6 pg/ml, $P < 0.05$ vs Sham). In mice transferred with SLIT Tregs, IL-5, IL-13 and IL-10 responses were not different from Sham (316.7 ± 72.3, 638.1 ± 191.4 and 25.2 ± 18.5 pg/ml respectively). After oral exposure to peanut, eosinophilic infiltration in esophagus was significantly lower in EPIT than Sham (14.8 ± 6.6 vs 50.8 ± 10.5 eosinophils/mm^2 (E/mm^2), $P < 0.05$), whereas SLIT was not different (30.8 ± 10.5 E/mm^2). When transferred, EPIT Tregs prevented eosinophil infiltration in esophagus whereas SLIT Tregs did not (15.5 ± 5.9 and 50.1 ± 18.8 E/mm^2 respectively).
Conclusion: EPIT and SLIT showed similar efficacy in decreasing allergen-specific Th2 responses but only EPIT decreased eosinophils infiltration in esophagus after peanut oral exposure. EPIT-induced, but not SLIT-induced, Tregs maintained suppressive activity during a long period after stopping treatment suggesting possible induction of tolerance by EPIT.

1296
Immunogenic activity of pollen allergoids

Haefner, D; Meyer, H; Weber, B; Kettner, J; Narkus, A
Allergopharma GmbH & Co. KG, Medical Department, Reinbek, Germany

Background: Chemically modified allergen preparations (allergoids) are characterized by reduced IgE-binding activity, but retained immunogenicity. Accordingly, allergoids bear the potential of performing specific immunotherapy (SIT) at high initial and maintenance doses. Numerous recent clinical studies have confirmed that sufficient allergen dosing is required for successful immunotherapy. The concept of high-dose allergoids was initially introduced for pollen-SITs. We analysed the kinetics of serum IgG_4 levels following SIT with grass and birch allergoids.
Method: Prospectively planned analyses of serum IgG_4 levels were performed using Allergopharma ELISA in two double-blind, placebo-controlled clinical trials. For randomisation patients (18–60 years) had to have a history of allergic rhinitis +/− asthma, positive skin prick and conjunctival challenge test results, serum IgE to grass and birch pollen allergens (EAST class ≥2), and symptoms at baseline (BL). IgG_4 levels were determined before and after 1 and 2 years in preseasonal SIT regimes.
Results: SIT using allergoids was generally associated with serum specific IgG antibody inductions, measurable after the first treatment year with further increases in the second year. Mean IgG_4 levels increased from [μg/l]: 386 (BL), 5767 (year 1), 20733 (year 2) for the birch pollen allergoid and [μg/l]:942 (BL), 61765 (year 1), 73433 (year 2) for the grass pollen allergoid. There were no relevant changes of serum IgG_4 levels in the placebo groups.
Conclusion: SIT using subcutaneous allergoid preparations is associated with serum specific antibody inductions. There is a continous increase in IgG_4 levels during treatment with a comparable percentage of change from baseline after two pre-seasonal treatments in both pollen allergoids.

1297
Epicutaneous and sublingual immunotherapy induce different phenotypes of regulatory T cells in mice sensitised to peanut

Dioszeghy, V[1]; Mondoulet, L[1]; Dhelft, V[1]; Ligouis, M[1]; Puteaux, E[1]; Dupont, C[2]; Benhamou, P-H[1]
[1]DBV Technologies, Bagneux, France; [2]Hôpital Necker, Université Paris Descartes, Paris, France

Background: Epicutaneous immunotherapy (EPIT) on intact skin increases Foxp3+ regulatory T cells (Tregs) in a model of mice sensitised to peanut. The aim of this study was to phenotypically and functionally characterise those Tregs in comparison to those induced by sublingual immunotherapy (SLIT).

Method: Thirty mice were sensitised orally to peanut protein extract (PPE) with cholera toxin, divided into three groups, EPIT, SLIT and Sham, and compared to naïve mice. After 8 weeks of treatment, spleen cells were harvested for flow cytometry analysis and cell culture. The phenotype of Tregs was analyzed using specific antibody for mouse CD4, CD25, foxp3, IL-10, CD304, CTLA-4, CD44 and CD62L. Suppressive activities were measured by cytokine production in PPE-restimulated splenocytes in presence or absence of anti-IL-10 or anti-CTLA-4 blocking antibody.

Results: EPIT and SLIT induced Tregs with different phenotypes. EPIT and SLIT both increased CD4+CD25+Foxp3+ cells compared to Sham (6.10 ± 0.11 and 4.75 ± 0.23 vs $3.18 \pm 0.21\%$ respectively, $P < 0.001$), significantly more with EPIT than with SLIT ($P < 0.001$). EPIT did not increase IL-10+ cells whereas SLIT did, compared to Sham (1.60 ± 0.11 vs 2.76 ± 0.24 vs $1.26 \pm 0.09\%$ respectively, $P < 0.001$). EPIT and SLIT both induced foxp3+ Treg with an effector phenotype (CD44hiCD62L-) ($P < 0.01$) and foxp3+ CD304+ nTregs ($P < 0.001$), but only EPIT induced Treg with a naïve phenotype (CD44loCD62L+) ($P < 0.001$), foxp3+ CTLA-4+ Tregs ($P < 0.01$) and foxp3+ CD304- iTregs ($P < 0.01$). A functional difference between EPIT- and SLIT-induced Tregs was also observed. PPE-specific IL-5 and IL-13 production of splenocytes was significantly decreased with EPIT and SLIT compared to Sham ($P < 0.05$), whereas IL-10 production increased only with SLIT ($P < 0.001$). The effect of SLIT on PPE-specific cytokine production, but not of EPIT, was abrogated in the presence of anti-IL-10 antibody, whereas the effect of EPIT, but not of SLIT, was abrogated in presence of anti-CTLA-4 antibody.

Conclusion: EPIT induced Foxp3+ iTregs which act through CTLA-4 mediated cell contact mechanisms, whereas SLIT induced IL-10+ Tr1 cells. These differences and the induction of naïve Tregs only by EPIT may have important consequences on long term tolerance and on further sensitisation to other allergens.

Poster Session 53

Developments in clinical research in allergen-specific immunotherapy

1298
A Cuban nation-wide pharmacovigilance study of allergen immunotherapy for asthma using allergen vaccines from tropical relevant house-dust-mite species

Mateo Morejón, M[1]; Castro Almarales, RL[1]; Reyes Zamora, MC[1]; Jimenez Lopez, G[2]; Alfonso Orta, I[2]; Labrada Rosado, A[1]
[1]Biocen, Allergen, Bejucal, Cuba; [2]Ministry of Public Healthcare, National Pharmaco-epidemiology Service, Habana, Cuba

Background: Efficacy and safety of allergen immunotherapy (AIT) for asthma, using House Dust Mite (HDM) vaccines has been widely evaluated in controlled clinical trials. However, relatively few studies address this problem in routine clinical practice with a large population sample, particularly in tropical countries. The aim of this study was to assess the efficacy and safety of AIT using HDM vaccines by sublingual (SLIT) or subcutaneous (SCIT) route in clinical practice, in Cuba.
Method: It was a prospective, open, nation-wide, Fase IV, pharmacovigilance, study of asthmatic patients undergoing AIT with standardised vaccines of Dermatophagoides pteronyssinus, Dermatophagoides siboney and Blomia tropicalis (VALERGEN, BIOCEN, Cuba). The last two species are tropical. The study was carried out in 27 allergy services in 10 different Cuban provinces. Both, adult and infant (5–16 years) asthmatic patients were included. Efficacy variables were assessed in 634 patients who completed 1 year treatment. For adverse reactions, the analysis comprised all included patients totaling 1805.
Results: Patients who abandoned the study reached 8.4%. SCIT was mostly used in adult (60.1%), while in adults both routes were used in a similar extent. Highly significant improvement ($P < 0.0001$) was reported for all efficacy variables as compared to baseline. In particular, increase of Quality of Life index by 1.93 units, decrease of asthma severity by 91.2% and medication intake by 47%, and increase of symptom-free days by 18.7%. The efficacy was similar between the two administration routes. Drug consumption was significantly ($P < 0.001$) lower in children, particularly by SLIT. The overall efficacy was similar for the three products. Eighty-seven adverse events were reported, including 60 systemic (Grade I–IV) and 27 local reactions; 4.76% of patients showed adverse events. The subcutaneous route predominated in both systemic and local reactions, with a frequency four times higher than the sublingual. No serious systemic events (Grade III–IV) were reported by SLIT.
Conclusion: The efficacy and safety of AIT for asthma, using tropical HDM vaccines was confirmed, in routine clinical practice, with a relatively large and diverse sample of Cuban patients. SLIT showed fewer and less severe adverse reactions. The efficacy of SLIT was similar as compared to subcutaneous route.

1299
Is clinical efficacy of sublingual immunotherapy comparable at different pediatric age ranges?

Occasi, F[1]; De Castro, G[1]; Castagna, G[1]; Cesoni Marcelli, A[1]; di Coste, A[1]; De Vittori, V[1]; Bardanzellu, F[2]; Duse, M[1]
[1]Sapienza University of Rome, Rome, Italy; [2]Department of Pediatrics, Sapienza University of Rome, Rome, Italy

Background: Sublingual immunotherapy is considered as a valid treatment for allergic respiratory deseases and the only aetiology-based therapy that has the potential for a disease modification. The aim of our study is to assess its clinical efficacy at different age ranges in pediatric patients.
Method: We selected 70 children, 43 boys (61.4%) and 27 girls (38.6%), aged between 6 and 14 years with a clinical history of allergic asthma and/or rhinitis nasal. Sensitisation was assessed by Skin Prick Test, S-IgE and by a clear relationship between exposure to sensitising allergen and symptom appearance. Within the 70 children group, 29(41.4%) underwent SLIT for House Dust Mite (HDM) and 41 (58.6%) for Grass mix. According to the age we divided children into three groups: 20 patients (28.6%) aged between 6 and 8 years (Group 1); 31 patients (44.3%) aged between 9 and 11 years (Group 2); 19 patients (27.1%) age >12 years old (Group 3). During the 3 years of treatment, clinical symptoms were evaluated by a Nasal and Bronchial Symptom score: a score of 1 was assigned to mild symptoms, 2 to moderate and 3 to severe. Drug consumption was assessed by a Medication Score (MS): a score of 1 was assigned to the use of CST and BLD, 2 to ANTILT, and 0 if no medication was needed.
Results: At the baseline Asthma was diagnosed in three children (15%) of Group 1, 4 (12.9%) of Group 2, 3 (15.8%) children of Group 3. Rhinitis in four children (20%) of the first group, 10 (32.3%) of second group, 6 (31.6%) of the third group; while both asthma and rhinitis were diagnosed in 13 (65%) patients of Group 1, in 17 (54.8%) of group 2, 10 (52.6%) of group 3. A significant clinical improvement was observed since the first year of treatment, assessed by a decrease of Symptom Score in all three age ranges. The reduction of asthma, rhinitis and medication scores over time did not show any statistically significant difference among different age ranges ($F = 2.160$ $P > 0.05$; $F = 0.904$, $P > 0.05$; $F = 1.702$, $P > 0.05$).
Conclusion: SLIT is effective in determining a significant reduction of both asthma and rhinitis symptoms in pediatric patients. The reduction of symptoms scores over time was similar in all the three age ranges considered in children aged between 6 at 14 years of life.

1300
Low frequency of systemic reactions in cluster up dosing regimens in subcutaneous immunotherapy

Peralta Filpo, G; Escobar Bolaños, C; Cimarra, M; Rodríguez Alvarez, M; González Gutierrez, ML; Robledo Echarren, T; Fernández-Rivas, M
IdISSC, Allergy, Hospital Clinico San Carlos, Madrid, Spain

Background: Specific subcutaneous immunotherapy (SCIT) is a proven, highly effective treatment for allergic diseases. The experience with cluster schedule has shown good tolerance and improvement in adherence and effectiveness of the treatment. However, cluster schedules in SCIT are not widely used due to the potential increase of systemic reactions.

Objective: To evaluate the frequency and severity of systemic reactions in patients with rhinoconjunctivitis and asthma treated with SCIT in cluster schedule.
Method: We performed a retrospective study of patients who received in our Immunotherapy Unit, from September to December 2012, SCIT to any inhalant with a cluster up-dosing schedule. We analysed the frequency and characteristics of systemic reactions.
Results: The analysis included 104 patients, with a mean age of 26 years (range 6–61 years), of whom 57 were females. Forty-four patients (51.9%) had rhinoconjunctivitis, and 50 (48.1%) rhinoconjunctivitis and asthma. The allergen composition of the vaccines included: grass pollen in 44 patients (42.3%), grass + cypress pollens in 18 (17.3%), cypress pollen in 12 (11.5%), olive pollen in 6 (5.8%), olive + cypress in 2 (1.9%), olive + grass pollen in 2 (1.9%), cypress + plane tree in 2 (1.9%), dog dander in 2 (1.9%), cat dander in 2 (1.9%), and D. Pteronyssinus in two patients (1.9%). The total number of injections was 727. There were six systemic reactions (5.7% of patients and 0.8% of injections), of which five were delayed, and only one had an early onset. In terms of severity, one systemic reaction was grade 2, and the remaining five were grade 1. Due to the low number of reactions we can not find an association between the allergen composition and the appearance of systemic reactions.
Conclusion: The frequency and severity of systemic reactions in patients with rhinoconjunctivitis and asthma treated with immunotherapy in cluster schedules is similar to the frequency of systemic reactions seen in slower build-up regimens.

1301
Safety of maintenance dose subcutaneous immunotherapy: the occurrence of local and systemic reactions in patients with seasonal allergic rhinitis during and outside the pollen season

Distler, A[1]; van Os, N[1]; Bubel, P[2]; Neumann, U[3]; Angelova, N[4]; Nienhuis, H[4]; Boot, D[4]
[1]HAL Allergy GmbH, Medical Department, Düsseldorf, Germany; [2]ENT Cooperative Sachsen-Anhalt, Eisleben, Germany; [3]ENT Cooperative Sachsen-Anhalt, Wolmirstedt, Germany; [4]HAL Allergy BV, Medical Affairs, Leiden, The Netherlands

Background: During the pollen season an increase in the severity of local and systemic reactions due to the increase in allergen exposure has been documented. As a result, dose reduction during the pollen season is advised in the leaflet of many subcutaneous allergen immunotherapy (SCIT) products and is often applied in clinical practice. The product under investigation is a suspension of a glutaraldehyde-modified allergen extract from pollen adsorbed onto aluminum hydroxide (allergoid). Such preparations are considered safer than non-modified allergen extracts and as a result dose reduction is not required during the pollen season. The current non interventional study in adults and children aimed to investigate if there is a difference in local and systemic reactions during and outside the pollen season when the maintenance dose is kept unchanged.
Method: Subjects with a history of allergic rhinoconjunctivitis with or without mild concomitant asthma due to pollen were treated with SCIT pollen allergoids for approximately 1 year, according to onsite routine. Patients received SCIT with the highest recommended maintenance dose (0.5 ml). At each visit, local (wheal and flare size) and systemic reactions were recorded. Patients filled in a diary after every injection. Before and after treatment the patient was asked to judge his/her allergy symptoms on a VAS scale. The duration of the respective pollen season was based on local pollen counts.
Results: Two hundred and thirty-three patients (105 female, mean age 33 years, 44 children) were included in the study analysis. 44.5% received grasses, 43.2% birch or trees, and 12.3% received a combination of grasses and trees. The number of injections during maintenance dose was 3.168 (617 during and 2.551 outside the pollen season). The average wheal size was 9.8 and 8.5 mm inside and outside the pollen season, respectively. The average flare size was 18.9 and 23.3 mm inside and outside the pollen season, respectively. Fifty-three percent of the patients reported an adverse event (AE). The vast majority (92.9%) was injection site related. No severe systemic AE (grade 2–4) occurred. The decrease in overall, lung, eye and nose symptoms compared to baseline was −45.3%, −16.7%, −48.7% and −45.2%, respectively.
Conclusion: Maintenance dose SCIT with pollen allergoids during the pollen season didn't show more local and systemic reactions compared to injections outside the pollen season. In addition, 1 year of treatment resulted in improvements in allergic symptoms.

1302
Compliance in subcutaneous specific immunotherapy: doctors' assessment vs real therapy duration on the basis of 11 473 German patient cases

Martin, E[1]; Thum-Oltmer, S[1]; Schmelcher, D[1]; Karadag, E[2]
[1]Allergopharma GmbH & Co. KG, Reinbek, Germany; [2]Pyramis Pharma-Marktforschung GmbH, Munich, Germany

Background: Three to five years duration of SCIT is recommended by allergological associations (guidelines). Current investigations on companies' sales data showed that compliance on subcutaneous specific immunotherapy (SCIT) seems to be lower than supposed. Aim of this research was to measure the compliance in daily practice on the basis of real patient cases.
Method: Patient cases were collected by 377 German physicians (allergy-focussed) via an independent market research company. To randomise the sample, the doctors were advised to list all patients receiving SCIT in 2008 (via accounting code for subcutaneous SIT) and then report the first-listed patients (Ø = 30/practice). Documentation included statistics, SCIT product, treated allergen, therapy concept, real therapy duration (calculated by 1. injection date/last injection date) and doctor's compliance rating.
Results: Of 11 473 cases from 377 practices were analyzed. Forty percent of participants were ear, nose and throat (ENT) doctors, 33% dermatologists, 11% pulmonologists, 7% pediatricians and 9% from other specialties. ENT doctors and dermatologists were over-represented vs the total market shares in Germany (29% and 25%). Half of the patients were female. Eleven percent were insured privately, reflecting nationwide average. Twenty-six percent of patients were firstly diagnosed in 2008. Perennial SCIT was planned in 70.3% of patients as well as continued perennially in 70.4% of cases. Therapy duration was 37 months in the average. The therapy duration was >36 month in 58% of perennially treated patients. In 87% of cases the duration was >24 month. Doctors rated 84% of their patients as compliant, 16% as 'non-compliant'. Mostly, the reasons for non-compliance were not known. Patients failed to appear without reason in 30% of 'non-compliant' cases.
Conclusion: Doctors' appreciation of patient compliance was clearly more positive than real therapy duration of SCIT. Only 58% of patients were treated more than the recommended minimum of 36 months, whilst doctors assessed 84% of patients as 'compliant'.

1303
Sustained efficacy of 300IR sublingual solution of birch pollen extract in adults with birch pollen-induced rhinoconjunctivitis

Worm, M[1]; Mélac, M[2]; Cadic, V[2]; Zeldin, RK[2]
[1]Charite – Universitätsmedizin Berlin, Berlin, Germany; [2]Stallergenes, Global Clinical Development, Antony, France

Background: Birch is the major allergen-producing tree in northern Europe. Approximately, 70% of birch-allergic patients exhibit symptoms consistent with oral allergy syndrome (OAS). Here, we report the efficacy and safety of 300IR birch pollen sublingual solution administered pre- and co-seasonally for two consecutive years to patients with birch-associated allergic rhinoconjunctivitis (ARC).
Method: Adults (18–65 years old) with at least a two birch-pollen season history of ARC, a retrospective symptom score ≥12 (scale 0–18) and a positive skin test and *in vitro* test for birch-specific IgE antibodies were stratified according to OAS status and randomised to receive placebo or 300IR sublingual solution of birch pollen extract beginning 4 months pre-seasonally and over the pollen season for two consecutive years. Patients scored each of their six rhinoconjunctivitis symptoms on a 0–3 scale and reported rescue medication use. The primary endpoint was the Average Adjusted Symptom Score (AAdSS, range: 0–18) during the second pollen period (ANCOVA analysis). Secondary endpoints included the AAdSS according to OAS status, Average Rhinoconjunctivitis Total Symptom Score and Average Rescue Medication Score. Safety was assessed by means of adverse event (AE) reporting.
Results: Five hundred and seventy-four patients were randomised to placebo ($n = 290$) or 300IR ($n = 284$) and 496 (86%) completed the study. In each group, 54% of patients had OAS. The AAdSS least-squares (LS) mean difference vs placebo during the second pollen period was −2.04 [$CI_{95\%}$ (−2.69; −1.40), $P < 0.0001$], corresponding to a relative LS mean difference vs placebo of −30.6%. In the subgroups of patients with and without OAS, similar reductions in AAdSS vs placebo were observed. Other secondary endpoints were significantly reduced in the 300IR group compared to placebo, in the overall population. Significant differences were also observed for these variables during the first pollen season. AEs were mainly application site reactions: oral pruritus, oedema mouth. Fewer patients in the active group (4.2%) than in the placebo group (6.6%) reported an AE of asthma.
Conclusion: Pre- and co-seasonal treatment with 300IR birch pollen sublingual solution over 2 years was efficacious in the overall population and in the subgroups with and without OAS. The treatment was well tolerated.

1304
Who will benefit from treatment with house dust mite allergy immunotherapy tablets?

Demoly, P[1]; Virchow, JC[2]; Simin Geertsen, M[3]; Seitzberg, D[4]; Holger, M[5]; Dahlgren, S[6]
[1]Allergy Division, Pulmonary Department, Hôpital Arnaud de Villeneuve, University Hospital of Montpellier, INSERM U657-EA2415, Montpellier, France; [2]Department of Pneumology/Intensive Care Medicine, Universitätsklinikum Rostock, Rostock, Germany; [3]ALK, Global Medical Affairs, Horsholm, Denmark; [4]ALK, Global Clinical Development, Horsholm, Denmark; [5]Allergy Clinic, Copenhagen University Hospital, Gentofte, Denmark; [6]ALK, Hoersholm, Denmark

Background: A randomised, double-blind, placebo-controlled, 4-arm trial with a house dust mite (HDM) allergy immunotherapy tablet (AIT) (ALK, Denmark) was conducted in subjects ≥14 years (EudraCT 2006-001795-20). The primary analysis gave proof of concept for efficacy of the 6 DU dose. Post-hoc efficacy analyses have been performed in two subpopulations, and a characterisation of the entire trial population with respect to GINA control has been presented. This abstract combines the results to identify the patients with the highest medical need who can be safely treated.
Method: Trial population: 604 HDM-allergic subjects, mild-moderate asthma, rhinitis, daily inhaled corticosteroid (ICS, budesonide) 100–800 μg, controlled asthma, FEV1 ≥ 70% at baseline. The 6 DU and placebo groups comprised 299 subjects (intent-to-treat, ITT), categorised into GINA 2010 control levels by translation of baseline ACQ individual domain scores. Subjects with a medical need in asthma ($N = 56$) were identified as those with non-optimal asthma control (ACQ = 1–1.5) despite ICS ≥ 400 μg. Subjects with a medical need in rhinitis ($N = 154$) were identified as those with a baseline total combined rhinitis score (TCRS) >0 and ICS ≤600 μg, with the latter inspired by an observed negative correlation for the entire population between baseline TCRS and ICS dose.
Results: 70% of the subjects were partly controlled, 11% were uncontrolled (GINA 2010). The benefit on asthma parameters was highest for patients partly controlled (ACQ) by medium-high ICS use, while the benefit in rhinitis was highest for patients with low-medium ICS use and symptomatic rhinitis at baseline.
Conclusion: The lack of observed safety concerns suggests that the HDM AIT could be applicable at all GINA control levels as long as FEV1 is ≥70%. The subgroup analyses led us to hypothesise that the most favourable benefit-risk profile is expected in patients with HDM respiratory allergic disease manifesting as partly controlled asthma despite medium-high ICS use or moderate-severe persistent rhinitis. Efficacy of the HDM AIT in these patient profiles is currently under investigation in two confirmatory trials (EudraCT: 2010-018621-19, 2011-002277-38).

1305
Effects of subcutaneous specific immunotherapy with recombinant hypoallergenic Bet v 1 folding variant (rBet v 1-FV) on asthma symptoms

Haefner, D; Tribanek, M; Kettner, J; Narkus, A
Allergopharma GmbH & Co. KG, Medical Department, Reinbek, Germany

Background: The recombinant folding variant rBet v 1-FV has been shown to be effective in SCIT of birch pollen allergic rhinoconjunctivitis, and its optimum therapeutic dose has been established. Although prevention of asthma or improvement of asthma symptoms have been recognised as a further target of subcutaneous specific immunotherapy (SCIT), evidence for the efficacy of SCIT in allergic asthma from randomised, well controlled studies is limited. We analyzed the effects of SCIT with rBet v 1-FV on seasonal asthma symptoms in asthmatic patients included in a birch pollen rhinoconjunctivitis study.
Method: A post-hoc analysis of a randomised, double-blind, placebo-controlled trial was performed. For entry, patients (18–60 years) had to have a history of birch pollen allergic rhinoconjunctivitis +/− asthma, symptoms during the baseline

	ITT			Subgroup		
6 DU vs placebo	Mean	95% CI	*P*	Mean	95% CI	*P*
ICS reduction, μg/day	81	27; 136	0.004	327	182; 471	<0.0001
Asthma quality of life questionnaire (AQLQ) score*	0.11	−0.03; 0.25	0.11	0.52	0.13; 0.92	0.01
TCRS, %*	16	−4; 35	0.11	28	6; 49	0.01

*End of treatment.

birch pollen season (BL) in total eye-nose-lung symptom score, a positive skin prick and conjunctival challenge test and serum IgE to birch (EAST class ≥2). Inhaled corticosteroids ≤400 μg budesonide-equivalent and salbutamol p.r.n in GINA I/II patients were maintained unchanged. The sum of cough, wheeze and dyspnoea scores at BL and after 1 and 2 years of perennial SCIT with rBet v 1-FV was assessed.

Results: The full analysis set comprised 104 actively treated and 98 placebo patients. Fifty-five rBet v 1-FV (placebo: 51) patients were known to have asthma at study entry. After one treatment year, a considerable median change in the lung symptom score of −58.0 for rBet v 1-FV (placebo: −51.0) was observed. After 2 years of treatment a significantly more pronounced treatment effect of −47.5 for rBet v 1-FV treated patients in comparison to a decrease of −22.0 in the placebo group was observed ($P < 0.05$).

Conclusion: SCIT with the hypoallergenic recombinant birch pollen major allergen vaccine rBet v 1-FV improved lung symptoms in patients with birch pollen induced allergic asthma. Prospective trials in bronchial asthma are needed to substantiate our findings.

1306
The specialists' choice for sublingual immunotherapy products is 'flight to quality'

Canonica, GW[1]; Passalacqua, G[1]; Dell'Albani, I[2]; Incorvaia, C[3]; Cadario, G[4]; Marengo, F[4]; Fiocchi, A[5]; Senna, G[6]; Rossi, O[7]; Romano, A[8]; Scala, E[9]; Romano, C[10]; Ingrassia, A[11]; Zambito, M[12]; Frati, F[2]; AIDA Study Group
[1]Allergy and Respiratory Diseases, DIMI, University of Genoa – IRCCS-Azienda Ospedaliera Universitaria San Martino, Genoa, Italy; [2]Stallergenes Italy srl, Medical and Scientific Department, Milan, Italy; [3]Allergy/Pulmonary Rehabilitation Unit, ICP Hospital, Milan, Italy; [4]Allergy and Clinical Immunology Unit, San Giovanni Battista (Molinette) Hospital, Turin, Italy; [5]Department of Pediatrics, Division of Allergy, Pediatric Hospital Bambino Gesù, Rome, Vatican City, Italy; [6]Allergy Unit, Verona General Hospital, Verona, Italy; [7]AOUC, Department of Biomedicine, University of Florence, Florence, Italy; [8]C.I. Columbus, Department of Internal Medicine and Geriatrics, UCSC-Allergy Unit, Rome, Italy; [9]IDI-IRCCS, Experimental Allergy Unit, Rome, Italy; [10]ASL Napoli 3 Sud, Naples, Italy; [11]ASP Trapani, Allergology Department, Trapani, Italy; [12]AUSL 6 Palermo, Palermo, Italy

Background: The most recent documents on sublingual immunotherapy (SLIT) introduced the concept of 'brand product' and defined which are the factors to be considered by physicians when they choose SLIT products. It was suggested to choose them depending on the available level of scientific evidence for a specific allergen. Furthermore, the WAO Position Paper comprised a set of recommendations for manufacturers, which should provide a good scientific documentation of efficacy, standardised quantities of allergen contents and the titration of major allergens in micrograms. The aim of this survey was to investigate which are the criteria used in real-life clinical practice by specialists in the choice of SLIT products for adult population.

Method: The AIDA survey (Allergen Immunotherapy Decision Analysis) was performed as an *e*-research by Lexis Ricerche Srl (Milan, Italy), using an on-line questionnaire validated by a Scientific Board of 12 experts. It was composed of a list of 14 items to be ranked by each physician according to the importance attributed when choosing SLIT products (most important = 1, less important = 14). The survey involved 444 Italian physicians, including allergists, Ear-Nose-Throat (ENT) specialists and pulmonologists. Complete questionnaires returned from 169 physicians (38.1%) were used for statistical analysis.

Results: The majority of sample was composed of 79% allergists, 11% pulmonologists and 3% ENTs, the most part aged between 45 and 64 years. The final rankings of 14 items were: Efficacy – Level of EBM validation (3.44), Safety – Level of EBM validation (4.30), Standardisation of product (5.37), Efficacy based on personal experience (5.82), Titration of major allergens in micrograms (5.96), Scientific evidence for single allergen (6.17), Safety based on personal experience (6.32), Easiness of administration scheme (8.08), Cost and terms of payment (e.g. installments) (9.17), Dose personalisation (9.24), Patients' preferences (9.25), Easiness of product storage (9.93), Reimbursement (10.12) and Availability of assistance by manufacturer (11.89).

Conclusion: In real-life clinical practice the driver of specialists' choice for SLIT products is the concept of quality, which means: level of EBM validation in terms of efficacy and safety, standardisation of product, efficacy based on personal experience and titration of major allergens in micrograms.

1307
Evaluation of clinical contraindications in allergen immunotherapy: preliminary report of an EAACI Task Force

Pitsios, C[1]; Bilò, MB[2]; Ruëff, F[3]; Valovirta, E[4]; Gawlik, R[5]; Sturm, G[6]; Rodriguez del Rio, P[7]; Paraskevopoulos, I[8]; Tsoumani, M[9]; Pfaar, O[10]; Demoly, P[11]; Calderon, MA[12]
[1]Department of Dietetics and Nutrition, Harokopio University, Athens, Greece; [2]Ospedali Riuniti di Ancona, Department of Allergy, Immunology and Respiratory Dis., Ancona, Italy; [3]Ludwig-Maximilians-Universität, Klinik und Poliklinik für Dermatologie und Allergologie, Munich, Germany; [4]Department of Pediatric Allergy, University of Turku, Turky, Finland; [5]Department of Allergy and Clinical Immunology, Silesian University of Medicine, Katowice, Poland; [6]Division of Environmental Dermatology and Venerology, Medizinische Universität Graz, Graz, Austria; [7]Allergy Department Hospital Infantil Universitario Niño Jesús, Madrid, Spain; [8]Allergy Department, 401 Army General Hospital, Athens, Greece; [9]Department of Allergy and Clinical Immunology, 'Laikon' General Hospital, Athens, Greece; [10]Center for Rhinology and Allergology, Universitäts HNO-Klinik Mannheim, Mannheim, Germany; [11]University Hospital of Montpellier, Clin. Maladies Respiratoires, Montpellier, France; [12]Imperial College – National Heart and Lung Institute, London, United Kingdom

Background: Currently, there are various National and International guidelines, reporting several clinical contraindications to allergen Immunotherapy (AIT). Some of them are not thoroughly verified by evidence-based-medicine data. The 'Contraindications in AIT' Task Force (TF) was created by the EAACI Immunotherapy Interest Group to evaluate and update the current literature aimed to propose recommendations on both sublingual and subcutaneous AIT for respiratory and venom allergy.

Method: An extensive literature research has been performed by TF members. All findings were cross-matched with the results of a librarian's research, using keywords commonly accepted. The contraindications addressed are: autoimmune diseases, cancers, mastocytosis, treatment with various drugs, cardiovascular diseases, severe/uncontrolled asthma, HIV, transplantations, history of anaphylaxis due to AIT, age (lower than 6, higher than 65 years of age) and mental diseases. The authors have addressed each topic with the following questions:

1 Are there any negative effects of AIT on the condition?
2 Are more frequent or more severe AIT-related side effects expected?
3 Is AIT expected to be less efficacious?
4 Which is the level of evidence?

Results: Severe asthma, HIV, history of anaphylaxis, cardiovascular diseases requiring the use of beta-blockers and cancers are the most common referred contraindications in AIT. No international consensus is established regarding the 'absolute' or 'relative' status of these contraindications.

Limitations in establishing evidence-based medicine guidelines have been accepted. Based on preliminary results, some current contraindications do not have any negative impact on the effectiveness or safety of AIT.
Conclusion: Future guidelines should be precise, assessing the different levels of evidence. An EAACI-position paper on 'Contraindications to AIT' is planned in due time. Legal implications of the Guidelines should be considered.

1308
The European survey on adverse systemic reactions due to allergen immunotherapy: study doctors' and patients' inclusion criteria

Rodríguez del Río, P[1]; Vidal, C[2]; Just, J[3]; Pfaar, O[4]; Passalacqua, G[5]; Demoly, P[6]; Calderon, MA[7]; EASSI
[1]Hospital Universitario Niño Jesus, Allergy, Madrid, Spain; [2]Hospital Clinico Universitario, Santiago de Compostela, Santiago de Compostela, Spain; [3]Centre de l'Asthme et des Allergies, Hôpital d'Enfant Armand Trousseau, Paris, France; [4]Zentrum für Rhinologie und Allergologie, Universitäts HNO-Klinik Mannheim, Wiesbaden, Germany; [5]University of Genoa, Genoa, Italy; [6]Hôpital Arnaud de Villeneuve, University Hospital of Montpellier, Clin. Maladies Respiratoires, Montpellier, France; [7]Imperial College – National Heart and Lung Institute, London, United Kingdom

Background: To collect the information on clinically relevant systemic reactions due to the regular use of subcutaneous or sublingual allergen immunotherapy (SCIT and SLIT, respectively), a methodological process needs to be properly designed. Within the framework of EAACI, a Task Force has been approved to prepare this electronic survey.
Methods: Two specific questionnaires with the criteria for the selection for recruiting study doctors and patients taking part in this prospective study were pre-established before initiation of the survey. The Survey Monkey® online survey instrument was used. Participant study doctors store all data in a centralised manner. The data stored is protected by an enhanced security system (SSL). The selection questionnaires were done in accordance with the CHERRIES guidelines.
Results: At least 30 study doctors from each participant country (France, Germany, Italy and Spain) have been recruited. Each doctor completed the 'Doctors' Questionnaire' once with nine questions (taking 3 min). The information about the study doctor prescribing AIT includes: speciality, clinical experience, setting of practice (social security system or private), number of new patients on AIT in the last year and the percentage of SCIT or SLIT treatments prescribed in the last year.

For every patient who has started receiving either SCIT or SLIT, a 'Patients' Questionnaire' of 25 questions was completed only once (taking 8 min). This questionnaire includes: demographic data of the patient (age, gender), baseline medical history (cardio-vascular problems and other clinically relevant chronic diseases), allergy history (asthma, rhinitis, urticaria, atopic dermatitis, conjunctivitis), current treatment, patient's allergic profile (SPTs, sIgE, clinically relevant), previous AIT (tolerance, composition) and details of the prescribed AIT that will be followed up along the survey (onset, composition, route, extract, formulation, schedule, premedication). As of Jan 15th 2013, within the four countries participating in this survey, 144 doctors and 1233 patients have been included.
Conclusion: Our methodology used to recruit study doctors and patients in a prospective European electronic survey has been successfully tested.

1309
The European survey on adverse systemic reactions due to allergen immunotherapy: reporting reactions using a harmonised MedDRA terminology

Rodríguez del Río, P[1]; Vidal, C[2]; Just, J[3]; Pfaar, O[4]; Passalacqua, G[5]; Demoly, P[6]; Calderon, M[7]; EASSI
[1]Hospital Universitario Niño Jesus, Allergy, Madrid, Spain; [2]Hospital Clinico Universitario de Santiago de Compostela, Allergy, Santiago de Compostela, Spain; [3]Centre de l'Asthme et des Allergies, Hôpital d'enfant Armand Trousseau, Paris, France; [4]Zentrum für Rhinologie und Allergologie, Universitäts HNO-Klinik Mannheim, Wiesbaden, Germany; [5]Allergy and Respiratory Diseases, University of Genoa, Italy, Genoa, Italy; [6]Hôpital Arnaud de Villeneuve, University Hospital of Montpellier, Montpellier, France; [7]Imperial College – National Heart and Lung Institute, London, United Kingdom

Background: Systematic reviews on systemic adverse reactions (SAR) due to AIT have indicated the lack of harmonisation in the terminology used. Published data regarding SAR due to AIT is mainly retrieved from three different sources: clinical development of commercialised products, clinical trials and post marketing surveillance (pharmacovigilance). To do so, several classifications can be used: medical dictionary MedDRA (mandatory in pharmacovigilance and clinical trials), WAO and EAACI SAR classifications. The aim of our survey was to harmonise and validate one classification during a pan-European prospective survey, aiming to estimate the frequency of these SAR and the possible risk factors involved.
Methods: An EAACI Task Force of European experts in AIT, pharmacovigilance, epidemiology and drugs regulation, prepared a harmonised terminology for SAR due to AIT based on the MedDRA classification. None of the MedDRA words were withdrawn or adapted but all were connected to WAO/EAACI SAR classification terminology. A specific questionnaire to collect details about every single SAR for all survey-patients was pre-established. All study doctors were thoroughly instructed. The Survey Monkey® online survey instrument has been used. Data is stored and protected by an enhanced security system (SSL) in a centralised manner.
Results: The harmonised MedDRA terminology was incorporated to a specific 'Adverse Reactions Questionnaire' with 21 questions (it takes 6 min). Only SAR of patients who started AIT (either subcutaneous or sublingual) in the study period will be collected. Data collected includes: treatment phase when the reaction occurred (up-dosing/maintenance), elapsed time from application to onset of SAR, description of symptoms, SAR' treatment, severity, seriousness, causality, duration, cofactors, final outcome, treatment discontinuation, or if any modification to the schedule was applied. As of Jan 15, only 15 systemic mild reactions have been reported in a population of 1233 patients over a period of 4 months.
Conclusion: Currently, a harmonised MedDRA classification of SAR due to AIT is being successfully tested. Our preliminary data could suggest that AIT has a good safety profile in real life clinical settings. Further analysis of risks factors and estimation of the frequency of clinically relevant systemic adverse reactions due to AIT will be conducted.

1310
Tolerability and efficacy of house dust mite injective rush immunotherapy with monomeric allergoid compared to sublingual administration in patients with allergic rhinitis

Brivio, G[1]; Boscolo, MA[1]; Compalati, E[2]
[1]Department of Allergy Unit, Hospital of Merate, Merate, Italy; [2]Allergy and Respiratory Diseases Clinic, University of Genoa, Genoa, Italy

Background: Monomeric carbamylated allergoids (monoids) have reduced IgE-binding activity resulting safe by sublingual or subcutaneous administration. They reduce allergic symptoms and the use of drugs. Injections of mite (HDM) and grass pollen monoid are well tolerated following 5 weeks build-up phase. We investigated the safety and efficacy of a treatment course with a rush induction phase.
Method: This open pilot study included 30 mono-sensitised patients receiving monthly for 12 months a preparation of 0.50 ml of 10 BU/ml containing modified HDM extract, starting with a single day induction (0.20 ml plus 0.50 ml after half an hour).

As control 30 patients were treated with HDM sublingual monoid (1000 AU twice a week). Visual analogue score (VAS) was used to evaluate the symptoms of rhino-conjunctivitis at baseline and monthly. Drug consumption was calculated with a monthly score. Adverse reactions (ARs) to treatment were recorded at each visit.

Results: All patients completed the study. No serious ARs occurred. The rush induction resulted safe. No ARs occurred in 21 patients (70%), nine patients (30%) referred local mild ARs not requiring interruption. During the maintenance phase the treatment was well tolerated. Mean monthly VAS progressively improved during winter months ($P < 0.001$) without significant difference between groups throughout the year ($P > 0.05$). Drug intake was comparable between groups.

Conclusion: The safety of monoid appears confirmed also through injective route with rush induction scheme. The improved health condition and drug usage were comparable with those achieved by patients treated with monoid administered sublingually.

1311
Effectiveness and impact on quality of life of sublinual inmunotherapy in allergic conjunctivitis

Gracía, IF
Hospital Hermanos Ameijeiras, Allergy, La Habana, Cuba

Background: Ocular allergy involves the inflammatory process of the eye's surface. Nowadays it is an on-growing global entity with an incidence between 25% and 40% in general population.

Purpose: To evaluate the effectiveness of sublingual immunotherapy for treatment of allergic conjunctivitis and its impact on the patient quality of life.

Method: A longitudinal prospective study was achieved for evaluating permanent allergic conjunctivitis of 30 patients. The variables to be studied were gathered from the personal history chart. An initial evaluation was made at the first consultation and a second one took place at 6 months after treatment. The results were evaluated by means of an analog visual scale for symptoms and signs. The patients answered a quality of life questionnaire (EAPIQ) before and after treatment. The qualitative variables were showed by percentages and the quantitative ones by average, median and standard deviation.

Results: Ocular symptoms (redness, edema, tearing, secretion, photophobia and visual acuity impairment) significantly decreased after administrating sublingual immunotherapy. Comparing both pre and post questionnaires a statistically significant reduction was observed in regards to patients' daily activities limitation and ocular symptoms. A 0.7% referred some adverse event, none was serious and the 100% of patients were able to reduce oral medication.

Conclusion: The VALERGEN sublingual immunotherapy demonstrates tolerability and effectiveness for all the symptoms of permanent allergic conjunctivitis in clinical practice, observing improvement in the patient's quality of life.

1312
INSIDE – safety and efficacy of an intraseasonal short-time subcutaneous immunotherapy with a modified depigmented birch-pollen extract

Klimek, L[1]; Sager, A[2]; Klein, C[3]; Pfaar, O[4]
[1]Center for Rhinology and Allergology, Department for Otorhinolaryngology, HNS, University Hospital Mannheim, Wiesbaden, Germany; [2]LETI Pharma GmbH, Witten, Germany; [3]Novartis Pharma GmbH, Nuremberg, Germany; [4]Center for Rhinology and Allergology, Department of Otorhinolaryngology, HNS, University-Hospital-Mannheim, Wiesbaden, Germany

Background: In current global guidelines initiation of SCIT is recommended before the beginning of the pollen-season due to safety concerns. Therefore, the immediate access of this causal treatment is refused to patients with acute allergic-symptoms during pollen-season.

Method: To our knowledge, this is the first DBPC-clinical trial investigating the safety and tolerability of an intra-seasonal SCIT-schedule with reaching the maintenance-dose within one treatment day followed by five maintenance-doses throughout 5 weeks in the birch-pollen-season. Two hundred and two birch-pollen allergic patients in 40 trial-centers in Germany were enrolled.

Results: The intra-seasonal treatment was not associated with an increased rate of adverse events. The rate of local and systemic reactions was comparable in both groups (Active: local/systemic reactions in 18.0%/7.0% of all patients; Placebo: local/systemic reactions in 20.6%/ 6.9% of all patients). Severe adverse events (grade III and IV due to EAACI-classification) were not observed. Furthermore, there was a highly significant increase of birch-pollen-specific IgG_4 levels in the active group (42.8 ng/ml vs 4.8 ng/ml; $P < 0.0001$). No statistical significant difference on the combined SMS was revealed between the treatment groups due to the short observation time.

Conclusion: The results confirm for the first time the safety and tolerability of the application of a fully implemented intra-seasonal (short-time) SCIT with achievement of the maintenance-dose at the first treatment day. Moreover, significant immunological effects of this intra-seasonal approach were clearly demonstrated.

1313
Symptoms and medication scores results in the ALUMITES study, a randomised, controlled, multicenter phase IV study with house dust mites subcutaneous immunotherapy for allergic rhinitis. Six months interim analysis

Hernandez, D[1]; Nuñez, B[2]; Sanchez, J[3]; Malek, T[4]; Pelaez, A[5]; Garcia, MA[6]
[1]Hospital Universitario y Politecnico La Fe, Valencia, Spain; [2]Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain; [3]Hospital Clinico Universitario, Barcelona, Spain; [4]Hospital General Castellón, Castellon, Spain; [5]Hospital Clinico Universitario de Valencia, Valencia, Spain; [6]Stallergenes Iberica, Barcelona, Spain

Background: ALUMITES study was designed to assess the efficacy of house dust mites (HDM: *D. pteronyssinus* + *D. farinae*) subcutaneous immunotherapy (SCIT) for the treatment of allergic rhinitis patients along 1 year. Here we report an interim analysis done after 6 months of treatment.

Method: In this controlled multicenter phase IV study, HDM allergic adult patients were randomised to receive SCIT with a 10 IR/ml depot extract plus symptomatic treatment (group A) or only symptomatic treatment (group B) (2:1). Symptom scores were collected at inclusion visit and once a month till the end of the trial, using a 0–3 scale for four nasal symptoms. Medication consumption was collected at inclusion visit and daily along the study, only controlled symptomatic medication was allowed. Medication score was calculated as following: antihistaminic 1 point, nasal decongestant 2 points and nasal corticoids 3 points.

Results: Forty-two of 57 patients completed the patient's symptom and medication diary completely. Total symptom score in active group at basal visit was 3.6 ± 2.75 and 6 months later 2.2 ± 2.17 ($P < 0.0001$). In control group the basal value was 3.5 ± 2.30 and 6 months later 2.6 ± 2.58 (P = NS). Each individual nasal symptoms score also showed a similar evolution: nasal congestion, sneezing, runny nose and nasal itching experienced a significant reduction in active group but not in control group.

Medication consumption decreased from 1.0 ± 0.93 to 0.3 ± 0.48 ($P < 0.0001$) in active control whereas in control group the decrease was from 1.5 ± 1.36 to 1.0 ± 0.82 (P = NS).

Conclusion: Efficacy is demonstrated with 6 months of SCIT with a 10 IR/ml depot HDM extract that resulted in a significant improvement in nasal symptoms and reduction of rescue medication intake. This improvement was not achieved by the control group.

Poster Session 54

Clinical studies in allergen-specific immunotherapy IV

1314
Patients with grass-induced allergic rhinitis and unresponsive to drugs achieve disease control through 5-grass pollen tablet treatment

Losappio, L[1]; Frati, F[2]; Dell'Albani, I[2]; Incorvaia, C[3]; Pastorello, EA[1]; ORS Study Group
[1]Allergology and Immunology Department, Niguarda Hospital, Milan, Italy; [2]Medical and Scientific Department, Stallergenes Italy Srl, Milan, Italy; [3]Allergy/Pulmonary Rehabilitation Unit, ICP Hospital, Milan, Italy

Background: An important subpopulation in allergic rhinitis (AR) is represented by patients with severe form of disease inadequately controlled by drug treatment. Furthermore, about 80% of patients with moderate-to-severe AR are unsatisfied with pharmacological therapy. We evaluated in real-life clinical practice the effectiveness of allergen immunotherapy (AIT) with a five-grass extract in tablets in AR patients unresponsive to drugs.
Method: We carried out a multicenter observational study in 47 patients (38 adults and nine adolescents) suffering from grass-induced AR, unresponsive to drug therapy and treated for the first year with five-grass pollen tablets according to a precoseasonal regimen. The clinical data collected before and after AIT dealt with ARIA classification of AR, onset of AR symptoms, clinically relevant allergen, rescue medication use, presence of comorbidities, response to therapy and patient's satisfaction.
Results: Of 47 patients, none (0%) had a mild intermittent AR, 3 (6.4%) had a moderate/severe intermittent AR, 10 (21.3%) a mild persistent AR, and 34 (72.3%) a moderate/severe persistent AR; 19 patients (40.4%) had also asthma. The response to treatment was judged as poor by 33 patients (70.2%) and very poor by 14 patients (29.8%). During the subsequent pollen season, after SLIT treatment, 33 (70.2%) patients had a mild intermittent AR, none (0%) had a moderate/severe intermittent AR, 7 (14.9%) had a mild persistent AR, and 7 (14.9%) had a moderate/severe persistent AR. Only 13 patients (27.6%) presented asthma as comorbidity. Compared to 2010 grass pollen season, medication score was significantly reduced (in 2010 4.2 ± 1.3 vs 2.4 ± 2.0 in 2011). The patients' satisfaction before and after AIT significantly increased ($P < 0.01$).
Conclusion: These findings show that most patients with grass pollen-induced AR unresponsive to symptomatic drug treatment obtain a control of AR after just one season of treatment with five-grass pollen tablets. Furthermore, five-grass pollen tablet treatment significantly reduced the presence of asthma, rescue medication use and produced a significant increase of patients' satisfaction.

1315
One season of treatment with five grass pollen tablets in adults demonstrated a reduction in disease symptoms and impacts. Findings of the SMILE study

Antolin, D[1]; Valbuena, T[2]; Valls, A[3]; Garrido, S[4]; Blanco, C[3]; Garcia, MA[5]
[1]Hospital Universitario Principe de Asturias, Alcala de Henares, Spain; [2]Hospital Universitario Infanta Sofia, San Sebastian de los Reyes, Spain; [3]Hospital Universitario de la Princesa, Madrid, Spain; [4]Complejo Hospitalario de Navarra, Pamplona, Spain; [5]Stallergenes Iberica, Barcelona, Spain

Background: The SMILE study was performed in Spain in 2012 to collected information about clinical characteristics of patients under grass pollen tablet sublingual immunotherapy (SLIT).
Method: In this observational, cross-sectional, multicenter study, allergy specialists collected information in a unique follow-up visit at the end of the grass pollen season 2012. Patients had to have moderate to severe allergic rhinoconjunctivitis (ARC) while being treated but not controlled with symptomatic drugs and they received a tablet for the first time with grass pollen according a precoseasonal scheme.
Results: We present here the results of 226 adult patients. Fifty-five percent of them had asthma and 60% were polysensitised. Mean time of evolution of their ARC was 10.4 ± 8.4 years. All patients were treated with a tablet of a five grasses extract (300 IR) for 4.9 ± 0.9 months.

Before the immunotherapy treatment 92% of the patients had a persistent moderate to severe ARC (according to ARIA classification), and they were only 30% after the precoseasonal therapy. Fourty-nine percent of patients had evolved to a mild ARC. Severity markers disappeared in a large proportion of patients patients: 44% of them had no longer sleep disturbances, 67% had no more impact on their daily activities, 64% had no longer work impairments and 57% had no troublesome symptoms anymore. Before immunotherapy, 92% and 77% of patients were treated with oral anti-histamins and nasal corticoesteroids, respectively. After 5 months of SLIT, these percentages decrease to 62% and 28%, respectively. Among those who still need symptomatic treatment, 80% declared a reduction in their consumption of anti-histamine drugs and 69% for nasal corticoesteroids.
Conclusion: In Spain, the precoseasonal treatment of grass pollen ARC with five-grass SLIT tablets (300 IR), demonstrated, in 'real life' settings, to be effective both in reducing ARC symptoms, as in diminishing the consumption of symptomatic medication in grass-pollen allergic adult patients, therefore, decreasing the disease impact.

1316
Clinical efficacy evaluation of subcutaneous and sublingual immunotherapy in patients monosensitised to house dust mites

Yildirim, O; Coskun, BU; Ucal, YO; Ucak, I; Tetik, F
Otolaryngolgy Department, Sisli Etfal Training and Research Hospital, Istanbul, Turkey

Background: Allergic rhinitis constitutes a major health issue worldwide. Being the only treatment modality to be able to modify the course of the disease, specific immunotherapy is mainly administered by two routes; subcutaneous (SCIT) and sublingual (SLIT). The aim of this study was to investigate and compare the clinical efficacy of subcutaneous immunotherapy and sublingual immunotherapy with standardised house dust mite extract (*D. pteronyssinus*, *D. farinae*) for allergic rhinitis.
Method: One hundred and eleven allergic rhinitis patients monosensitised to house dust mite allergens who completed SCIT (*Phostal*, *Stallergenes*, France) (52 patients) or SLIT (Staloral 300, *Stallergenes*, France) (60 patients) regimen for 5 years were enrolled in this study. The symptom scores (sneezing, rhinorrhea, nasal obstruction, pruritus, postnasal drip, wheezing,

rashes, eye symptoms, cough, loss of smell) were evaluated using a 4-point rating scale (from 0 = absent to 3 = severe) before and after the treatment as well as the medication scores, life quality and treatment satisfaction questionnaires. The efficacy of SCIT and SLIT was assessed using the mean change from baseline in symptom and medication scores.

Results: Both SCIT and SLIT significantly reduced the individual symptom scores of sneezing, rhinorrhea, nasal obstruction, postnasal drip, pruritus, cough, loss of smell after 5-years treatment when compared with the baseline ($P < 0.05$). There was no significant difference in decreased mean scores of the symptoms between SCIT and SLIT groups ($P > 0.05$). A significant reduction in eye symptom scores was found in SCIT group compared with SLIT group. There was no significant difference in wheezing scores of SCIT patients, whereas symtom severity was significantly reduced in SLIT group. In the quality of life assessments, and treatment satisfaction questionnaires, there were no statistically significant differences between groups. SLIT and SCIT demonstrated a significant reduction in medication scores. During the treatment course only one systemic reaction was observed in the SCIT group which was successfully treated by early recognition. Local reaction rates were higher in the SCIT group.

Conclusion: Both SLIT and SCIT demonstrated clinical improvement in allergic rhinitis patients sensitised to HDM after 5 years of treatment. The overall clinical efficacy was simillar with SCIT and SLIT.

1317
Decreased specific basophil responses following treatment with grass allergy immunotherapy tablets as well as subcutaneous *Phleum pratense* immunotherapy

Aasbjerg, K[1]; Lund, G[2]; Holm, J[2]; Nielsen, NC[2]; Holse, M[2]; Wagtmann, VR[2]; Backer, V[1]; Würtzen, PA[2]
[1]Department of Respiratory Medicine, Bispebjerg Hospital, Copenhagen, Denmark; [2]ALK, Immunology, Hoersholm, Denmark

Background: No effect on basophil responsiveness was recently reported after 4 months of receiving tablet based immunotherapy with grass pollen extract. In contrast, several groups have shown that subcutaneous treatment (SCIT) leads to decreased basophil response – although never tested in parallel treatment groups. We therefore wanted to investigate longitudinal influence of grass allergy immunotherapy tablet (AIT) as well as SCIT immunotherapy on basophil response to *P. pratense* in the same study.

Method: In total 40 individuals with grass pollen allergic rhinitis (AR) were randomised into three groups receiving SQ-standardised grass AIT (Grazax 75,000 SQ-T), SCIT (Alutard SQ) or no treatment (controls). Treatment was continued for 15 months. Blood samples were regularly collected and basophil response to challenge with different doses of *P. pratense* was compared by induced expression of basophile surface markers CD63 and CD203c measured by flow cytometry. Allergen sensitivity for induced surface marker expression (EC50) was evaluated.

Results: Basophil responses to *P. pratense* were progressively decreased compared to the control group as well as compared to baseline in the group receiving grass AIT daily, becoming significant from 10 months treatment. For SCIT treated patients basophil sensitivity were significantly decreased from 3 months treatment when reaching maintenance dose. In contrast to both treatment groups, basophil sensitivity to *P. pratense* was increased after grass pollen season in untreated control patients.

Conclusion: Treatment with SQ- standardised grass allergy immunotherapy tablets lead to significantly decreased basophil response to *P. pratense* following 10 months treatment. This indicates that changes in basophil sensitivity are involved in the immunological effect of both Grass AIT and Grass SCIT treatment.

1318
Management and characteristics of sublingual allergen immunotherapy in children suffering from respiratory allergies induced by house dust mite allergens

Trebuchon, F[1]; David, M[2]; Demoly, P[3]
[1]Private Office, Montferrier-sur-Lez, France; [2]Stallergenes, Antony, France; [3]University Hospital of Montpellier, Montpellier, France

Background: The objective was to retrospectively describe the paediatric use of house dust mite (HDM) sublingual immunotherapy (SLIT) as part of an observational study of children and adults in France.

Method: A total of 1289 patients were recruited, 553 adults and 736 patients aged 5–17 years old, by 139 allergy specialists in two stages (with initiation of SLIT in 2002 or 2005). The physicians filled out a case report form covering clinical symptoms, health-related quality of life and the observed efficacy and safety of SLIT. The average of the treatment duration was 3 years. Here we compared also the results between children and adolescents vs adults.

Results: On the surveyed children, 62.3% were polysensitised (62.8% in adults), and 59.3% were suffering from both rhinitis and asthma (39% in adults). Thirty-six percent of the paediatric patients had isolated rhinitis and 4.5% had isolated asthma. The HDM-allergic children also had troublesome (83%), with impairments in sleep (39%), school work (36%) and daily activities/leisure (34%).

Overall, 78% of the paediatric patients considered their SLIT to be effective and, likewise, 78% were satisfied with their therapy. Sixty-three per cent of the patients experienced less severe asthma symptoms (with the greatest reductions in coughing and wheezing) and 61% had less severe rhinitis symptoms (mainly reductions in sneezing, nasal congestion and rhinorrhoea). Initiation of SLIT was associated with reduced rescue medication use by patients with rhinitis (with decreases of 35% for oral antihistamines and 23% for nasal corticosteroids) and those with asthma (with decreases of 31% for oral antihistamines, 29% for nasal corticosteroids and 17% for beta-2 adrenergic agonists). Eighty-two percent of the paediatric patients were treatment-compliant. SLIT was well tolerated and most of the reactions were mild and local.

Conclusion: Paediatric patients having been prescribed HDM SLIT for moderate to severe allergic rhinitis (and, in some cases, isolated or concomitant asthma) were satisfied with their treatment and reported a decrease in symptom severity and in the consumption of symptomatic medication.

1319
Clinical profiles of house dust mite allergic patient initiating sublingual immunotherapy, results from the ACTIVE study in real world settings

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, St Jean, France; [2]Private Office, Boulogne Billancourt, France; [3]Stallergenes, Antony, France; [4]Private Office, Issy les Moulineaux, France; [5]Private Office, Marseille, France; [6]Liautey Hospital, Strasbourg, France

Background: House dust mites allergy is the leading cause of perennial allergic respiratory symptoms in the world. Its prevalence and severity can markedly affect the quality of life of patients and, due to an insufficient control of the disease with symptomatic treatments, lead to treatment by sublingual immunotherapy (SLIT).

Method: ACTIVE is an observational, prospective study conducted in France aimed at describing patients' course, clinical profile of patients requiring SLIT with

house dust mites (HDM) extracts, treatment regimen, and the benefits on allergic symptoms during the first year of treatment. The study is ongoing and the baseline characteristics of the included patients are presented below.

Results: Eight hundred and fifteen HDM allergic patient initiating SLIT patients were included; their mean age was 25 years (4–63 years). Of 71.1% had an allergy to HDM only (mono-allergic patients), 15.7% associated with grass pollen allergy, 13% with animal dander and 9.2% with trees pollens. All patients suffered from allergic rhinitis (AR), most frequently (70.4%) rated as persistent moderate to severe, 53.6% associated with asthma most often partially or totally controlled (81%) and 40.5% with conjunctivitis.

While in adults, rhinitis and conjunctivitis severity and impact on daily activities were the main reported outcomes; in children asthma symptoms were more prevalent.

98.4% of patients were treated with HDM SLIT with an initiating dose of 10 IR (88%), followed by a maintenance phase at 300 IR (99.0%). The planned average duration of AIT was 3 years.

Conclusion: In this real life study in French practices, HDM SLIT is mainly prescribed to patients suffering from persistent moderate to severe AR frequently associated with asthma.

1320
Safety and efficacy of tree pollens specific immunotherapy on ultra-rush method

Bozek, A[1,2]; Jarzab, J[1,2]
[1]Medical University School of Silesia, Katowice, Poland; [2]Clinical Department of Internal Disease, Dermatology and Allergology, Zabrze, Poland

Background: Specific immunotherapy (ITS) with ultra-rush in the treatment of allergy to hymenoptera venom constitutes a safe and effective therapeutic option. ITS with Purethal (HAL Allergy) for tree pollens (birch, hazel, alder) allergens has been evaluated to assess its effectiveness and safety.

Method: The study group consisted of 18 patients with symptoms of allergic rhinitis and confirmed allergy to tree pollens. Patients were randomised to ultra-rush therapy or conventional pre- seasonal ITS. Treatment was carried out during three consecutive years.

Results: After 3-year treatment a similar reduction in nasal symptoms was observed – according to the visual analog scale (VAS) decrease from 3.991 ± 0.804 points to 1.634 ± 0.540 in the ultra-rush group and from 3.845 ± 0.265 points to 1.501 ± 0.418 in the group desensitised by the conventional method ($P > 0.05$) There was also a comparable reduction in reliever drugs use during pollen season. No significant differences in safety profile were observed – only local reactions in both groups.

Conclusion: Ultra-rush ITS with Purethal is effective and safe in the treatment of tree pollens allergy. However, further investigations on larger groups of patients are required.

1321
Reduction of local and systemic reactions to housedust mite subcutaneous immunotherapy using premedication with montelukast vs levocetirizine among Filipino children 6–18 years old with IgE mediated bronchial asthma and/or allergic rhinitis: a double blind randomised controlled trial

Valerio, MRB
University of the Philippines-Philippine General Hospital, Department of Pediatrics, Manila, Philippines

Background: Although the benefits of specific immunotherapy (SIT) are well established in certain allergic diseases, local and systemic reactions can occur. Traditionally, antihistamines were the only option for premedication to reduce reactions. Recent studies show that antileukotrienes can be an alternative drug for premedication.

Objectives: Our objective is to compare Montelukast and Levocetirizine in their ability to reduce local skin reactions and to prevent systemic reactions during immunotherapy.

Methodology: Twenty two Filipino children, 6–18 years old, diagnosed with bronchial asthma and/or allergic rhinitis with positive skin prick test to house dust mite and who underwent immunotherapy were enrolled into a double-blind, randomised, controlled study to receive either levocetirizine 5 mg and montelukast 5 mg as premedication. Local skin reactions in mm, itching scores measured via a visual analogue scale (VAS) were compared. Tests of significance at P 0.05 level were done.

Results: Demographic data of the two groups were comparable at baseline. There was no significant difference in terms of total number, volume and maximum concentration of allergen injections. Mean length and width of the wheal were lesser, although not significant, in the montelukast group (mean = 3.87 vs 5.65, $P = 0.82$) adjusting for different allergen concentrations. The itching severity using VAS was slightly higher in the montelukast group but this was not statistically significant within groups ($P = 0.18$). Systemic reactions (anaphylaxis and generalised urticaria) were noted only in the montelukast group.

Conclusion: Premedication with montelukast is comparable with levocetirizine in preventing local reactions among Filipino children undergoing specific immunotherapy to house dust mite. However, the use of montelukast in premedication for immunotherapy was more associated with systemic reactions.

1322
Subcutaneous immunotherapy with single or multiple allergens in monosensitised or polysensitised patients: clinical efficacy and the effects on health-related quality of life

Erdoğan, AP[1]; Sin, AZ[1]; Koç, ZP[2]; Kose, T[3]; Gürlek, F[2]; Ardeniz, O[2]; Gulbahar, O[2]; Gokmen, NM[2]; Kokuludag, A[2]
[1]Ege University Faculty of Medicine, Internal Medicine, Izmir, Turkey; [2]Ege University Faculty of Medicine, Internal Medicine Division of Allergy & Immunology, Izmir, Turkey; [3]Ege University Faculty of Medicine, Medical Statistics, Izmir, Turkey

Background: In this study we aimed to evaluate the clinical effects and health-related quality of life by using single or multiple allergen immunotherapy (AIT) in monosensitised or polysensitised patients.

Method: Randomly selected 145 patients with allergic rhinitis with/without asthma complaining seasonal and/or perennial symptoms were included to this retrospective study. The patients who had been receiving AIT for at least 1 year with the relevant allergen(s) according with the skin test results, pollination and clinical symptoms were separated into six different groups. Receiving AIT with: One or two or three or four pollens, mites or pollen+mite separately. Standardised allergen extracts of different manufactured (Allergopharma, ALK-Abello, Stallergenes) were used. At most two different pollen allergens were into one vial in polysensitised patients. Mite and pollen allergens were into separated vials. Clinical efficacy was evaluated before and after IT by using symptoms and drug scores, visual analog scale (VAS) and subjective global recovery (SGR) surveys. Turkish validated health-related quality of life questionnaire for rhinoconjunctivitis (RQLQ) and asthma (AQLQ) were used for assessment of different AIT extracts.

Results: Characteristics of 145 patients and AIT contents were shown in Table 1.

	Number	Per cent
Allergic Rhinitis (AR)	102	70.3
AR + Asthma	43	29.7
Seasonal/Perennial	44/20	30.3/13.8
Seasonal + Perennial	81	55.9
AIT Contents		
1 pollens-2 pollens	27–27	18.6–18.6
3 pollens-4 pollens	23–21	15.9–14.5
Pollen + Mites	23	15.9
Mites	24	16.6

AIT periods of patients were as follows: for 1 year 11 pts (7%), 2 years 38 (26%) 3 years 33 (23%), 4 years 33 (23%) 5 years 30 (21%).

The improvement of symptoms scores and the values of VAS and SGR surveys were obtained significantly both in single and multiple AIT groups ($P < 0.05$). In some subgroups, although symptom scores were improved dramatically, values could not reached statistical significant because of the small numbers of patients. All items of RQLQ and AQLQ were significantly improved in all patients receiving single or multiple allergen IT

Conclusion: Both single and multiple allergen has similar improvement effects on symptoms and qualty of life.

1323
Non-interventional 2-year-study of sublingual immunotherapy (SLIT) in patients with allergic rhinoconjunctivitis caused by grass pollen

Karagiannis, E[1]; Hadler, M[1]; Shah-Hosseini, K[2]; Titova, K[2]; Moesges, R[2]
[1]Stallergenes GmbH, Kamp-Lintfort, Germany; [2]University of Cologne, Institute of Medical Statistics, Informatics and Epidemiology (IMSIE), Cologne, Germany

Background: The aim of this non-interventional study was to document the impact of a sublingual allergen immunotherapy (AIT) with five-grass pollen tablets (Stallergenes, France) on symptom severity and use of symptomatic medication as well as tolerability in patients with grass pollen-induced allergic rhinoconjunctivitis (RC) over 2 years of routine medical practice treatment.

Method: This prospective, open, non-controlled, non-interventional, multicenter study was conducted from Sep. 2010 to Oct. 2012 in Germany. Overall 1482 patients (752 female, 722 male, 8 unknown; mean age: 28 ± 15 years) participated in the study.

The patients rated their symptoms (rhinitis, conjunctivitis, asthma) as a combined scores of severity [scale: 0 (none) – 3 (severe)] and frequency [scale: 0 (none) – 4 (very often)]. In the combined RC score, the severity of rhinitis and conjunctivitis were pooled (scale: 0–6).

Results: During the grass pollen season preceding SLIT treatment, 84% of the patients had used symptomatic medication. This rate dropped to 52% in the 1st season and to 42% in the 2nd season under AIT treatment. Likewise the RC score decreased from a mean value of 4.06 ± 1.47 to 1.84 ± 1.76 during the 1st year and to 1.27 ± 1.49 during the 2nd year of treatment.

Among patients with concomitant asthma symptoms (38%), the asthma symptom score decreased from 3.56 ± 1.58 to 1.42 ± 1.48 (1st year) to 1.08 ± 1.37 (2nd year).

All scores decreased significantly ($P < 0.001$).

An improvement in health status after the 2nd year of treatment was documented by 94% of the patients at study completion. The assessment made by the treating physicians was nearly identical.

AIT with five-grass pollen tablets was well tolerated. Adverse events occurred in 15% of the patients during the 2 years of treatment. The incidence of non-fatal serious adverse events was 0.6%.

At the end of the second grass pollen season, 97.7% of the patients overall evaluated the tolerability of the five-grass pollen tablets as 'very good' or 'good.' Only 0.9% of the study participants rated tolerability as 'poor.'

Conclusion: Based on the study results, AIT with five-grass pollen tablets was well tolerated by patients in routine medical practice. All symptom scores were reduced significantly after the 1st and 2nd year under treatment with five-grass pollen tablets compared to the previous season. Overall a significant reduction in symptomatic medication use was documented in the patient population.

1324
Absence of IgE neosensitisation in allergic patients undergoing sublingual immunotherapy

Batard, T[1]; Baron-Bodo, V[1]; Horiot, S[1]; Bergmann, K-C[2]; de Beaumont, O[1]; Moingeon, P[1]
[1]Stallergenes, Antony, France; [2]Charité - Universitätsmedizin, Berlin, Germany

Background: Allergenic extracts intended for allergen immunotherapy (AIT) contain multiple major and minor allergens. Since patients receiving such extracts might be sensitised to few allergens only, AIT carries a theoretical risk of neosensitisation to allergens against which no IgE are detected prior to treatment. The aim of the present study was to evaluate the occurrence of neosensitisation during sublingual AIT (SLIT) with grass pollen and house dust mite (HDM) extracts.

Method: In a first double-blind placebo-controlled (DBPC) study, 82 European grass pollen-allergic patients were treated sublingually for 4 months with a daily five-grass pollen tablet or a placebo tablet. Seric IgE titers to recombinant Phl p 1, 2, 5b, 6, 7, 11, 12 and natural Phl p 4 were assessed before and after treatment, by using ImmunoCAP assays. In a second DBPC study, 509 European HDM-allergic patients were sublingually treated for one year with a daily HDM tablet or a placebo tablet. Seric IgE titers to purified natural or recombinant Der p 1, Der p 2 and Der p 10, and Der f 1 and Der f 2 were assessed before and after treatment, and one year after treatment cessation, by using ImmunoSolid-phase Allergen Chip (ISAC) assays.

Results: In grass pollen-allergic patients who had no detectable IgE specific to given allergen(s) before treatment, there was no IgE induction to such allergen(s) during SLIT. This absence of IgE neosensitisation was confirmed by western blotting. In the HDM cohort, similarly, we observed neither *de novo* IgE responses to group 1, 2 and 10 allergens in patients who were unsensitised prior to immunotherapy, nor IgE neosensitisation to wheat germ or yeast components contained in the HDM culture medium.

Conclusion: Based on seroepidemiological analyses performed in large cohorts of patients receiving SLIT based on either grass pollen or HDM extracts, we conclude that sublingual administration of allergens does not lead to any IgE neosensitisation. This further documents the superior safety profile of SLIT, when compared to SCIT.

1325
Epicutaneous immunotherapy for treatment of cockroach allergy

Kesornsukhon, N
Department of Otorhinolaryngology, Samut Sakhon Hospital, Samut Sakhon, Thailand

Background: Allergen immunotherapy has demonstrated promising results in treatment of IgE-mediated disease. Sublingual and other alternative route may improve patient compliance and safety. But some allergens aren't comfortable for patients to use orally. Epicutaneous route is effective for food and pollen allergies and may effective in other allergens.

Objective: To evaluate the clinical efficacy and safety of cockroach epicutaneous allergen immunotherapy.

Method: Case-controlled study was conducted from November 2011 to December 2012 at the Samut Sakhon Hospital. Thirty persistence allergic rhinitis patients who have positive reaction of skin prick test and specific IgE to cockroach were received adhesive tape stripping and allergen wet patches ($n = 15$) or placebo ($n = 15$) three times a week for 16 weeks. The outcomes were evaluated at 6 months by nasal symptoms score (NSS), skin prick test (SPT) and specific IgE.

Results: The NSS showed significantly decreased in allergen patch group ($P = 0.005$) but no improvement in placebo group ($P = 0.1$). There was no statistical difference of skin prick test and specific IgE after treatment in each groups and comparison between both group. Minimal eczema and erythema were noted at patch site in almost treated patients. No systemic adverse events were observed. The treatment result wasn't better in monosensitised patients.

Conclusion: Epicutaneous allergen immunotherapy improve the clinical symptoms of cockroach allergic rhinitis patients with no systemic reaction. Further study may follow with more sample size and better study design.

1326
Effectiveness and tolerability of a 2-year sublingual allergen immunotherapy in routine medical practice in birch pollen allergic patients

Hadler, M[1]; Karagiannis, E[1]; Shah-Hosseini, K[2]; Moesges, R[2]
[1]Stallergenes GmbH, Kamp-Lintfort, Germany; [2]University of Cologne, Institute of Medical Statistics, Informatics and Epidemiology (IMSIE), Cologne, Germany

Background: Clinical trial results have shown clinical efficacy of AIT with birch pollen extract (Stallergenes, France). However, published data on routine medical practice are still scarce. In this two-year non-interventional study we documented the impact of birch pollen AIT both on symptoms and use of symptomatic medication as well as the tolerability of treatment in a large population of patients with rhinoconjunctivitis (RC) related to birch pollen.

Method: This open, prospective, non-controlled, multicenter study across Germany was conducted over two consecutive birch pollen seasons between fall 2010 and fall 2012. A total of 176 physicians included 716 patients (409 female, 300 male, seven unknown; mean age: 38 ± 16 years) in the study. Ninety-one percent of the patients suffered from rhinitis symptoms, and 76% had conjunctival symptoms (median duration of RC symptoms: 7.0 years). Patients were treated according to the SmPC of the product.

Allergic symptoms were analyzed as combined scores of severity [scale: 0 (none) – 3 (severe)] and frequency [scale: 0 (none) – 4 (very often)]. In the combined RC score, the severity of rhinitis and conjunctivitis were pooled.

Results: The rhinitis score decreased from an average of 4.83 in the birch pollen season prior to the study to 3.17 in the 1st study year and to 2.31 in the 2nd study year (−52%), while the conjunctivitis score showed a 55% reduction from 3.74 to 2.11 (year 1) and to 1.69 (year 2). The RC score used in routine practice was reduced by 53% [from 3.76 to 2.29 (year 1) and 1.76 (year 2)]. The percentage of patients requiring symptomatic medication decreased from 81% to 59% and 48%.

In general, treatment was well tolerated throughout the study. Adverse events (AE) were documented for 10% of the patients. The most frequent AEs were local reactions in the mouth (itching, tingling, burning). The majority of AEs occurred during the 1st study year.

Conclusion: In a large population of patients treated in routine medical practice, AIT with birch pollen extract reduced allergic symptoms as well as symptomatic medication use during the 1st treatment year. There was a sustained and further reduction in symptoms and symptomatic medication use during the 2nd treatment year. Treatment was well tolerated.

1327
Perlingual spray application with a mixture of mite allergen extracts is a safe treatment option for patients allergic to house dust mites

Dichmann, R
Medical Practice, Pneumology, Allergology, Witten, Germany

Background: For the assessment of the overall treatment adherence to sublingual immunotherapy (SLIT) the patients' compliance is one important aspect and is among other criteria related to the safety especially at the beginning of the treatment. In this context the use of perlingual spray application with an aqueous solution of mite allergens has been analysed in relation to the safety during the first weeks after starting the therapy and the compliance of patients sensitised to house dust mites.

Method: Using a 5-point-scale from *very bad* to *very good* the patients' compliance during the first 8 weeks after start of treatment was assessed on evaluable data from 23 patients (18–66 years of age; 13 female; 10 male). The patients received an aqueous allergen extract of a mixture (50% *D. pteronyssinus*, 50% *D. farinae;* SULGEN Spray; 30 000 TU/ml) according to their allergic disposition. They started the treatment with two puffs on the first day and continued daily with the same dose. Any side effects during the observation period were documented.

Results: For 60.9% of the patients the compliance was described as very *good*, 26.1% as *good*, for 8.7% as s*atisfactory*. One patient showed a *bad* compliance and stopped the therapy. No side effects at all were reported during the evaluated time period.

Conclusion: A good patients' compliance and a good safety profile were seen for patients treated with an aqueous extract of house dust mite allergens using the perlingual spray application.

1328
What is the opinion of the patients under five grass pollen tablets immunotherapy? First season assessment in adults. Findings of the SMILE study

Fernandez-Nieto, M[1]; Alvarez, JA[2]; Alvarado, MI[3]; Reaño, MM[4]; Blanco, C[5]; Garcia, MA[6]
[1]Fundación Jiménez Díaz, Madrid, Spain; [2]Clinica de Alergia y Asma, Oviedo, Spain; [3]Hospital Ciudad de Coria, Caceres, Spain; [4]Hospital Puerta de Hierro de Majadahonda, Madrid, Spain; [5]Hospital Universitario de la Princesa, Madrid, Spain; [6]Stallergenes Iberica, Barcelona, Spain

Background: The SMILE study was performed in Spain in 2012 to collect information about satisfaction with grass pollen tablet sublingual immunotherapy (SLIT) and the quality of life (QoL) of grass pollen allergic patients.

Method: In this observational, cross-sectional, multicenter study, allergy specialists collected information in a unique follow-up visit at the end of the grass pollen season 2012. Patients had to have uncontrolled moderate to severe allergic rhinoconjunctivitis (ARC) while being treated with symptomatic drugs and received a tablet for the first time with grass pollen according to a precoseasonal scheme. Satisfaction with treatment and QoL were assessed by two validated questionnaires, the ESPIA and RQLQ, respectively. Results are mean ± SD.

Results: We present here the results of 226 adult patients. Fifty-five percent of them had asthma and 60% were polysensitised. Mean time of evolution of ARC was 10.4 ± 8.4 years. All the patients were treated with a tablet of five grasses extract (300 IR) for 4.9 ± 0.9 months.

The ESPIA questionnaire ranges from 16 to 80 points (the higher, the more satis-

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

fied). At the end of the treatment period, the global satisfaction was 62 ± 15 points (72 points adjusted over 100). ESPIA domains included: perceived efficacy (84 adjusted points), activities and environment (76 adjusted points), cost-benefit balance (76 adjusted points) and general satisfaction (82 adjusted points).

The RQLQ ranges from 0 to 6 points (the higher, the more affected). At the end of the treatment period, the global score was 1.28 ± 1.10 points. RQLQ includes seven domains: activity limitation (1.90 ± 1.52), sleep problems (1.00 ± 1.39), nose symptoms (1.70 ± 1.49), eye symptoms (1.15 ± 1.26), non-nose/eye symptoms (1.09 ± 1.19), practical problems (1.74 ± 1.55) and emotional function (0.77 ± 1.10).

An *ad hoc* questionnaire was administered to collect information about beliefs and attitudes of patients, using a 5-point Likert-like scale (from 'totally agree' to 'totally disagree'). The percentage of patients who either totally agreed or agreed with the following statements: It is important to administer the treatment properly (i.e. precoseasonally) (93%), an intermittent treatment is easier to follow than a continuous one (85%).

Conclusion: In Spain, adult patients are highly satisfied with the precoseasonal treatment of grass pollen ARC with five-grass SLIT tablets (300 IR). The RQLQ scores are low in all domains confirming the beneficial impact of the therapy.

1329
Subcutaneous-specific immunotherapy in patients with perennial allergic rhinitis induced by house dust mite: an observational case-control study

Mijakoski, D[1]; Karadzinska-Bislimovska, J[2]; Stoleski, S[2]; Minov, J[2]; Marsenic, M[1]
[1]Institute for Occupational Health of RM-Skopje, GA2LEN CC, WHO CC, Allergy Center, Skopje, FYR Macedonia; [2]Institute for Occupational Health of RM-Skopje, GA2LEN CC, WHO CC, Skopje, FYR Macedonia

Objective: To evaluate the efficacy of subcutaneous-specific immunotherapy (SCIT) in the reduction of symptoms, and rescue medications in patients with perennial allergic rhinitis (AR) induced by house dust mite (HDM).

Methods: A case-control study was conducted and 28 patients with perennial AR due to HDM were analyzed. Patients with perennial AR due to HDM treated for at least two consecutive years with SCIT with *Dermatophagoides pteronyssinus* extract were defined as cases ($N = 14$). Increasing doses were administered in the initial phase until the maintenance dose was reached. This dose was then given once a month for 24 months. Patients with perennial AR matched for all relevant parameters who were never treated with SCIT were defined as controls ($N = 14$). Clinical efficacy was evaluated by rhinoconjunctivitis symptom score (SS) (nasal congestion, sneezing, rhinorrea, nasal itching, ocular itching and watery eyes) with a scale ranging from 0 (no symptoms) to 3 (severe symptoms), the medication score (MS) assessing symptomatic drug intake (antihystamine and inhaled corticosteroids), and the size of immediate skin reaction to HDM. SS and MS were evaluated at the end of the observational period in relation with the period, considering the last 12 months, in which patients suffered the highest symptoms levels (during the period of maximum allergen exposure). Skin test reactivity was re-measured after 24 months of treatment to HDM.

Results: The data obtained showed that mean SS was 3.5 ± 1.3 in cases and 6.9 ± 1.6 in controls (−49.3%) ($P < 0.01$) while mean MS was 2.1 ± 1.1 and 3.2 ± 1.6 among cases and controls, respectively (−34.4%) ($P < 0.05$).

Conclusion: Our study showed that a 2-year SCIT treatment in patients with perennial AR is associated with lower symptom and medication scores in comparison with subjects treated with symptomatic drugs only.

1330
Quality of life improvement results in the ALUMITES study, a randomised, controlled, multicenter phase IV study with house dust mites subcutaneous immunotherapy for allergic rhinitis. Six months interim analysis

Valero, A[1]; Hernandez, D[2]; Rodriguez, V[3]; Malek, T[4]; Cadavid, S[5]; Garcia, MA[6]
[1]Hospital Clinico Universitario de Barcelona, Barcelona, Spain; [2]Hospital Universitario y Politecnico La Fe, Valencia, Spain; [3]Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain; [4]Hospital General Castellón, Castellon, Spain; [5]Hospital Clinico Universitario de Valencia, Valencia, Spain; [6]Stallergenes Iberica, Barcelona, Spain

Background: ALUMITES study was designed to assess the efficacy of house dust mites (HDM: *D. pteronyssinus* + *D. farinae*) subcutaneous immunotherapy (SCIT) for the treatment of allergic rhinitis patients along one year. Here we report an interim analysis done after 6 months of treatment.

Method: In this controlled multicenter phase IV study, HDM adult allergic patients were randomised to receive SCIT with a 10 IR/ml depot extract plus symptomatic treatment (group A) or only symptomatic treatment (group B) (2:1). Quality of life (QoL) was recorded at basal visit and after 6 months of treatment. ESPRINT-15 questionnaire was used. The ESPRINT-15 consisting of 15 items on a 7-point Likert scale (lower score indicating a better quality of life) is composed of four domains: symptoms, daily activities, sleep and psychological affectation.

Results: Forty-five of 57 patients completed the ESPRINT-15 questionnaire. Active group experienced an improvement in their QoL score being at basal visit 2.6 ± 1.55 and 6 months of treatment later 1.5 ± 1.12 ($P = 0.0004$). Control group also experienced a significant improvement since they scored 2.6 ± 1.21 at basal visit and 1.9 ± 1.35 ($P = 0.0128$) 6 months later. By domains, also symptoms −1.2 [95% CI: (−1.8, 0.6), $P < 0.0001$], daily activities −0.9 [95% CI: (−1.6, 0.3), $P = 0.0081$], sleep −0.7 [95% CI: (−1.4, −0.1), $P = 0.0131$] and psychological affectation −1.4 [95% CI: (−2.1, 0.7), $P < 0.0001$] improve in active group whereas only symptoms −1.0 [95% CI: (−1.6, 0.4), $P = 0.0070$] and sleep −1.3 [95% CI: (−2.3, −0.3), $P = 0.0236$] affectation domains improve in the control group.

Conclusion: Patients report improvement of their quality of life (ESPRINT-15) already 6 months after beginning SCIT with a 10 IR/ml depot HDM extract in all domains demonstrating the clinical meaningfulness of the efficacy of this treatment.

1331
Effectiveness of subcutaneous high-dose modified pollens extract in daily practice

Chivato Pérez, T[1]; Rodríguez Mosquera, M[2]; Izquierdo Calderón, JP[3]; Hernández Peña, J[3]
[1]School of Medicine, CEU San Pablo, Madrid, Spain; [2]University Hospital Puerta de Hierro, Allergy Unit, Majadahonda, Spain; [3]MERCK SL, Medical Department, Madrid, Spain

Background: Immunotherapy is the only treatment that may affect the natural course of allergic disese and prevent the development of asthma in patients with allergic rhinitis. Efficacy and safety of subcutaneous high-dose hypoallergenic pollen preparations have been documented in several clinical trials. The objective of this study was to confirm these results in daily practice through the determination of the efficacy perceived by patients treated with this subcutaneous high-dose modified allergens after the first pollen season after starting treatment.

Method: A multicenter observational study with the participation of 29 researchers. From September to December 2011, 256 patientes with pollen IgE mediated allergic rhinitis and/or bronchial asthma were included in the study. Date was collected via structured questionnaries.

Patients assessed their conditions on a visual analogue scale from 0 (worst condition) to 100 (best condition). A clinical relevant improvement was defined to be an improvement by at least 20 points to compare the results of their self-assessment in the pollen season before starting the immunotherapy treatment.

Results: Hundred and thirty-three patients (52%) were male. The mean age was 32.9 (SD 11) years old and 27% of the population were pediatric patients with a mean age of 10.2 (SD 3) years old.

81.6% of the preparations contained grasses, 36.6% olive and 7% other allergens (trees and weeds).

The overall score of the patients improved by 33.5 points (40–74.5 points; $P < 0.001$) being the improvement between 30 and 50 points on 51.6% of the total population (132 patients) and was clinically significant (>20 improvement points) in 234 patients (92.4% of the total population).

Two patients (0.8%) interrupted treatment by bad-tolerance without medical advice. the treatment was well tolerated by the 254 remaining patients (99.2% of the total population).

Conclusions: Subcutaneous immunotherapy with high-dose modified allergens of pollen is effective and well tolerated in real life practice.

The patiens' conditions improved remarkably being this effect objectified in the first pollen season after starting the treatment.

1332
High rate of desensitisation and tolerance induction by rush oral immunotherapy for anaphylactic children with egg allergy: a randomised controlled trial

Itoh-Nagato, N[1,2]; Fujisawa, T[3]; Shimojo, N[4]; Nagao, M[3]; Iwata, T[5]; Japan Oral Immunothrapy Group (J-OIT)
[1]RIKEN Center for Integrative Medical Sciences, Laboratory for Immune Regulation, RCAI, Yokohama, Japan; [2]The University of Tokyo, Department of Pediatrics, Graduate School of Medicine, Tokyo, Japan; [3]Mie National Hospital, Institute for Clinical Research, Tsu, Japan; [4]Chiba University, Department of Pediatrics, Graduate School of Medicine, Chiba, Japan; [5]Tokyo Kasei University, Department of Child Health and Development, Tokyo, Japan

Background: Current standard of care for childhood food allergy is dietary avoidance while awaiting natural outgrow. Recently, efficacy and safety of oral immunotherapy (OIT) as a disease-modifying treatment has been reported with varying protocols. However, induction rate of desensitisation and tolerance is not high enough to be recommended for routine clinical practice.

Methods: This randomised controlled trial (RCT) of rush OIT enrolled 45 children (5–15 years old) with severe IgE-mediated hen's egg allergy confirmed by double-blind, placebo-controlled food challenge (DBPCFC). Twenty-three children received rush OIT and 22 control children continued avoidance of egg for further 3 months after randomisation. The second DBPCFC was performed to compare threshold doses in the two groups at 3 months. Then the control patients received treatment with the same protocol (a delayed control design). The protocol consisted of two phases: a rush build-up phase to achieve ingestion of one medium-sized lightly cooked egg (60 g) and a maintenance phase during which the participants continued to take the same dose of egg every day. At 12 months of the maintenance, they avoided egg again for more than 2 weeks before the third DBPCFC to determine whether they achieved tolerance.

Results: At the first DBPCFC, the median threshold dose of egg was 0.8 g. At the second challenge after the rush phase, the threshold doses significantly increased in treatment group and no changes were observed in control group. Overall, including the delayed controls, 85% of the subjects were able to eat more than one whole egg without allergic reactions after the rush phase which took 16 days (median) and it was interpreted that they gained desensitisation. At the third DBPCFC, 50% of the subjects passed, or achieved tolerance, and the rest failed. Specific IgG, IgG4 and IgA to egg white and ovomucoid significantly were elevated after the rush phase and continued to increase at 12 months. Specific IgE significantly decreased at 12 months. QOL of their families significantly improved after the treatment. Five patients (11%) withdrew from therapy because of severe allergic reactions.

Conclusions: The present rush OIT was highly efficacious and relatively well-tolerated for severe egg allergy achieving desensitisation in 85% and tolerance in 50% of the participants. Small proportion of the subjects, however, had significant adverse events and safer protocol is still to be established.

1333
Push up the adherence: the impact of patients' knowledge on adherence in allergen-specific immunotherapy

Grando, K; Musholt, M; Ballmer-Weber, B; Schmid-Grendelmeier, P; Beeler-Truessel, S
Allergy Unit, Department of Dermatology, University Hospital of Zurich, Zurich, Switzerland

Background: The adherence of patients is a critical prerequisite for the success of a desensitisation therapy. However, patients' adherence is not always assured to the same extent and is subject to factors such as effectiveness of treatment, side effects, time, cost, doctor/patient relationship, care and targeted dissemination of information. Hence, based on the concept of the micro-instruction, an intervention for patients with desensitisation therapy has been developed.

Methods: Presentation of micro training as an intervention to increase patients' adherence in desensitisation therapy.

Results: The initial action in micro training consists in discussing the expectations of the patient and the nurse with respect to the planed treatment and to present the available information materials to the patient. As a next step the patient will be instructed in details in terms of course and time frame of the desensitisation treatment. Most importantly various aspects are discussed such as the affection of the everyday life by the treatment, the responsibility in dealing with the desensitisation therapy and other organisational planning. The micro training finishes with a review of whether the patient has understood all the important information. The main discussion techniques during the micro training are 'Ask-Tell-Ask' and 'Closing the loop'.

Conclusions: The concept of micro-training can be easily and quickly integrated into the clinical routine. The intervention is limited to 15 min, needs little preparation and is easy to learn.

Poster Session 55

Immunological mechanisms of allergen-specific immunotherapy

1334
Protocols measuring CD63 or CD203c with or without IL-3 priming lead to comparable conclusions for changes in specific activation of basophils from grass pollen allergic donors following specific immunotherapy

Lund, G[1]; Aasbjerg, K[2]; Skovsgaard, J[1]; Backer, V[2]; Holm, J[1]; Holse, M[1]; Wagtmann, VR[1]; Würtzen, PA[1]; Andersen, PS[1]
[1]ALK, Immunology, Hoersholm, Denmark; [2]Bispebjerg Hospital, Department of Respiratory Medicine, Copenhagen, Denmark

Background: Basophil surface markers CD63 and CD203c are widely used for addressing basophil activation. Different groups prefer different markers and protocols, and it is not clear whether one marker or protocol is superior for evaluating relevant changes over time following specific immunotherapy (SIT). For this reason we evaluated data obtained by different basophil activation test (BAT) protocols with respect to responses to *P. pratense* in basophils from grass pollen allergic donors measured at different time points before and after receiving SIT.
Method: Forty grass pollen allergic individuals receiving SQ-standardised grass-allergy immunotherapy tablet (AIT, Grazax 75,000 SQ-T), SCIT (Alutard SQ) or no treatment (controls) were followed for 1½ years. Basophils in whole blood were stimulated with *P. pratense* (0.00015–1500 ng/ml) w/wo IL-3 and activation markers measured by flow cytometry. Allergen- and/or IL-3-induced CD63 and CD203c expression were compared in terms of the percentage of double positive cells with high expression of CD63 and CD203c (degranulated cells) or mean fluorescence (MFI). Dose response curves were evaluated regarding sensitivity (EC50), max reactivity and a combination.
Results: Basophil activation performed by the different methods was comparable with respect to sensitivity and max reactivity. Immunotherapy resulted in decreased basophil sensitivity as well as decreased max reactivity when measuring CD203c as well as CD63 with or without IL-3 priming. Some variations in the ranking of individual patients based on the different protocol/BAT readouts were seen, but relative changes were comparable. Both sensitivity and max reactivity were transiently increased in the untreated patient group post pollen season. No correlation was found for sensitivity and reactivity.
Conclusion: Selection of basophil protocol is not critical for evaluating basophil activation over time; decreased basophil response to *P. pratense* is observed following tablet based as well as subcutaneous immunotherapy in all assays used and relative results are comparable. The presented data indicate that the pollen season affects both sensitivity and reactivity of the basophils in several months after the season. This has to be considered when evaluating data from BAT experiments performed at different time points.

1335
Hypo-responsive state of mast cells induced by IgE/antigen desensitisation

Giavina-Bianchi, P; Mezzano, V; Picard, M; Sancho-Serra, MDC; Castells, M
Harvard Medical School, Brigham and Women's Hospital, Clinical Immunology and Allergy, Boston, MA, United States

Background: Rapid desensitisation (DST) protocols used for drugs and foods have been developed based on clinical evidence, but few *in vitro* studies have provided a mechanistic approach. Better understanding of the mechanisms involved in the early stages of DST can lead to safer treatment protocols, protecting patients against anaphylaxis and identifying markers of therapeutic efficacy. The aim of this study is to demonstrate the induction of a state of hypo-responsiveness in mast cells by an IgE/antigen *in vitro* model of rapid desensitisation.
Methods: Mouse bone marrow derived mast cells (BMMCs) were sensitised with DNP (2,4-dinitrophenol)-IgE and stimulated with DNP in single dose (1 ng) or through rapid desensitisation by sequential progressive doses over 110 min to reach the activating dose (1 ng). After activation or desensitisation, BMMCs were rechallenged with DNP (1 ng) to assess hyporesponsiveness. BMMC activation was determined by b-Hexosaminidase (b-Hex) release and IL-13 production in the supernatants, and the expression of LAMP-1 (CD107a), as a surface marker of degranulation, by FACS analysis.
Results: Desensitised BMMCs released significantly less b-Hex than their activated controls (reduction of 58.89 ± 5.37%) and became hyporesponsive. Hyporesponsiveness was assessed by rechallenge with 1 ng DNP after DST or activation, showing reduced B-hex release (reduction of 63.53 ± 11.1% after DST and 72.05 ± 6.34% after activation). Mediator depletion was ruled out as calcium ionophore (A23187) induced a 35.83% and 41.13% release in previously activated and desensitised cells, respectively. LAMP-1 surface expression was 8.42% after DST and 30.45% after activation, indicating a decreased expression by 72.35% after DST. IL-13 production measured 4 h post-DST was reduced by 27% compared to activation.
Conclusion: Desensitisation induces a hypo-responsive state in mast cells in this *in vitro* model, which is not due to mediator depletion. Further studies are needed to clarify the cellular mechanisms involved in desensitisation.

1336
Induction of desensitisation in human basophil granulocytes in grass allergic and non-allergic subjects

Witting Christensen, SK; Thuraiaiyah, J; Kortekaas Krohn, I; Schmid, J; Hoffmann, HJ
Department of Respiratory Medicine, Aarhus University Hospital, Aarhus C, Denmark

Background: Desensitisation is a process where increasing doses of an allergenic substance is administered without triggering considerable hypersensitivity reactions.The mechanism remains to be established. We investigated an *ex vivo* protocol with increasing allergen concentration intervals for inducing desensitisation in human basophil granulocytes from grass allergic and non-allergic subjects.
Method: We recruited seven grass allergic subjects (positive skin prick test or >0.35 kU/l grass specific IgE) and seven controls without allergy. A basophil activation test of 8 log-concentrations was performed to determine allergen concentration at which maximal response occurred. For non-allergic subjects we used anti-FcεRI antibody, for grass allergic subjects we used grass allergen or anti-FcεRI to acti-

vate cells. Basophils in whole blood were desensitised by incubating with 2–2.5 fold increasing doses of allergen in 10 steps until maximal response concentration was reached. At each step we drew a sample to measure desensitisation level (DS) and a sample to stimulate with a max dose allergen to measure maximum activation (DSM). Activated ($CD63^+$) $CD193^+$ basophils were identified by flow cytometry. To analyze reproducibility, some desensitisation tests were repeated the same day or on different days. Desensitisation was considered successful when the ratio DSM/DS after desensitisation was significantly lower than before desensitisation. The ratio DSM/DS is a relative measure describing how desensitisation affects the basophils' ability to react to a max dose allergen. During the process the ratio decreases as the cells become increasingly desensitised and hence less reactive to allergen stimulation. Basophil sensitivity was measured by EC20; the allergen concentration at 20% activation. We calculated medians and compared values with Wilcoxon Signed Rank test.

Results: Desensitisation significantly reduced DSM/DS of all subjects and all tests from 17.1 to 1.1 ($n = 26$, $P \leq 0.0001$). DSM/DS for grass desensitisation was reduced from 15.8 to 1.1 ($n = 16$, $P = 0.002$). DSM/DS for anti-IgE desensitisation was reduced from 26.3 to 1.0 ($n = 10$, $P = 0.0003$). Basophil sensitivity by EC20 was reduced from 5.0E-7 to 2.5E-10 g/l ($n = 21$, $P = 0.0006$).

Conclusion: We successfully desensitised human basophils from grass allergic and non-allergic subjects. Desensitisation significantly reduced DSM/DS by decreasing DSM for all groups, and reduced basophil sensitivity by EC20.

1337
Immunological changes in saliva with sublingual immunotherapy

Saiz Sanchez, V; Guerra Pasadas, F
Reina Sofia Hospital, Allergy, Cordoba, Spain

Background: Immunotherapy is the only effective treatment for allergic rhinitis. Sublingual immunotherapy is used to reduce the risk of adverse effects provoked by subcutaneous immunotherapy and to increase the compliance.

The objective of this pilot study was to measure citocines in saliva in patients recibing daily grass and olive sublingual immunotherapy.

Randomised placebo controlled trial was conducted. Saliva was collected and stored at −20°C to determine immunological changes before, during and after the treatment. Citocines measured were IL-4, IL-5, IL-10, IL-2, INF-gamma, TNF-alfa.

Method: A randomised placebo controlled trial conducted during 10 months.

Immunotherapy was administered daily by sublingual route. The extract was biologically standarized by major allergens (grass Group 5 and Ole e 1) and quantified in micrograms. Placebo was similar in taste and appearance.

Determination of citocines Th1/Th2 in saliva by Cytometric Bead Array.

Results: Pilot study in which 33 patients started the study but only 29 finished (13 actives and 16 controls). One was retired for pregnancy, another because presented oral aphthas and two of them withdrew voluntarily.

Patients age was between 18–55 years old.

Ninety-three compliance in both groups. High tolerance.

Citocines in saliva were measured in four times (before immunotherapy, between 3 and 5 months, between 6 and 8 months and after the treatment).

In the comparisons intragroup, differences were not situated in the value of the citocines detected in T0 and T3.

Conclusion: Our aim has consisted of explaining the effect to local scale of this immunotherapy measuring citocines in saliva. Is an innocuous and new system since we have not found in the literature similar studies. Citocines detected in saliva will reflect systemic changes, so it was not necessary a blood extraction.

We do not find immunological changes in saliva. The measure of citocines in saliva is not a good system to detect modification of immunological parameters in the oral mucosa.

Further studies will be needed.

1338
Parasites and allergy: challenging humans with worms

Pritchard, DI; Immune Modulation Research Group
University of Nottingham, Pharmacy, Nottingham, United Kingdom

Necator americanus, a hookworm parasite of humans, stimulates IgE production and promotes eosinopoiesis, immune responses which in turn are associated with reduced worm fecundity.

This suggests that 'allergy' has value. The fact that the worm survives this assault also suggests worm-driven counter inflammatory strategies, which may be exploited therapeutically.

However, challenging humans with worms cannot be undertaken lightly, and this presentation describes how safety studies were conducted in allergic patients, and how worms are now manufactured to GMP standards for use in future trials.

1339
The allergen-specific immunotherapy in patients with atopic bronchial asthma are used in various ways

Novikau, D; Vykhrystsenka, L; Smirnova, A; Novikov, P; Yanchanka, U; Siamionava, I; Novikov, D
Department of Clinical Immunology and Allergology, Vitebsk State Medical University, Vitebsk, Belarus

Background: Development of various methods of allergen-specific immunotherapy (SIT) needs the highlighting research in allergy.

Aim: The main purpose of research was to study the action of various methods of SIT on specific and nonspecific hypersensitivity of patients with atopic bronchial asthma.

Methods: A randomised, open pragmatic in the parallel- group study was performed. Two hundred and eighty-nine 289 patients with atopic bronchial asthma have undergone SIT in three various ways. We used subcutaneous, inhalation and oral administration of allergen. The study had a 12 months of randomised treatment. The primary end points were clinical signs and medication of asthma, and secondary variables were skin and bronchial hypersensitivity, serum level of specific IgG, IgE and sIgA.

Results: Substantial decline of clinical signs of asthma in all groups was demonstrated.The application of SIT in atopic bronchial asthma results in distinct improvement of general conditions of patients, decrease of asthma exacerbation rates, lowering of night attacks of the disease, reduction of β_2-agonists usage with parallel rise of patients' asymptomatic days number together with improvement of quality of life of the patients.

An equivalent safety of inhalation and oral ways of SIT administration in comparison with subcutaneous one was confirmed, but oral SIT was proven to be of a highest safety.

Successful SIT reduced the severity of astmatic reaction in response to inhalation of allergen or acetylcholine, causing the growth of PD_{10} and PC_{20} that correlated with treatment efficacy in all groups. The decline of dermal sensibilization to an allergen in 12 months of SIT was determined in 20.8% of patients; moderate negative correlation with efficacy of treatment was established ($r = -0.42$; $P < 0.05$). SIT was accompanied with positive immunological changes – for majority (73.6%) of patients reduction of specific IgE levels was observed and the increase of IgG level (57.5%) was

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

demonstrated, but strong correlations between these findings and patient clinical state were not determined. It is worthy to note reliable interrelationships between specific salivary sIgA level and clinical efficiency of inhalatory ($r = 0.54$; $P < 0.05$), and oral SIT ($r = 0.65$; $P < 0.05$).

Conclusion: The SIT confirms considerable symptom prevention and reduced medication use. The obtained results confirm inhibitory influence of SIT on specific and nonspecific tissue hypersensitivity.

1340
Basophil sensitivity to allergen decreases rapidly after starting subcutaneous immunotherapy but increases slowly again during maintenance therapy

Schmid, JM[1]; Dahl, R[2]; Wuertzen, PA[3]; Hoffmann, HJ[1]
[1]Department of Respiratory Medicine, Aarhus University Hospital, Aarhus C, Denmark; [2]Aarhus University, Institute for Clinical Medicine, Aarhus, Denmark; [3]ALK-Abelló, Department of Experimental Immunology, Hoersholm, Denmark

Background: Subcutaneous immunotherapy reduces the specific type-1 allergic response and reduces allergic symptoms. Basophil sensititivity measured by basophil activation test (BAT) reflects the effect of SCIT on effector cell level.

Methods: In january 2010, we randomised 24 subjects suffering from seasonal rhinoconjunctivitis due to grass pollen allergy to receive standard SCIT ($n = 18$) or to an open control group ($n = 6$). Basophil sensitivity, defined as the allergen concentration leading to half-maximum basophil activation was measured by flow cytometry as the percentage of CD63 expression on the surface of $CD193^+$ blood basophils activated by 8 $\log_{10}$ dilutions of grass pollen extract (0.000256–25600 SQU/ml), both on washed cells and cells reconstituted with plasma from the present visit and plasma from the baseline visit.

Results: Basophil sensitivity decreased rapidly in cells reconstituted with present plasma in treated patients, from a median allergen concentration to cause half-maximum basophil activation of 1.2 SQU/ml (0.003–3.9 SQU/ml) at baseline to a maximum of 93.1 SQU/ml (28.7–616.6, $P = 0.0002$) after 9 months and then slowly increasing again to reach half-maximum activation at an allergen concentration of 5.7 SQU/ml (1.4–21.9; $P = 0.0019$) after 27 months of treatment.

Humoral changes,defined as the difference in the basophil sensitivity of cells reconstituted with present vs baseline plasma, related to a ca. 10-fold decrease in basophil sensitivity after 3 months (13.4: 8.1–30.6, $P = 0.0005$), remaining stable throughout the observation period.

In washed cells, we found a slower decrease in basophil sensitivity from 0.5 SQU/ml to 9.5 (3.9–34.7; $P = 0.0097$) after 9 months, falling back to baseline level at 2 years, reflecting changes in basophil sensitivity owing to cellular changes.

Conclusion: We found a fast developing decrease in basophil sensitivity during SCIT with a slow increase after 2 years of treatment. This increase seems to be mostly related to changes in the cellular mechanisms, while humoral changes reach a plateau after updosing and remain stable throughout the maintenance phase.

1341
Dermatophagoides pteronyssinus immunotherapy induces immunological changes among the time in B cells and plasma cells populations

Fernandez, TD[1]; Gomez, E[1]; Doña, I[2]; Rondon, C[2]; Blazquez, AB[1]; Herrera, R[2]; Melendez, L[1]; Blanca, M[2]; Torres, MJ[2]; Mayorga, C[1]
[1]Research Laboratory, Allergy Service, Malaga General Hospital, Malaga, Spain; [2]Allergy Service, Malaga General Hospital, Malaga, Spain

Background: The mechanism involved in the immunotherapy is still not well known, but it is established that immunological changes appear in a chronologic way and are related to B-lymphocytes and IgE production.

Objectives: To monitor the immunological changes occurring in different subpopulations of B lymphocytes and plasma cells during the immunotherapy in allergic rhinitis patients sensitised to *Dermatophagoides pteronyssinus* (DP).

Method: Peripheral blood was collected at five time points during the immunotherapy (before and after 1, 3, 6 and 12 months of treatment) and the phenotypical analysis of different cell subpopulations performed using a FACSCanto II cytometer.

Results: After 6 months of treatment we found a decrease of total B-cells ($P = 0.021$) with a progressive increase of DP-specific B-cells along the time ($P = 0.012$ at 12 months). We found also an increase of DP-specific B-cells expressing IgE in the first month ($P = 0.036$) that rapidly decrease returning at basal levels after 3 months. Total plasma cells increased after 1 month ($P = 0.005$) returning to basal levels at month 12. Similarly an increase in plasmablasts has observed at 1 month ($P = 0.012$) that return to basal levels at 3 months. However, both peripheral (CXCR3+) and long-lived (CXCR4+) plasma cells show a decrease after three first months ($P = 0.036$ and $P = 0.012$) that is maintained along immunotherapy. Regulatory B-cells decrease at 3 and 6 months ($P = 0.005$ and $P = 0.012$ respectively) but we observed an increase of those producing IL10 ($P = 0.035$ at 6 month).

Conclusion: DP-specific immunotherapy induces changes in the different B-cell subpopulation in very early stages. With a significant decrease of plasma cells both peripheral and long-lived and an increase of IL10 regulatory B-cells which will influence the low production of IgE antibodies and treatment effectiveness.

1342
Invariant natural killer T cells could influence B cells changes in *Dermatophagoides pteronyssinus* immunotherapy

López, S[1,2]; Fernández, TD[3]; Doña, I[4]; Gómez, E[3]; Gómez, F[4]; Rodríguez-Bada, JL[3]; Pozo, D[1,2]; Mayorga, C[3]; Blanca, M[4]; Torres, MJ[4]
[1]CABIMER, NIR Lab, Department of Cell Signalling CABIMER-Andalusian Center for Molecular Biology and Regenerative Medicine, Seville, Spain; [2]Department of Medical Biochemistry, Molecular Biology and Immunology, University of Seville Medical School, Seville, Spain; [3]Malaga General Hospital, Research Laboratory, Málaga, Spain; [4]Malaga General Hospital, Allergy Service, Málaga, Spain

Background: Human invariant natural killer T (iNKT) cells are specialised T-cell subsets characterised by phenotypic and functional properties of NK and T cells. It is known that immunotherapy induces immunological changes along the time. These changes are related to B-lymphocytes and IgE production. Some works have shown that the interaction between B lymphocytes and NKT cells can drive enhanced antibody responses.

Objective: To characterise iNKT cells interactions with different subpopulations of B lymphocytes and plasma cells in allergic rhinitis patients sensitised to Dermatophagoides pteronyssinus (DP) receiving immunotherapy.

Method: Peripheral blood was collected at five time points during immunotherapy (before and after 1, 3, 6 and 12 months of treatment) and the phenotype of different cell subpopulations was determined using a FACSCanto II cytometer.Peripheral blood was collected at five time points during immunotherapy (before and after 1, 3, 6 and 12 months of treatment) and the phenotype of different cell subpopulations was determined using a FACSCanto II cytometer.

Results: We found a positive correlation between the changes induced by immunotherapy in iNKT cells and Dp-specific B-cells ($r = 0.530$), and between CD4+ iNKT cells and DP-specific B-cells expressing IgE ($r = 0.480$). Regarding the influence of iNKT cells in plasma cells, we have observed a positive correlation between

total iNKT cells and plasma cells ($r = 0.681$), mainly induced by the long-lived (CXCR4+) plasma cells ($r = 0.638$). On the other hand, the CD8+ iNKT cells showed negative correlations with both total ($r = -0.764$) and long-lived ($r = -0.713$) plasma cells.

Conclusion: The correlations found between the immunological changes induced by DP immunotherapy on iNKT cells, B-lymphocytes, and plasma cells could reflect the presence of an interaction that could influence the differentiation of naive B-cells to memory B-lymphocytes or plasma cells. However, further studies are needed to clarify the existence of this interaction and the mechanisms underlying the iNKT cell influence to serological memory.

1343
Specific immunotherapy modified T cells responses in a Spanish population of Der p1 allergic patients

Gómez, E[1]; Fernández, TD[1]; Blazquez, AB[1]; Doña, I[2]; Rondón, C[2]; Rodríguez-Bada, JL[1]; Galindo, L[2]; Mayorga, C[2]; Blanca, M[2]; Torres, MJ[2]
[1]Research Laboratory – Allergy Service – Carlos Haya Hospital, Malaga, Spain; [2]Allergy Service – Carlos Haya Hospital, Malaga, Spain

Background: Although specific immunotherapy (SIT) is actually the only aetiological treatment for allergic disorders, the underlying mechanism is still not fully understood. SIT induces changes in lymphocyte Th subsets from Th2 to Th1-Treg profile. This study aim to analyse the changes occurred in the main T cell subsets during *Dermatophagoides pteronissynus* (Der p) SIT in a south-Spanish population.

Methods: We included 10 patients with Der p allergy that receive SCIT for 1 year. Six allergic subjects that refused the treatment were included as controls. Peripheral blood mononuclear cells (PBMCs) were obtained from all subjects at different time-points along the SIT (T0 = basal, T1 = 3 months, T2 = 6 months, T3 = 12 months) and stored in liquid-nitrogen for later study. All samples were unfrozen and processed at the same time. PBMCs were cultured in different conditions (non-stimulated; Der p1 at 10 ug/ml) during 6 days. After this, cells were analysed by flow cytometry. The main T cells subsets were defined as follow: Th1: $CD4^+IFNg^+$; Th2: $CD3^+CD4^+CD27^-CRTH2^+$; Th9: $CD4^+CRTH2^-IL9^+IL10^+$; Th17: $CD4^+RORgt^+IL17A^+$ and Treg: $CD4^+CD127^-CD25^{hi}$. Results are expressed as stimulation index calculated as (%-Der p1 stimulated cells/%-Non-stimulated cells). In all cases, samples results from each time were compared with the basal.

Results: SIT with Der p1 induced changes in T cells subsets at different time-points. Th1 cells were increased from T1 being significant only at T2 ($P = 0.035$). In Th2 there was a progressively decrease being significant at T2 ($P = 0.011$) and T3 ($P = 0.005$). In Th9 cells an increased occurred from T1 of SIT ($P = 0.026$, $P = 0.026$ and $P = 0.035$). In the case of Th17cells, a decrease was observed at T1 (n.s.) and at T3 ($P = 0.035$). Regarding Treg cells data showed a increase tendency along the SIT. No differences for any of the subpopulation analysed were observed in the control group.

Conclusion: SIT with Der p1 induced immunological changes at very early stages. Der p1 allergic patients disclosed changes in frequencies of different T-cells subsets, with a reduction of T effector cells (Th2 and Th17) and an increase of Th1, Th9 and Treg cells. All these data demonstrated that our group of patients modified the allergic response from Th2 toward Th1/Treg profiles, detectable at the 3 months of SIT.

1344
A model of subcutaneous priming plus sublingual boosting using two nano-particulate adjuvant formulations for immunotherapy of respiratory allergy

Tamargo Santos, B[1]; Ramírez González, W[2]; Martinez, D[2]; De Leon, Y[3]; Infante, JF[4]; Morejón, A[2]; Bourg, V[5]; Torralba Averoff, D[2]; Ayala, A[6]; Fresno, M[6]; Labrada, A[2]; Sierra, G[4]
[1]Pharmacy and Foods Faculty, Havana University, Technology, Havana, Cuba; [2]National Center of Bioproducts, Allergens, Bejucal, Cuba; [3]Molecular Immunology Center, Havana, Cuba; [4]Finlay Institute, Havana, Cuba; [5]National Center of Bioproducts (BioCen), Allergens, Havana, Cuba; [6]Center for Molecular Biology 'Severo Ochoa', Autonome University of Madrid, Madrid, Spain

Background: Novel adjuvants are being increasingly investigated in order to improve conventional allergen-specific immunotherapy, regarding efficacy, shrinking of treatment duration and safety. The aim was to evaluate the immune modulating effect of a novel experimental nano-particulate vaccine formulations in a therapeutic murine model of respiratory allergy, using a priming-boosting strategy.

Method: Adjuvanted vaccine formulations were prepared by combining purified native allergens of the mite *Dermatophagoides siboney* (Der s 1 and Der s 2) with immune modulating molecules in nano-proteoliposomes (nPL) and nano-cochleates (nCH). C57/Bl6 mice were sensitised administering *D.siboney* allergen by IP route and exposing mice to allergen aerosols, and then, treated with the experimental formulations, first with subcutaneous priming with nPL-Ds and later, with sublingual daily doses during 4 weeks (boosting) with nCH-Ds. Finally, mice were subjected to inhalation allergen challenge.

Results: Subcutaneous priming induced a moderate pro-Th1 allergen-specific response with mixed IgG1 and IgG2a antibodies, moderate amount of IFN-gamma, besides Th2 and Tr1 cytokines. After the boosting intervention by sublingual route, IgG2a values, similar to those of healthy animals, were achieved. In treated mice subjected to allergen challenge, IgE antibodies showed a decrease, together with an increase of the IgG/IgE ratio, increase of IL-10 and CD4+FoxP3+ cells, as well as, decrease of local and systemic eosinophilia and peribronchial inflammatory infiltrate and mucus secretion, as assessed by lung histology.

Conclusion: This novel vaccine formulation, using the priming-boosting strategy by systemic and mucosal routes, showed promising results by modulating the allergic response first to a Th1 cytokine profile and later to a regulatory and tolerogenic pattern.

1345
Reaching maintenance dose with two injections in one week using an allergoid preparation for subcutaneous immunotherapy in children and adolescents

Kassir, S
MVZ Teltow-Fläming GmbH, Luckenwalde, Germany

Background: SCIT with different dosage schedules to reach the maintenance dose are on the market. Initial treatment ranges from one day up to 16 weeks. By the use of an individual up-dosing scheme within one week the time to reach the maintenance dose can be reduced. In the last years several data with rush or cluster schemes for the initial treatment were presented or published. Especially chemically modified allergens (allergoids) show the potential for very fast up-dosing treatment.

Method: Nineteen children and adolescents from 7 up to 17 years (17 male, ø 10.1 years) with an allergy against tress (hazel, elder, birch), grasses (incl. rye) or house dust mites (HDM) were treated with SCIT allergoid (20 000 AUM/ml) after a positive anamnesis, skin prick test and nasal provocation test. The registered dosage scheme for this product requires six injections in weekly intervals to reach the maintenance dose (starts with 0.05 ml up to 0.5 ml after 5 weeks). We examined a new scheme composing two injections within 1 week for trees, grass or HDM allergic patients. Two patients received

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

SCIT with trees and grasses in different weeks. With our individual initial treatment scheme we injected 0.2 ml and then after 7 days 0.5 ml. After 4 weeks we gave the next injection with the maintenance dose.

Results: Nine patients (8–15 years) received trees, eight children and adolescents (6.5–17 years) grasses and four patients (8–11¾ years) HDM. All patients reached the maintenance dose of 0.5 ml within 7 days. No systemic reactions were reported. Only slight local reactions (LR) on the injection site with redness and swelling <5 cm occurred. One child with trees developed a LR with <2 cm in ø following the second injection with 0.5 ml. Two children with grasses developed a small LR <1 cm in ø following the first injection with 0.2 ml. After the second injection only one child had again a small LR. No LR was documented concerning the patients with HDM injections.

Conclusions: With this allergoid preparation an individual dosing scheme with two injections in 7 days was possible using different allergens. The safety and compliance was very good. This new dosage scheme is timesaving and an alternative to the registered up-dosing procedure.

1346
Influence of phosphate salts and alum content on to the immunogenicity and stability of a *Dermatophagoides siboney* adjuvanted vaccine

Samalea Díaz, R[1]; Ramirez, W[1]; Tamargo, B[2]; Torralba Averoff, D[1]; Morejón, A[3]; Martinez, D[1]; Mas, A[1]; Oliva Diaz, Y[1]; Infante, JF[2]; Bourg, V[3]; Huergo, L[3]; Cruz Jimenez, R[1]; Mateo, M[1]; Espinosa, B[1]; Perez, O[2]; Labrada Rosado, A[1]
[1]Biocen, Allergen, Bejucal, Cuba; [2]Finlay Institute, Havana, Cuba; [3]Biocen, Bejucal, Cuba

Background: Allergen adsorption can be relevant for clinical efficacy and safety of alum-adsorbed allergen vaccines. AFPL1 is a novel combination adjuvant containing alum-adsorbed Outer Membrane Proteoliposomes from *Neisseria meningitidis*. Phosphate ions interfere with adsorption of *Dermatophagoides* allergens to alum hydroxide. On the other hand, alum is associated to the development of granulomas in the injection site, so its content should be minimised. The aim of this work was to evaluate the immunogenicity and stability of formulation variants of a novel *Dermatophagoides siboney* AFPL1-adjuvanted vaccine, using different content of phosphate salts and alum hydroxide.

Methods: A 2^3 experimental design was used for evaluating the effect of phosphate (0–8.5 mmol/l) and alum content (0.5–2 mg/ml) on to adsorption of Der s1. Stability during storage was assessed following standard ICH guidelines for biopharmaceuticals. Four formulation variants were further selected for immunogenicity testing in Balb/C mice, using three weekly injections (2 μg Der s 1). Allergen-specific serum antibodies (IgG, IgG1, IgG2a, and IgE) were measured by ELISA; IL-13, IL-5, IL-10 and INF-g cytokines were measured in cell culture supernatants by ELISA.

Results: Best variants regarding Der s1 adsorption were in the range 0–4.26 mmol/l of HPO_4 and 0–2.5 mmol/l of H_2PO_4, achieving values up to 99.9%. Decrease of $Al(OH)_3$ content from 2 to 0.5 mg/ml did not significantly ($P > 0.05$) influenced adsorption. All variants (even the one lacking phosphate salts) were able to maintain Der s1 and protein adsorption levels during 6 months of storage at 4°C. Regarding immunogenicity, all tested variants induced a mixed response of IgG1 and IgG2a antibodies in a similar extent, as well as, a moderate increase of IFN-g, and decrease of Th2 cytokines and lung tissue inflammation as compared to allergic control, particularly, after the aerosolized allergen challenge. However, the low alum variant showed slightly significant reduction of both IgG1 and IgG2a, although with less IgE and eosinophils in challenged mice. In addition, this variant substantially decreases the size and persistence of granulomas in the injection site.

Conclusions: Reduction of alum and phosphate content could be considered as pharmaceutical improvements with no disadvantages regarding immunogenicity and safety of this experimental allergy vaccine.

1347
Depigmented allergoids induce the synthesis of additional IgG epitopes respect to native extracts

Carnés, J; López-Matas, MA; Gallego, MT; Iraola, V; Moya, R; Leonor, JR
Laboratorios LETI, R & D, Tres Cantos, Spain

Background: Different markers have been suggested over the years to measure the immunological effect of SIT and to predict clinical benefits. The synthesis of allergen-specific IgG antibodies (mainly IgG4 subclass) blocking interaction with IgE after immunotherapy has been related to clinical efficacy. Mapping sequential and conformational IgG epitopes is becoming a potent tool for the exploration of the immunological response to allergens. The objective of the study was to detect and compare the linear IgG epitopes induced by depigmented allergoids (Dpg-pol) and native unmodified allergen extracts.

Method: Rabbits were immunised with native and Dpg-pol extracts of birch pollen and subsequent serum samples obtained. Sequences of Bet v 1 and Bet v 2 were obtained from the UniProtKB/Swiss-Prot data bank (www.uniprot.org). Linear synthetic peptides covalently bound to a cellulose membrane by the C-terminus (SPOTs) were commercially obtained. A total of 26 peptides were prepared with Bet v 1 and 25 peptides with Bet v 2. All of them were synthetized overlapping by six aminoacids, and contained 12 aminoacids except the last one for both allergens. Recognition of linear IgG epitopes of Bet v 1 and Bet v 2 was analysed by immunoblot.

Results: Serum samples from rabbits immunised with native birch pollen extracts recognised 11 linear epitopes, while serum samples from Dpg-Pol-immunised animals recognised eight epitopes from Bet v 1. For Bet v 2 peptides, eight epitopes were recognised by IgG from animals immunised with native extracts, and nine epitopes from Dpg-Pol immunised animals. Dpg-Pol immunised serum samples recognised some of the epitopes identified by serum samples form native extracts but they also recognised new synthetized epitopes.

Conclusion: Dpg-pol birch pollen allergen extract stimulates the synthesis of specific IgG antibodies which recognise common but also novel epitopes compared with native extracts from the major allergens Bet v 1 and Bet v 2.

Poster Session 56

Insect venom hypersensitivity

1348
Efficacy of bee venom immunotherapy during the first year of treatment

Galani, M; Vovolis, V; Mikos, N; Kalogiros, L; Tsoumani, M
Laikon General Hospital, Allergy & Clinical Immunology, Athens, Greece

Background: Venom immunotherapy (VIT) is the treatment of choice for patients allergic to bee venom. Literature review supports VIT efficacy after having reached the maintenance dose (MD) of 100 μg. Our purpose was to examine efficacy right after the build-up phase, in our bee venom allergic patients undergoing VIT.
Method: Patients were submitted to treatment with a modified ultra rush protocol during initial phase and reached MD after a month. We recorded incidents of field stings, reported from our patients, within a year after patients had reached the MD of 100 μg. Additionally we performed bee sting challenges in our hospital. Thirty-six bee venom allergic patients were included in this study, while 12 patients did not consent to a sting challenge. Thirteen (36.1%) of these patients, were professional beekeepers. A total of 41 bee stings were recorded 17 of which were field stings, whereas 24 stings were conducted in our Hospital. Challenges are performed in a fully equipped room provided with adequate resuscitation facilities.
Results: Reactions during bee VIT were observed in 15 patients, five of whom reported only subjective symptoms. The MD for nine patients was increased at 200 μg. Out of 17 patients who reported field stings, only 2 (11.8%) reacted. In 5 (20.8%) of 24 challenges, reactions occurred. Out of nine patients treated with the MD of 200 μg bee venom, one person (11.1%) reacted on a subsequent in-Hospital challenge.
Conclusion: The efficacy of bee venom immunotherapy during the first year of treatment amounts to 80.6%. However, results seem to vary considerably between the group of field stings and that of in-Hospital bee sting challenges. Performing these challenges in appropriate in-Hospital settings seems to be the most reliable method for detecting VIT efficacy in these patients. In the minority of patients who are not protected with the dose of 100 μg, tolerance is gained with an increased 200 μg MD.

1349
Negative predictive value of the basophil activation test in hymenoptera venom allergy

Bokanovic, D[1]; Laipold, K[1]; Pickl-Herk, B[1]; Griesbacher, A[2]; Aberer, W[1]; Vollmann, J[3]; Crailsheim, K[3]; Sturm, GJ[1]
[1]Department of Dermatology and Venerology, Medical University of Graz, Graz, Austria; [2]Division of Biostatistics, Center for Medical Research, Medical University of Graz, Graz, Austria; [3]Department of Zoology, University of Graz, Graz, Austria

Background: Insect stings can cause severe, life threatening allergic reactions. Double positive results for bee and wasp venom are frequent, but often clinically irrelevant. Therefore it can be difficult to find the relevant venom for therapy. The basophil activation test (BAT) is generally recognised as an additional and reliable tool in the diagnosis of hymenoptera venom allergy. Several studies have confirmed its usefulness in determining the culprit venom. The aim of the current study was to examine its ability to identify clinically irrelevant sensitisations and to demonstrate that a negative result in the BAT has a high negative predictive value.
Methods: Sixteen insect venom allergic patients with dual sensitisation in sIgE testing and intradermal testing but mono-sensitisation in BAT were included. Two different BAT protocols were used; an in-house protocol and a commercially available kit (Flow Cast®, Bühlmann, Switzerland). Negative results of the BAT were confirmed by sting challenges with the respective insects. The negative predictive value of the BAT was calculated as number of true negatives / (number of true negatives + number of false negatives).
Results: Of 93.8% (15/16) tolerated sting challenges without systemic symptoms. Of 37.5% (6/16) developed normal local reactions and 56.3% (9/16) large local reactions. In one patient a weak grade I reaction (according to Ring and Messmer) was observed. Thirty minutes after the bee sting an isolated wheal appeared at the neck together with two wheals nearby the large local sting reaction on the forearm. Another 30 min later a small periumbilical exanthema developed. However, vital signs were not altered and tryptase serum levels were normal. Therefore, negative predictive value of the BAT among these double-sensitised patients was 93.8% with the commercial kit and 87.5% with the in-house protocol.
Conclusion: These findings clearly show that the basophil activation test is a useful additional diagnostic tool for determining clinically irrelevant sensitisation. In the case of a negative BAT result no severe symptoms after a deliberate sting challenge were observed. However, predicting isolated skin symptoms is a weak point of the BAT.

1350
Risk factors for hymenoptera stings – a case-control study

Silva, D[1]; Santos, N[1]; Pereira, A[1]; Pereira, AM[1]; Delgado, L[1,2]; Coimbra, A[1]
[1]Hospital de São João, Porto, Portugal; [2]Faculty of Medicine of the University of Porto, Immunology, Porto, Portugal

Background: Avoidance measures for bee and wasp stings are critical in patients with hymenoptera allergy. However, no strong evidence exists to support the usual recommendations.

The aim of this study was to evaluate risk factors for hymenoptera sting in a group of individuals with history of hymenoptera sting and to compare them with controls that witnessed the event.
Method: Case-control study including individuals with known history of hymenoptera stings (cases) and those that were at the same place and at the same time, witnessed the event, but were not stung (controls). A convenience sample was obtained from:

1 subjects with known history of allergic reaction to hymenoptera venom followed in the Hymenoptera Allergy Unit of a Portuguese University Hospital in November 2012 ($n = 35$);
2 individuals with hymenoptera sting history who had specific IgE measurements in the same hospital from 2008 to 2011 ($n = 120$).

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

Each case was asked to include its own control. Data was collected using a written self-assessment questionnaire that was mailed home and it included questions about environmental factors, clothes worn and activity at the time of the event. The questionnaire was answered by 42% ($n = 65$) of the selected individuals; 22 were excluded due to lack of a control. No differences were detected between respondents and non-respondents. Comparisons between cases and controls were performed with McNemar and Marginal Homogeneity test.

Results: A total of 43 cases and correspondent controls were included. Cases had bee allergy suspicion in 70% and wasp in 30%, 67% were under venom-immunotherapy. Upper limb (51%) and head (40%) were the most reported stung body parts. The great majority of stings occurred in rural (88%) and outdoor areas (80%) with fruit trees nearby (50%); 25% of the participants were gardening or farming when they were stung. No significant differences were seen between cases and controls on the type or color of clothing, type of shoes and the degree of movement of the activities. The great majority of cases and controls were wearing dark-colored clothes, 62% and 69%, respectively.

Conclusion: In this sample, most of the stings occurred in rural environments, during outdoor activities and when wearing dark-colored clothing. Avoidance of hymenoptera stings can be life-saving in venom-allergic patients; however more studies are needed to in order to establish correct secondary prevention advice.

1351
Hymenoptera venom allergy and mastocytosis, an association to keep in mind!

Fagedet, D[1]; Jacquier, J-P[2]; Chandesris, M-O[3]; Boccon-Gibod, I[1]; Deroux, A[1]; Sarrot-Reynauld, F[1]; Bouillet, L[1]
[1]Grenoble University Hospital, Grenoble, France; [2]Chambery Hospital, Chambéry, France; [3]Mastocytosis French Reference Center, Paris, France

Background: Mastocytosis is a myeloproliferative syndrome with variable clinical burden from indolent to aggressive disease.

Indolent systemic mastocytosis (ISM) was found to be more prevalent among allergic patients to hymenoptera (Bonadonna et al.).

Method: From January to December 2012, serum tryptase level was measured in patients consulting for hymenoptera allergy at our institution. When it was superior to 20 ng/ml without any clinical sign of mastocytosis, a bone marrow examination with cytology, molecular biology and flow cytometry was performed looking for ISM.

Results: Among 169 patients consulting for hymenoptera allergy, five (3%) had a serum tryptase level superior to 20 ng/ml (27.7–91.5; mean 45.0 ng/ml), four male and one female, four being allergic to wasp and one to bee.

Allergic reaction according to Müller's classification was grade III ($n = 3$) or IV ($n = 2$) with a mild cutaneous and biologic sensitisation (IgE being always inferior to 2 kUA/l). Clinical exam was considered normal for all patients.

Diagnosis of ISM and clonal mast-cell activation syndrome (cMCAS) was confirmed after bone marrow exploration for three and one patient respectively. One patient had asymptomatic hepatosplenomegaly and three had osteoporosis.

Conclusion: Four out of five of the patients assessed had either ISM or cMCAS without any evocating clinical sign. This diagnosis implies a lifelong immunotherapy, anesthesics precautions, mast-cell degranulation prevention (lifelong anti-histaminic drugs and epinephrin) and assessment and treatment of osteoporosis. Our results must encourage practitionners to investigate patients with high serum tryptase level, even without clinical sign of mastocytosis, especially as clinical allergic reaction was severe with low sensitisation.

1352
The risk factors of hymenopthera venom allergy in north-eastern Poland population

Oledzki, B; Zietkowski, Z; Tomasiak-Lozowska, M; Pampuch, A; Bodzenta-Lukaszyk, A
Medical University of Bialystok, Poland, Allergology and Internal Medicine, Bialystok, Poland

Background: The aim of this study was to analyze the potential risk factors on severity of systemic allergic Hymenopthera sting reaction among patients admitted to The Department of Allergology and Internal Medicine of Medical University in Bialystok in years 2008–2011.

Method: In retrospective analysis, 144 patients with systemic allergic sting reaction were assigned into one of four groups depending on severity of sting reaction according to H.L. Mueller. The number of patients in each group was comparable; group I-34, II- 36, III- 36, IV-38 respectively. In particular groups the risk factors such as age, stinging insect, time interval between stings, part of the body affected by sting, total IgE and serum basal tryptase concentration were analyzed and compared.

Results: The mean age was higher in grade III and IV reaction. However, significant statistical difference was shown only between grade II and III ($P = 0.005$). In analyzed population wasp was the culprit of 83, honey bee of 55 and hornet of six stings but no significant correlation was shown between the groups. Overall 39 patients were re-stung in time interval <12 months, among them 15 were found in group IV, besides 37 of 144 patients reported to be stung in head/neck, 28 of them found in groups III and IV. No correlation between any groups was found in total IgE concentration but statistically significant difference in basal tryptase concentration between groups I-III, I-IV, II-III and II-IV was observed.

Conclusion: We proved that such factors as older age, shorter time interval between stings, sensitive body parts as head and neck and serum basal tryptase concentration influence severity of systemic reaction to Hymenopthera sting.

1353
'Double sensitisation' to wasp and bee venoms in insect sting allergy – are specific IgE tests to recombinant major allergens of wasp and bee clinically useful?

Nagendran, V; Bansal, A
Department of Immunology, Epsom & St.Helier University Hospitals NHS Trust, Carshalton, United Kingdom

Background: Some patients with a history of insect sting allergy may show 'double positive' specific IgE antibody results to both wasp and bee venom. This may be due to cross reactivity (of amino acid sequence identity or cross reactive carbohydrate determinants) or true double sensitisation to both insects. Differentiating between these two possibilities is clinically important in selecting the appropriate venom preparation for immunotherapy in a patient who has experienced systemic allergic symptoms. Recent studies indicate that component resolved analysis with recombinant species specific major allergens is helpful in distinguishing true double sensitisation from cross-reactivity.

Method: We studied seven patients with a history of anaphylaxis/systemic allergic symptoms after suspected insect sting, who showed double positive results to bee and wasp venoms. All patients were questioned about the suspect insect that caused the sting, clinical features of co-existing allergy and symptoms of mastocytosis. Specific IgE to recombinant component proteins of wasp (Ves v1 and Ves v5) and bee (Api m1) were measured.

Results: Six of the seven patients with double positive results were positive to wasp component proteins Ves v1 and/or

Ves v5, and negative to bee component protein Api m1. One patient was negative to Api m1, Ves v1 and Ves v5 (subsequently found to have food dependent exercise induced anaphylaxis).

On reviewing the double positive (whole venom) results, the majority had one result 2–9 times higher than the other, and in six patients the higher Specific IgE result (for whole venom) correlated with the occurrence of positive Specific IgE to the relevant component protein.

Conclusion: It was noteworthy that the higher whole venom Specific IgE appeared to predict the outcome of component protein testing. However, the positive component protein results certainly guided our choice of venom immunotherapy in these patients. When there is doubt about the offending insect in patients with double positive results, component protein testing may help to avoid unnecessary dual (bee and wasp) venom desensitisation.

1354
Recurring nearly deadly mosquito bites in a patient with systemic mastocytosis

Reiter, N; Maurer, M; Siebenhaar, F
Charité – Universitätsmedizin Berlin, Dermatology, Berlin, Germany

Background: Mosquito allergy is an entity that causes severe large or atypical vesicular and even necrotic local allergic reactions at bite sites in some individuals. Systemic reactions including urticaria, angioedema, dyspnea, or hypotension are less common.

Objective: We report a unique case of systemic mastocytosis and recurring grade IV allergic reactions to mosquito bites.

Methods: To confirm the mosquito allergy we performed skin prick testing with whole-body extract of two mosquito species that are common in middle Europe (*Culex pipiens* and *Aedes communis*). Further, CD63 up-regulation in basophils as a measure of basophil activation was determined by flow cytometry after stimulating patient's and control's basophils with whole-body extract of both mosquito species. To show that specific IgE in patient's serum mediated the severe allergic reactions we looked for specific IgE against mosquito via CAP.

Results: Skin prick test with histamine as a positive control showed a wheal of 5 mm at the maximum diameter and a flare. Prick test with *Culex pipiens* gave a positive result with a wheal of 7 mm. *Aedes communis* skin testing showed a small papule of 2 mm. The negative control did not produce any reaction. The skin prick test was negative in all four healthy controls for both mosquito species. The patient's basophil activation with CD63 up-regulation to *Culex pipiens* extract was 90%. This was clearly elevated compared to the highest basophil response of a healthy control (38%). Sixty-two percent of the patient's basophils were activated by the *Aedes communis*. The highest response of the basophils of a healthy control to *Aedes communis* extract was 24%. Specific IgE against mosquito was not detected by CAP. The total IgE was normal with 48.4 kU/l (<100 kU/l).

Conclusion: We confirmed the patient's mosquito allergy by prick test and basophil activation test (BAT) and thus identified the first case worldwide with a grade IV allergic reaction to mosquito.

1355
Venom-dependent vibration-induced anaphylaxis: a new risk after following large local reactions from Hymenoptera stings

Savoia, A; De Bartolomeis, F; Astarita, C
Allergy Unit, Department of Internal and Experimental Medicine and Surgery, Second University of Naples, Naples, Italy

Background: Large local reactions (LLRs) from Hymenoptera stings are frequent and some acquired physical disorders like cold urticaria or anaphylaxis are increasing after LLRs. Vibratory angioedema/urticaria is a rare physical disorder and its association with insect stings and vibratory anaphylaxis have never been reported. We evaluated vibration hypersensitivity and mast-cell degranulation in a case of anaphylaxis occurring in connection with vibration exposure 18 h after a wasp sting. A 49-year-old tailor had been suffering for 18 h from a tolerable LLR on his right forearm due to a wasp sting, when he developed severe anaphylaxis with dyspnea, collapse, and loss of consciousness 30 min after exposure to vibratory forces of a buttonholing-machine. Medical records of Emergency Department registered Epinephrine treatment, and serum tryptase levels of 75 μg/l at admission and of 3.6 μg/l at discharge after 28 h. Patient's medical story was notable for his last well-tolerated sting that had occurred 7 weeks before anaphylaxis, and for a widespread pruritus with the swelling of his right forearm that had occurred while he was riding a scooter one week after anaphylaxis. Clinical investigations evidenced only an IgE-mediated sensitisation to wasp venom.

Method: Vibratory challenge tests (VCTs) were performed by exposing patient's right forearm to the Vortex mixer at 1200-rpm for 10 min and his body to high-speed 3600-rpm buttonholing-machine for 60 min. Serum tryptase levels were determined before and after VCTs.

Results: Fifteen minutes after Vortex-VCT, a swollen area with peripheral itchy urticarial wheals appeared on patient's forearm in same site as previous LLR. Thirty-minutes after buttonholing-machine-VCT, widespread hives and swollen itchy areas around the point of the sting and the point of wasp skin test on left forearm appeared.

There were no other symptoms. Serum tryptase levels before and after VCTs suggested a prominent role of vibrations in triggering symptoms due to mast-cell degranulation. After 5 weeks, VCTs were negative.

Conclusion: Specific IgE to wasp venom and exposure to two stings in about 2 months may have induced LLR and the transient hypersensitivity of mast cells to vibrations by still unknown mechanisms. Our data suggest that we must prudently ban exposure to intense vibration until normalisation of lesions in patients with active LLRs and then check vibration hypersensitivity to prevent delayed physical disorders.

1356
Importance of biopsy technique in the diagnosis of systemic mastocytosis: a case report

Verdú, M[1]; Cano, MDM[2]; Serrano, P[2]; Fernández, L[2]; Ruiz, B[3]; Moreno, C[2]; Guerra, F[2]
[1]Hospital Universitario Reina Sofía, UGC Alergología, Córdoba, Spain; [2]Hospital Universitario de Reina Sofía, Córdoba, UGC Alergología, Córdoba, Spain; [3]Hopital La Mancha Centro, Servicio de Alergología, Alcázar de San Juan, Spain

Background: Systemic mastocytosis (SM) is a rare disease characterised by abnormal proliferation and accumulation of mast cells (MC). Approximately 90% of SM are benign forms, known as indolent systemic mastocytosis (ISM); it is common in adults that the clinical features of ISM do not include skin symptoms such as urticaria or edema. The diagnosis of this pathology is performed according to the World Health Organization (WHO) criteria, being the major criterion the presence of mast cell aggregates in a bone marrow biopsy. Here we present the case of a patient diagnosed of ISM who did not initially fulfill the WHO criteria.

Method: Our patient is a 36 year old man who presented with generalised erythema, eye irritation, dizziness, intense asthenia and weakness without loss of consciousness, inmediately after suffering a wasp sting. The symptoms were treated urgently with intramuscular corticosteroids. He

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497

refers milder symptoms with the previous wasp stings; he is highly exposed to hymenoptera stings due to his occupation and habitat.

Results: We performed our standard protocol for the diagnosis of hymenoptera venom allergy: skin tests to Apis mellifera, Polistes dominulus and Vespula were all negative, as well as the results for specific IgEs to the same allergens, including recombinant profile. Serum triptase levels were 18'4 and 23'1 ng/ml in two different analysis. However, the bone marrow biopsy done at our hospital showed no abnormalities. With a high suspicion of SM we referred our patient to a reference center: 'Istituto de Estudio de Mastocitosis de Castilla la Mancha (CLMast), Spain, where the second bone marrow biopsy showed perivascular MC aggregates, as well as a mutation of the c-kit (CD 117) restricted to MC and an aberrant phenotype. Complementary studies revealed moderate osteopenia in the lumbar spine. Several months after starting treatment with cromoglycate and antihistamines, the patient suffered another wasp sting and no symptoms were noticed.

Conclusion: The presence of MC aggregates is determinant for reaching the diagnosis of SM. The biopsy thecnique is very important in these cases. When a mastocytosis is suspected it is necessary to study cells associated to stroma; it is advisable to do the bone marrow aspirate with a needle of a certain gauge (8–11 g) and to aspirate strongly using a syringe of 20 ml. This exam should be carried out by experienced professionals who know the hystologic characteristics of this disease.

1357
Venous thrombosis after hymenoptera sting

Quecchia, C[1]; Braga, M[1]; Di Gioacchino, M[2]
[1]University of Brescia, Allergology and Clinical Immunology, Brescia, Italy; [2]G. d'Annunzio University, Specialization Course in Allergology and Clinical Immunology, Chieti, Italy

Background: Hymenoptera sting can result in the classical IgE mediated reactions, or in non immunological reactions due to substances present in their venom as thrombogenic substances that might be responsible for cardiovascular events, as reported in a few case reports.

Methods: Here we describe the case of a 22 year old man, in good health, who was stung on the knee in the afternoon by a hymenoptera, likely a wasp. He developed a distrettual edema during the night that in few hours diffused to the entire leg. An echography demonstrated a posterior tibial vein thrombosis. He had no familiarity for coagulation disorders and all specific thrombosis exams (coagulation factors and gene mutations) and the routine resulted negative. The patient was prescribed low molecular weight heparin. Finally, he referred to the outpatient clinic of the Allergology and Clinical Immunology unit of Brescia Hospital.

Methods: Skin prick test and intradermal tests for *Polistes* spp., *Vespula* spp. and Apis m., using venom extracts from Stallergenes (Milan, Italy) and venom specific IgE (ImmunoCAP Phadia, Barcelona, Spain) for *Polistes* spp., *Vespula* spp., *Dolichovespula arenaria* and *maculata*, Apis m., *Blatella germanica*, *Aedes communis* and *Tabanus* spp, were executed along with the determination of serum tryptase and total IgE.

Results: Skin prick test and intradermal test with Apis m., *Vespula* spp. and *Polistes* spp. venoms were negative. Venom specific IgE for *Polistes* spp., *Dolichovespula arenaria* and *maculata*, Apis m., *Blatella germanica*, *Aedes communis* and *Tabanus* spp. were <0.1 KUA/l, IgE for *Vespula* spp. was 0.15 KUA/l. Serum tryptase level was normal (6.3 ug/l), and total IgE were 127 KU/l.

Conclusions: The negativity of immunological *in vivo* and *in vivo* tests for hymenoptera antigens supports the hypothesis that the thrombosis occurred after the hymenoptera sting is due to a direct action of thrombogenic substances contained in the venom. Therefore, local reactions of edema that follow a hymenoptera sting should be carefully evaluated for their pathogenesis.

1358
Tolerance of rush protocol of hymenoptera venom immunotherapy: a case report

Hernández-Mondragón, LO[1]; Del Rio-Navarro, BE[1]; Del Rio-Chivardi, J[1]; Navarrete-Rodriguez, EM[1]; Lievano-Perez, MA[1]; Rosas-Vargas, MA[1]; De Baro-Álvarez, P[1]
[1]Hospital Infantil de Mexico 'Federico Gomez', Allergy and Clinical Immunology, Mexico City, Mexico

Background: Reactions to Hymenoptera stings can be classified as local, extensive (LLRs), systemic (SRs) and toxic. Patients with SRs should be diagnosed and give an adrenaline autoinjector device. The venom immunotherapy (VIT) is the only specific treatment available, and is indicated in patients with severe SRs. Accelerated schedule (Rush and ultra-rush) are reserved for cases that require a maintenance dose reach in a short time to provide protection.

Method: We report a case of 14 years old female without atopy history, whose family is dedicated to floriculture, living in a subtropical region of Mexico, who 15-min after sting of Vespula vulgaris developed generalised urticaria, angioedema of face and extremities, dysnea, wheezing, vomiting, abdominal pain and loss of alertness: treated at rural clinic and discharged 24-h later; she was referred to our service, where we request specific IgE Apis mellifera <0.35 KU/l (negative), paper wasp 67.9 kU/l, yellow-jacket wasp 5.32 kU/l, whiteface wasp 1.79 kU/l (positive in moderate to high range); positive skin prick test with a 1 cm wheal diameter at [1/10 000] concentration corroborating hypersensitivity to Vespidae super-family. Serum tryptase levels 4 ng/ml was normal. We decided to perform Rush hymenoptera VIT with weight/volume allergen extract mixture of wasp-venom. The procedure was performed in 4 days in a pediatric intensive care unit, starting at a 1/10 000 000 concentration (1000-fold lower concentration than positive SPT), with gradual increase in volume, administered subcutaneously (0.1, 0.2, 0.3 to 0.9 ml) at intervals of 30 min.

Results: The projected dose for maintenance was achieved (0.9 ml at a 1/100 concentration). She manifested local reaction (wheal and erythema) during the last dose treated with ice and 2nd generation antihistamine with complete resolution, with no presence of systemic adverse reactions. The maintenance dose will be 0.5 ml [1/100] every week for 6 months and then we will try to administrate progressively every 2, 3 or 4 weeks.

Conclusion: The accelerated schedule type 'Rush' with allergen extract weight / volume with wasp venom mixture was clinically well tolerated by the patient, reaching the maintenance dose (protective) within 4 days without the presence of adverse reactions.

1359
Sting challenge test: a tool to assess efficacy of hymenoptera immunotherapy, also in pediatric population

Verdú, M[1]; Cano, MDM[1]; Serrano, P[1]; Fernández, L[1]; Ruiz, B[2]; Moreno, C[1]; Guerra, F[1]
[1]Hospital Universitario de Reina Sofía, Córdoba, UGC Alergología, Córdoba, Spain; [2]Hopital La Mancha Centro, Servicio de Alergología, Alcázar de San Juan, Spain

Background: The sting challenge test has been executed during the last years as a tool to assess the level of protection of patients ongoing immunotherapy with hymenoptera venom. We want to show the utility of these tests in a group of pediatric patients.

Method: Our study population is a group of patients under 18 years-old, with a demonstrated allergy to hymenoptera venom who are receiving allergen-specific inmunotherapy. We perform the sting challenge test to check the efficacy of the provided treatment. Previous to the realisation of the mentioned sting challenge, several data is collected (symptoms of the allergic reaction, number of previous stings, results of skin tests, values of specific IgE, serum tryptase, type of extract used, age of the patient at the beginning of treatment and other relevant aspects, such as relation with apiculture) to analyse if any of these characteristics could influence the outcome of the test.

Results: Our population consisted on 26 pediatric patients, 22 boys (74′6%) and 4 girls (15′4%). All of them presented with different grades of anaphylaxis after the sting of bee or wasp, and after the diagnostic process 15 of them (57%) began treatment with Apis mellifera venom extract and 11 (42%) with Polistes venom extract, using a cluster build-up phase. The bee or wasp sting challenge was carried out at least once during a period from 6 months to 7 years since the maintenance dose was reached. We have performed the test 38 times (nine patients took the test twice or more times). All of them had a negative outcome, with the patients presenting no systemic reaction to the venom; the negativity of the test didn′t imply the early discontnuation of the immunotherapy, planned to last at least 5 years.

Conclusion: The sting challenge test constitutes a reliable way of valuate the efficay of the hymenoptera specific immunotherapy. Even though it has its limitations as a test, a negative result givesboth the physician and the patient some positive information about the efficacy of the treatment. A positive outcome of the test would reveal that the level of protection reached is not enough, and we should carry out other estrategies to reinforce it.

1360
Specific immunotherapy may prevent anaphylactic reaction to sting by one but not to several wasps

Djuric, V[1,2]; Stosovic, R[1]; Arandjelovic, S[1]; Lalic, D[1]
[1]Clinic Center Serbia, Clinic of Allergology and Immunology, Belgrade, Serbia; [2]Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Background: Specific immunotherapy is highly efficient for anaphylactic reaction to insect venoms.

Method: We present a case of a 45 year old male patient who had repeated anaphylactic reactions to wasp sting. Skin prick test with wasp venom was positive and specific IgE to wasp venom was present in his serum. Specific immunotherapy with wasp venom was carried out for 4 years.

Results: Skin test to wasp venom became negative and specific IgE to the same allergen was undetectable. During the course of immunotherapy he was stung by wasps on several occasions. He had anaphylactic reaction when he was stung by 5 wasps. However, there was only a mild reaction to a single wasp sting both before and after the described reaction.

Conclusion: This case shows that in some cases immunotherapy may protect against anaphylactic reaction to usual but not large amounts of allergen. Negative skin test and undetectable specific IgE are also not a guarantee that such reactions may not occur.

1361
Stability of baseline serum tryptase concentration in venom sensitised children

Cichocka-Jarosz, E[1]; Brzyski, P[2]; Rogatko, I[3]; Jedynak-Wąsowicz, U[1]; Lis, G[1]; Pietrzyk, JJ[1]
[1]Jagiellonian University Medical College, Department of Pulmonology, Allergy and Dermatology, Krakow, Poland; [2]Jagiellonian University Medical College, Department of Medical Sociology, Krakow, Poland; [3]Jagiellonian University Medical College, Department of Biochemistry, Krakow, Poland

Background: Baseline serum tryptase (BST) concentration reflects the constitutive, individual mast cell load or activity. BST values higher than 11.4 μg/l are considered to be a marker of mast cell clonal disorders, including occult systemic mastocytosis[1,2]. Studies of BST were performed in healthy and atopic children[3]. We attempted to evaluate the stability of baseline serum tryptase concentration in venom-sensitised children.

Method: In a prospective study, BST levels were measured by UniCAP method, twice – during first diagnostic procedure performed at the median of 7 months (Q1 = 5, Q3 = 10) after systemic reaction to field insect sting (FIS) and just before introduction of incremental protocol of venom immunotherapy (VIT). In further analysis, type of sensitisation, number of stings, Mueller's grade of IFS reaction and time interval after IFS, gender, age, and venom specific IgE concentration were regarded. Linear regression with gamma distribution of dependent variable and identity linking function was estimated using Generalised Estimating Equations.

Patients: 66 children (47 boys – 71%), mean age 10.8 years, (SD = 3.8) were qualified to VIT due to systemic reactions to FIS (41 bee venom (62%), 25 wasp venom (38%)). The history of severity of venom reaction were as follows: grade II - 20 (30%), III - 28 (43%), IV- 18 (27%), respectively. None of the patients experienced FIS during follow-up.

Results: Mean level of BST upon diagnosis equaled 3.81 (SD 0.30), whereas before beginning of VIT it decreased to 3.64 (SD 0.28) with difference equal to 0.17 (95% CI = (0.01, 0.33), $P = 0.032$). Multivariate model, after adjustment for number of FIS before treatment and kind of culprit insect showed that the difference between first and second measurement was no longer significant ($P = 0.235$). The level of BST increased by 2.80 [95% CI = (1.66, 3.94), $P < 0.001$] with each subsequent FIS during first measurement, whereas it increased by 2.51 with each IFS during second measurement – the difference between impact of number of IFSs between measurements equaling −0.28 (95% CI = (−0.52, −0.04), was significant ($P = 0.022$). There was no relationship between BST and other aspects of clinical characteristics.

Conclusions: BST concentration in venom allergic children before VIT introduction remains stable regardless of the time after systemic FIS reaction and other factors.

1362
Ultrarush venom desensitisation after systemic reactions during conventional venom immunotherapy

Patella, V[1]; Florio, G[1]; Giuliano, A[2]; Oricchio, C[3]
[1]Division of Allergy and Clinical Immunology, ASL Salerno, Agropoli, Italy; [2]Department of Public Health, ASL Salerno, Vallo della Lucania, Italy; [3]Division of Immunohaematology, ASL Salerno, Agropoli, Italy

Background: Rush and ultrarush venom immunotherapy (VIT) protocols are safe and effective in patients with Hymenoptera hypersensitivity. However, these protocols have typically been used instead of conventional VIT, but not usually in patients who have experienced adverse reactions during conventional VIT. The few reports that using an ultrarush VIT protocol to desensitise patients with a history of severe systemic reactions during conventional VIT have been done with a premedication with steroids and anti-H1 and anti-H2. To determine whether ultrarush VIT can be safely administered to a high-risk patient with a history of severe systemic reactions to conventional VIT without use of steroids and anti-H1 and anti-H2, we performed this study.

Method: Three patients (2M, 1F, ± 38.5 yrs) received VIT. An aqueous preparation of the venom from *Vespula* sp., Hymnox (ROXALL), was administered by

subcutaneous injections to patients. The cumulative dose, administered in 13 fraction doses each 15 min (3 h), at the end of the incremental phase the cumulative dose was 111.101 μg of Hymenoptera venom. The values of basal serum tryptase and basal serum ICAM-1 were obtained before and after VIT.

Results: The patients tolerated the procedure with minimal adverse effects. All patients received subcutaneously a maintenance dose of 100 μg on fifteenth day and then they subsequently received maintenance dosing in the outpatient clinic weekly for 4 weeks. The values tryptase (before 3.4 ± 1.8 μg/l; after 3.6 ± 1.9 μg/l;) were not modified during VIT while the ICAM-1 values changed from 356.4 ± 48.2 ng/ml before VIT to 264.7 ± 32.9 after VIT, $P < 0.0016$.

Conclusion: We report the successful use of ultrarush VIT in a high-risk patients with a history of severe systemic reactions during conventional VIT. Ultrarush venom desensitisation, after systemic reactions during conventional venom immunotherapy, does not modify values of basal serum tryptase but it improves values of basal serum ICAM-1. This protocol should be considered in patients with a history of allergy to Hymenoptera who require VIT but cannot reach a maintenance dose with conventional VIT owing to systemic reactions.

1363
Hymenoptera-venom basophil activation test should be routinely performed in patients with positive history and negative skin and specific IgE results

Silar, M[1]; Erzen, R[2]; Čelesnik Smodiš, N[1]; Zidarn, M[2]; Bajrovic, N[2]; Kopac, P[2]; Kosnik, M[2]; Korosec, P[1]
[1]University Clinic for Respiratory and Allergic Diseases Golnik, Lab.for Clinical Immunology and Molecular Genetics, Golnik, Slovenia; [2]University Clinic for Respiratory and Allergic Diseases Golnik, Golnik, Slovenia

Background: Significant number of patients with a clear history of insect allergies have a negative skin and sIgE tests. The aim of our study was to show the applicability of the CD63 basophil activation test in detecting hypersensitivity to Hymenoptera venom.

Method: Twenty-one patients with anaphylactic reactions to Hymenoptera sting (median grade III) and negative venom-specific IgE were routinely and prospectively tested with BAT.

Results: We diagnosed 81% (17 of 21) of patients with basophil activation test (BAT) and 57% (12 of 21) with intradermal skin testing. Three wasp venom-allergic patients showed IgE positivity to rVes v 5. Four patients (19%) were negative for all tests. In the case of double-positive BAT, the culprit insect correlated with the venom that induced a significantly higher basophil response.

Conclusion: BAT allows the identification of severe Hymenoptera venom allergic patients with negative skin and sIgE tests. The routine use of this cellular test should facilitate prescription of venom immunotherapy in complex cases with inconclusive diagnostic results.

1364
Sensitisation to hymenoptera venom in pollen allergic patients: frequency, reason and relevance

Bergmann-Hug, K[1]; Fricker, M[2]; Hausmann, O[2]; Helbling, A[1]
[1]Allergiestation Spital Bern Ziegler, Bern, Switzerland; [2]Allergie Poliklinik Inselspital, Bern, Switzerland

Background: About 20% of the Swiss population show specific Immunoglobuline E (sIgE) against honeybee or *Vespula* spp. venom even without a remarkable history to hymenoptera stings. On the other side 3% of the Swiss population react with systemic allergic reactions to bee or wasp stings. This high rate of sensitisation can be explained with the presence of specific IgE against cross-reacting carbohydrate determinants (CCD) on glykoproteins in plants and insects. The aim of the study was to investigate how many patients with pollen allergy have specific IgE to hymenoptera venoms, and whether the sensitisation is due to CCDs or to a primary sensitisation.

Method: Sera were collected from patients with a history of a seasonal allergy with positive skin prick tests to commercially available pollen extracts (ALK-Abello). *s*IgE against bee and wasp venom, bromelin (MUXF3) and the recombinant major allergens of hymenoptera venom (rApi m 1, rVes v 5, rVes v 1) were analysed by ImmunoCAP (Thermo Fisher Scientific Inc.). In a selected group of venom IgE positive patients a basophil activation test (BAT) is to be performed.

Results: So far, 97 patients (53 f., 35 m. with a mean age of 32 years) have been included. Complete laboratory analyses were done in 88 patients: 36/88 (41%) showed sIgE to bee venom, 25 (28%) to vespula venom, and 16 (18%) to bromelin. Moreover, 7 (8%) had sIgE to rApi m 1, 13 (15%) to rVes v 5, and 9 (10%) to rVes v 1. Nineteen (22%) patients were positive to both, bee and Vespula venoms, 14 to bee and 1 to Vespula venom only. In the bromelin-specific subgroup all various combinations could be detected.

Conclusion: Since >20% of pollen allergic individuals were sensitised to the major venom allergens one may suggest that more patients than expected are primarily sensitised to hymenoptera venom, or other factors than CCDs may contribute to cross-reactivity.

1365
The prevalence of hymenoptera venom allergy in general adult population in Istanbul

Gelincik, A[1]; Unal, D[1]; İssever, H[2]; Demirturk, M[1]; Isık, E[2]; Gul, H[2]; İliaz, R[1]; Kara, E[1]; Ertek, B[1]; Ozseker, F[1]; Colakoglu, B[1]; Buyukozturk, S[1]
[1]Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Allergy, Istanbul University, Istanbul, Turkey; [2]Istanbul Faculty of Medicine, Department of Public Health, Istanbul University, Istanbul, Turkey

Background: The aim of the study was to determine the prevalence of Hymenoptera venom allergy in general adult population since little is known in our population.

Method: The data of the telephone questionnaire which had been applied to a total of 11 816 (69.25%) out of 17 064 randomly selected individuals from both sides of Istanbul in 2005–2006 was reevaluated. Of 1171 individuals who were suspected of having hypersensitivity to hymenoptera venom according to the first questionnaire were applied a second questionnaire in 2010–2011 and those who agreed to be investigated personally were invited to our allergy clinic. Prick tests were applied with standard extracts of Apis mellifera, *Vespula* spp., *Polistes* spp. Intradermal tests with the same extracts were applied incrementally to a maximum dosage of 1 μg/ml until positive results were observed. *In vitro* sIgE levels of the same allergens and tryptase levels were also detected.

Results: The flow sheet of the study and the investigations performed were shown in Figure 1. According to the first questionnaire 1171 individuals (9.9%; 95%CI: 9.38–10.46%) were considered as having hypersensitivity to hymenoptera venom. Hundred and eighty-seven individuals (1.6%) reported systemic reactions, 984 (8.3%) reported local reactions. Apis mellifera, *Vespula* spp. and *Polistes* spp. were responsible in 838 (71.6%), 274 (23.4%) and 3 (0.3%) of the reported reactions, respectively. Seventeen individuals (1.5%) couldn't remember the culprit insect. Individuals aged ≥40 years old, the ones without having familial atopy were found to be more affected. Hundred and twenty-eight subjects out of 606 were considered as having hypersensitivity to hymenoptera venom (1.1%; 95%CI: 0.9–1.29%). During

personal investigations skin tests and/or sIgE levels revealed positive results in 24 individuals out of 44 subjects. Accordingly, the confirmed prevalence of hymenoptera hypersensitivity was found as 0.2% (95% CI: 0.14–0.30%) and 16 subjects had systemic reactions (0.14%; 95% CI: 0.08–0.22%), whereas eight experienced large local reactions (0.07%; 95%CI: 0.03–0.13%).

Conclusion: The results of this study show that prevalence of hymenoptera venom allergy was found infrequent in adults in our population when compared to other studies.

Allergy © 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, **68** (Suppl. 97), 337–497