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Poster Discussion Session PDS 1

Mechanisms and treatment of chronic rhinosinusitis

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Increased group 2 innate lymphoid cells in the peripheral blood of house dust mite allergic rhinitis in southern China may induce type 2 inflammation

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Background: Group 2 innate lymphoid cells (ILC2s) are essential in initiating and driving allergic immune responses. Elevated number of ILC2 in human peripheral blood (PBMC) of allergic asthma has been reported. However, there were inconsistent findings about the prevalence of ILC2s in PBMC of house dust mite (HDM) allergic rhinitis (AR) patients.The purpose of this study was to investigate whether there is an increase of ILC2s in the PBMCs of HDM-AR patients in southern China and their ability to induce Th2-skewed inflammation.
Methods: Flow cytometry and magnetic cell sorting were used to identify, isolate, and quantitate ILC2s in PBMCs from HDM-AR patients and healthy control subjects. The PBMCs and isolated ILC2s were cultured *in vitro* with a cocktail of cytokines (IL-2, IL-25 and IL-33) or allergen (Derp1). ELISA was used to measure the Th2 cytokine (IL-5 and IL-13) in plasma or cell-free supernatants.
Results: The number of ILC2s in PBMCs is increased in the HDM-AR group compared to healthy controls (HC) in southern China ($P < 0.001$). The AR patients symptom score (VAS) and the concentration of the Th2 cytokine (IL-13) in plasma were measured and was associated with the prevalence of ILC2s in HDM-AR patients ($P < 0.001$, $R^2 = 0.39$ for VAS and ILC2s; $P < 0.001$, $R^2 = 0.70$ for IL-13 and ILC2s). The concentrations of IL-5 and IL-13 increased in the supernatants of both PBMCs and isolated ILC2 cultured with the cytokine cocktail or allergen (Derp1) in the HDM-AR group vs the healthy control group. The effect of the cytokine cocktail was stronger than Derp1 ($P < 0.01$).
Conclusions: The number of Group 2 innate lymphoid cells (ILC2s) is increased in the HDM-AR patients in southern China and ILC2s could produce Th2 cytokines (IL-5 and IL-13) in the presence of IL-2, IL-25 and IL-33, which may contribute to type 2 inflammation in allergic immune responses.

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Effect on differentiation of human dendritic cells co-culture with primary nasal epithelial cells *in vitro*

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Background: Human nasal epithelial cells (hNECs) co-culture with human monocyte-derived dendritic cells (mo-DCs) to mimic the cells linking *in vivo* and study the effect on DCs differentiation, then investigate the possible mechanisms.
Method: Separate mo-DCs from human blood, then co-culture with hNECs. MGG staining and flow cytometry detected the primary cells. And flow cytometry detected the DCs' markers including CD86、HLA-DR、CCR7、CD11c and CD14 with or without co-cultured.
Results: The co-culture cells model was established successfully. After 24 h co-culture of hNECs with DCs, the percent of mo-DCs(CD14−, CD11c+) was 32.2% decreased compared with DCs single culture, and HLA-DR was less expressed compared with single culture($P < 0.05$).
Conclusion: The hNECs may be involved in inducing of DCs differentiation to monocyte in the co-culture system by some molecules.

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IL-2 contribute the remodeling in chronic allergic rhinitis murine model

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Background: IL-2 acts as an negative autocrine regulator of EOS migration, but in allergy process, and that this inhibitory effect may be downregulated as result of in allergy, allowing an increased migration of EOS towards chemotactic factors. The aim of our study is to investigate the function IL-2 of in the remodeling in the mice model of allergic rhinitis.
Method: The Masson, PAS staining and HE staining was done in OVA-stimulated allergic rhinitis murine model. IL-2 protein level of nasal mucosa was evaluated by ELISA. The number of esinophils cells, subepithelial collagenization, and thickening of the nasal mucosa epithelium were recorded.
Result: IL-2 was found lower in allergic rhinitis than normal control group ($P < 0.05$). And the number of esinophils was found increased in allergic rhinitis together with subepithial collagenization and thickening of epithelium. ($P < 0.05$).
Conclusion: Decreased IL-2 may play a role in aggravating airway inflammation and remodeling in chronic murine model of allergic rhinitis by attracted the accumulation of esinophils.

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Expression level and significance of IL-17A, IFN-γ and IL-23 in serum and nasal secretion of patients with chronic rhinosinusitis

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Background: Chronic Rhinosinusitis is a heterogeneous disease, it has recently been divided into two subgroups: chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP). CRS patients are both characterized by T-cell activation and impaired regulatory T-cell function; Reserch showed T-effector cells in samples from Asian demonstrated a TH1/TH17 polarization.
Method: Selected 56 cases of patients with chronic rhinosinusitis with nasal polyps (CRSwNP group) and 51 cases of patients with chronic rhinosinusitis without nasal polyps (CRSsNP group), 30 cases of healthy people (control group).All cases in CRSwNP group and CRSsNP group were evaluated by a visual analog scale (VAS) score of nasal synptoms. Collected peripheral blood and nasal secretions in all cases

and detected IL-17A, IFN-γ and IL-23 expression levels.
Results: There was no significant difference in VAS score of CRSwNP group and CRSsNP group ($P > 0.05$). IL-17A, IFN-γ and IL-23 levels of serum and nasal secretions in CRSwNP group and CRSsNP group were both higher than control group, it was with a highly significant difference ($P < 0.05$). It showed a clear correlation between expression of IL-17A and IL-23 both in serum and nasal secretions of CRSwNP ($P < 0.05$), whereas IFN-γcorrelated with CRSsNP ($P < 0.05$).
Conclusion: IL-17A and IL-23 may be important cytokines and IL17A/IL-23 pathway may paly a significant role in the pathogenesis of CRSwNP. IFN-γmay be actived to CRSsNP

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The anti-inflammation effect of alcohol extractive of the fruit of *physalis pubescens L.* in CRS

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Background: Alcohol extractive of the fruit of physalis pubescens L.(AEFPP) exhibited anti-tumor and anti-inflammation activities. However, the effects of AEFPP on CRS have not yet been fully elucidated. In this study, we evaluated the anti-inflammation effects of AEFPP on CRS rabbit model and we found that AEFPP is a drastically inhibitor to the bacterial strains collected from the CRS with functional endoscope sinus surgery.
Method: Sixty largeear white rabbit CRS models, half male and half female, 2.0–2.5 kg, were divided into three groups randomly. Group A was irrigated the rhinosinus with AEFPP liquid (15%),Group B was irrigated with normal saline and Group C was irrigated with clindamycin twice a day, 5 ml per time. Then we tested the nasal secretion about IL-6 level by ELISA before and after treatment. We also collected and cultured the bacterial strains of CRS before the functional endoscope sinus surgery to determine the minimum bacteriostatic concentration(MIC).
Results: After treatment, there was a significant difference between A and B ($P < 0.05$), but no significant difference between A and C ($P > 0.05$). We collected 16 specimens, 14(87.5%) showed positive results in bacteria culture. Stagphylococcus epidermidis were 5 specimens, stagphylococcus aureus were 8 specimens and efaecalis was 1 specimen. The MIC were 31–62.5 mg/ml, 62.5–125 mg/ml and 62.5–125 mg/ml respectively.
Conclusion: AEFPP exhibited anti-inflammation activity in CRS both *in vitro* and *in vivo*.

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The effect of hypoxia on nasal polyps and normal inferior turbinate derived nasal epithelial cells

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Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) was a disease with high incidence and recurrence rate, however its pathogenesis was unclear. Recently, the sinus and nasal middle meatus, where the most common part of the CRSwNP, was found in a hypoxic environment. Hypoxia may increase the release of multiple cytokines in other cell lines.
Method: HNPECs and HNECs were collected at the time of surgery from control patients ($n = 5$) and patients with CRSwNP ($n = 9$). Both HNPECs and HNECs were cultured under normoxic (21%) and hypoxic (1%) condition for 12 h, 24 h, 48 h, respectively. After the cell supernatants and lysates were collected, the concentration of IL-17A, IFN-γ, TSLP were measured using enzyme-linked immunosorbent assay and real time PCR.
Results: In control HNECs, hypoxia increased cytokine production ($P < 0.05$), measuring by TSLP and IL-17A, but expression of IFN-γ seemed the opposite. In CRSwNP patients, hypoxia decreased cytokine production ($P < 0.05$), measuring by TSLP, IL-17A and IFN-γ.
Conclusion: Human nasal polyps epithelial cells was inactive in response to Th17/Th1 polarized inflammation derived by hypoxia comparing with human nasal epithelial cells. Hypoxia may participate in the pathogenesis of CRSwNP by inhibiting the Th17/Th1 polarization T-effector cells.

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Immunopathologic features in antrochoanal polyps

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Background: Antrochoanal polyps (ACP) are benign lesions that arise from the mucosa of the maxillary sinus, grow into the maxillary sinus and reach the choana. Although great effort has been made during the last two decades on the molecular and cellular basis of bilateral polyposis (BNP), data on the distribution of inflammatory cells in antrochoanal polyps is lacking. This study aims to investigate the presence, distribution and degree of various inflammatory cells within antrochoanal polyps and compare to those in bilateral polyposis.
Method: Patients with ACP ($n = 33$) and BNP ($n = 50$) who underwent endoscopic nasal surgery for tumor resection were recruited. Inferior turbinate (IT) biopsies were obtained from subjects ($n = 50$) with symptomatic nasal septal deviation requiring septoplasty surgery and served as controls. Eosinophils were highlighted by H&E staining. Neutrophils, macrophages, mast cells, CD4+ T cells, CD8+ T cells and Foxp3+ T-Rreg cells were analyzed by Immunohistochemical staining. Sections were examined under microscopy to determine the percentages of different types of inflammatory cells.
Results: 88%(29/33) of patients with ACP displayed evidence of airway neutrophilia. The percentage of neutrophils was significantly increased in ACP tissues (median of 38.3% of all inflammatory cells) compared to BNPs (10.2%, $P < 0.001$) and controls (2.0%, $P < 0.001$). Although the number of eosinophils was increased in ACP samples (3.0%) compared to controls (0.34%, $P < 0.001$), it is significantly lower than those in BNPs (22.5%, $P < 0.001$). As compared to controls (6.6%), the percentage of macrophages was significantly increased in ACPs (13.8%, $P < 0.001$) and BNPs (11.5%, $P < 0.001$), but there is no difference between ACPs and BNPs ($P = 0.122$). The percentage of mast cells was decreased in ACP tissues (3.6%) compared to controls (7.0%, $P < 0.001$) and BNPs (5.5%, $P < 0.05$). CD4+T-cell and CD8+T-cell percentages were not significantly different in 3 groups. The number of Foxp3+ T-Rreg was increased in BNPs compared to ACPs and ITs.
Conclusion: Our data indicates a significantly upregulation of neutrophils in antrochoanal polyps. The different distributions of eosinophils, neutrophils, mast cells and Foxp3+ T-Rreg cells in antrochoanal polyps and bilateral polyposis suggest heterogeneity in their pathogenesis.

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Evaluation of the safety of antimicrobial photodynamic therapy (aPDT) for refractory chronic rhinosinusitis

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Introduction: Antimicrobial photodynamic therapy (aPDT) is proposed as a new treatment modality for the management of refractory chronic rhinosinusitis (CRS). Mechanistically, aPDT directly causes the eradication of bacteria in both planktonic and biofilm forms, as well as local mucosal immunomodulation. Operationally, a fiberoptic light diffusing catheter is introduced into a previously operated sinus cavity to activate an applied photosensitizing agent. Given the relationship of the paranasal sinuses to other critical structures and organs, a patient safety evaluation of the therapy was performed.
Methods: Specific safety evaluations were performed of patients undergoing aPDT therapy during a multicenter randomised clinical trial. These evaluations included pre and post treatment endoscopic visualization, CT imaging, ophthalmologic evaluation, olfactory testing using the University of Pennsylvania Smell Identification Test (UPSIT) and any reported adverse events recorded during the 24 h following aPDT therapy.
Results: Of the 44 trial patients, 31 were randomized to receive aPDT and a total of 43 treatments were delivered to 154 sinuses (52 frontal, 48 maxillary, 54 ethmoid). Thirteen patients underwent two treatments sessions. In no instances did any ocular dysfunction or visual loss occur. There was no trauma at the level of the surrounding sinus mucosa and in several patients there was resolution of disease. The most frequent post-treatment non-ocular symptom was transient mild pressure over the treated sinus(es), which rarely required analgesia.
Conclusions: This study demonstrates that there is a safe therapeutic window whereby antimicrobial Photodynamic Therapy (aPDT) of the paranasal sinuses can be safely performed in post endoscopic sinus surgery sinus cavities.

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Association of fungal hypersensitivity and their presence in the air with development of fungal rhinosinusitis

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Background: The relationship of the mycobiome of sinonasal mucosa, immune response on fungal presence in environment and development of FRS is not yet revealed. The aim of our study was to evaluate the relationship between the presence of fungi on sinonasal mucosa with their presence in the air of patient's homes and patient's clinical and allergological characteristics.
Method: The prospective study with 136 patients with chronic rhinosinustis/CRS was conducted at the Clinic for Allergology and Clinical Immunology, Clinical Centre of Serbia. Patients with molds mix specific IgE Ab classes 1–6 belonged to the group sIgE+, while patients with molds mix specific IgE Ab class 0 belonged to the group sIgE-. After mycological analyses, patients were divided in to patients with positive sIgE and positive fungal finding (AFRS group) and patients with negative sIgE and positive fungal finding (FRS group). Study design included:history data; measurements of molds specific IgE/sIgE and total IgE Ab, absolute eosinophile count and skin prick test; rhinologic and CT observation; mycological finding of sinonasal nasal aspirate and air sampling from the patient's bedroom.
Results:

1 Patients from sIgE+ group (30.4%) had more often repeated functional endoscopic surgery of sinuses ($P = 0.005$), presence of NP ($P = 0.025$) and more severe forms of CRS;
2 46.4% of patients from sIgE+ group had positive fungal finding on nasal mucosa and could be considered as AFRS;
3 prevalence of AFRS in the study was 1.3%, while prevalence of FRS was 2.8%;
4 patients with AFRS had statistically more frequent asthma ($P = 0.024$) and chronicity of CRS more than 10 years ($P = 0.000$)
5 out of all fungal isolates ($n = 225$) from air samples obtained from homes of patients with CRS, 41 fungi was isolated from home samples (HS) of AFRS patients, 24 from HS of FRS and 159 from HS of non-FRS patients with the most common species *Aspergillus niger* (57%) and *Penicillium* sp.(26%).

Conclusion: This is the first study which analyzes association of fungal presence in patients with FRS, with clinical and allergological findings, as well as with air sample from patients home. Huge amount of fungal spore in the air of patient's living area should be threat for development of FRS in predisposing patients, especially dose with prolonged CRS and recalcitrant NP. Next studies should clarify the mechanism by which airborne fungi turn from 'normal flora' into triggers of immunological reactions, resulting in FRS.

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Serum eosinophilia >0.3 × 10^9/l in CRS is associated with severe recurrent disease and has an influence on the dysbiotic sinus microbiome

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Background: Identification of serum biomarkers in chronic rhinosinusitis (CRS) would be of benefit in 'personalizing' therapy,yet this remains an elusive target. Serum eosinophilia >0.3 × 10^9/l has been used to predict response to to anti-IL5 therapy in asthma. We wished to explore whether serum eosinophilia >0.3 × 10^9/l could identify different endotypes of CRS and whether these had an impact on 1) evolution of CRS after surgery and ii) bacterial carriage in the sinus cavity.
Method: Previously phenotyped populations were assessed according to serum eosinophilia >0.3 × 10^9/l. Demographics were compared in two distinct populations previously phenotyped for genetic association studies:

1 204 subjects with refractory CRS and
2 500 subjects with CRS with nasal polyposis.

Impact on post operative evolution was assessed in 21 patients with CRS undergoing ESS and followed for four months. Impact on microbiome composition was assessed using 16 s bacterial sequencing in a second population of 20 patients undergoing ESS.
Results: In both populations of CRS patients previously phenotyped for genetic association studies, high eosinophil levels identify an increased frequency of asthma, allergy and ASA intolerance. Patients with pre-surgery serum eosinophilia ≥300 cells/mm^3 trended to more frequent recurrence of disease at four months (failure: 40.0% vs 18.2%, $P = 0.08$), and a higher culture rate for S Aureus. (68.0% vs 9.1%; $P = 0.018$) Microbiome profiling at time of

ESS showed a higher percentage of Gram-positive species, Firmicutes and Staphylococcus Aureus in the eosinophilia ≥300 cells/mm^3 group. (S Aureus: High Eos = 23.2%; Low Eos = 2.8%; $P = 0.033$).

Conclusion: When applied to CRS populations, serum eosinophilia $>0.3 \times 10^9$/l identifies a distinct population of CRS with potentially different underlying disease mechanisms. and predict i) increased likelihood of poor evolution following surgery, and ii) different contribution of S Aureus to the sinus microbiome. It is thus possible that this simple serum-based marker may predict patients with an increased benefit from anti-cytokine treatments targeting components of the Th2 system. This remains to be validated in prospective trials.

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The local immunity in the tissues of various forms of nasal polyps

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Background: Chronic polypoid rhinosinusitis is one of the most difficult forms of chronic rhinosinusitis, which has more relapses.

The aim of this study was to examine the state of white blood cells in the tissues of nasal polyps.

Method: Morphometry and immunohystochemistry was performed on paraffin-embedded surgical specimens, which remote during endoscopic sinus surgery in 45 patients who were hospitalized in the ENT department of 3-rd clinic of the Tashkent Medical Academy in 2013.

Results: Morphometry of postoperative materials showed that 33 preparations (73.3%) stated the prevalence of eosinophilic infiltration, 12 preparations (26.7%) noted the predominance of neutrophil infiltration. That's why patients were divided into two groups: with chronic 'eosinophilic' and 'neutrophil' polypoid rhinosinusitis. In immunohystochemical research we noted that there is a high expression of CD45 in mesenchymal clusters, which suggests that these clusters are a place of mesenchymal growth formations. This token is absent in the epithelium, but stroma also has a relatively high expression. Immunohistochemical study using CD45, showed good expression of the marker. It promotes the stimulation of T and B lymphocytes, stromal cells acting as the necrotizing factor. In 'neutrophil' polypoid rhinosinusitis, its expression was more pronounced. Markers CD45 accumulate around endothelia of vessels like ring, suggesting a poor prognosis of the disease.

Conclusion: The tissue of nasal polyps present rapid growth of white blood cells, which indicates that immunological changes that contribute to the development of relapses.

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The application of high definition and special imaging in endoscopic sinusitis surgery of chronic rhinosinusitis patients

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Background: Storz professional image enhancement system (Spies) has a powerful imaging function in nasal endoscopic sinusitis surgery. It can very useful in identification and diagnosis of nasal sinus mucous image characterisitics, especially in edentify the vessel and inflammation. Clinical histophologic characteristics may present the inflammation degree of Chronic rhinosinusitis (CRS). The change of blood vessels in nasal mucosa may reflect the degree of imflammation of the mucous membrane.

Method: From March 2015-June 2015, 53 cases of chronic rhinosinusitis patients were recruited with ESS surgery with STORZ Spies endoscoe in China- Japan Union Hospital of Jilin University. Of them, 13 patients were chronic rhinosinusitis without nasal polyps (CRSsNP), 40 patients were chronic rhinosinusitis with nasal polyps (CRSwNP). Collect the clinical imformation. All cases in CRSwNP group and CRSsNP group were evaluated by a visual analog scale (VAS) score of nasal synptoms. Reference to Lund-Kennedy endoscopic score, add special imaging (SPECTRA of A/B), according to the image of nasal mucous characters and blood vessal characters, we got the endoscopic score (0–20 points). From the CT examination we got the Lund-Machay Score (0–24 points). According HE staining and analyzing histopathology, including epithelial hyperplasia, epithelial squamous metaplasia, density of small blood vessels, goblet cell hyperplasia, collagen layer thickening, gland hyperplasia and eosinophil infiltration, we got pathology grade score (0–21 points). Analyz the correlation of endoscpic score with Lund-Machay score and pathologic grading scores.

Results: In gender, age and VAS score, it has no statistical difference sigificance between CRSsNP with CRSwNP ($P > 0.05$). In endoscopic score, Lund-Machay Score and pathology grade score, it has statistical difference sigificance between CRSsNP with CRSwNP ($P < 0.05$). In cell count, eosinophils and plasma cells were statistical difference significance between CRSsNP with CRSwNP ($P < 0.05$) whereas in lymphocyte, neutrophil and fibroblasts ($P > 0.05$). It was positively correlated between endoscopic score with Lund-Machay ($r = 0.675$, $P < 0.05$) and pathology grade score ($r = 0.897$, $P < 0.05$).

Conclusion: Storz professional image enhancement system (Spies) could improve the ability of observation of nasal endoscopic diagnosis. Through HD special endoscope imaging system could preliminary classified pathology diagnosis of chronic rhinosinusitis.

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Affection of pain level under nasal endoscopic examination: anesthetic, vasoconstrictor medicines ratio and personal history

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Background: Nasal endoscopic examination has attracted considerable attention as a regular intrusive inspection in Otolaryngology Department. Recently, several attempts have been developed to make the operating procedure more confortable. However the variable effects of the nasal spray of anesthetic and vasoconstrictor medicines with different ratios has been rarely reported. The Visual analogue scale (VAS) is also performed to evaluate their effects after spraying.

Method: One hundred and eight patients under nasal endoscopic examination were divided at random into 3 groups ($n = 36$ for each group) according to the different nasal spray ratio. The first group received 0.2 ml of 0.1% tetracaine hydrochloride and 0.2 ml of oxymetazoline spray (A group). The second group received 0.3 ml of 0.1% tetracaine hydrochloride spray and 0.1 ml of oxymetazoline spray (B group). The third group received 0.1 ml of 0.1% tetracaine hydrochloride spray and 0.3 ml of oxymetazoline spray (C group). The 4.0 mm diameter rigid Rigid Endoscope (Henke-sass wolf, Germany) was operated by two doctors, who have at least 2 years' experience in endoscopic examination. Visual analogue scale (VAS) (0–10) was used to evaluate the pain level of the patients. Lund-Kennedy endoscopic score was used in the trail. Personal history including sex, age, cigarate, alcohol abuse and drug-action time were collected. Double-blind design was used in the trial.

Results: The A group showed lower pain levels compared with B and C group. Moreover, higher pain level was observed in the non-smokers and male patients among the same group. In comparison, the smokers and female patients got lower ones. Furthermore, there was a positive correlation between the pain level and the Lund-Kennedy endoscopic score.
Conclusion: The pain level of nasal endoscopic examination is not only depended on the spray ratio of anesthetic to vasoconstrictor medicines, but also on the personal history. As observed, the optimum ratio of anesthetic and to vasoconstrictor medicines should be 1:1(0.2 ml: 0.2 ml), which make the procedure more comfortable. In addition, the personal history, such as smoke and age, should be collected before the endoscopic examination, because the non-smokers and male patients should be treated more gently during the endoscopic examinations.

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Pollen counts and its possible influence on the number of surgical nasal procedures performed

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Background: Hayfever is a common condition in the UK and presents with with symptoms of allergic rhinitis (AR). AR should be medically treated, but sometimes the condition may be masked by existing chronic rhinosinusitis (CRS), or AR may exacerbate the symptoms of existing CRS. The aim of this study was to assess a possible correlation between pollen counts and the number of surgeries on nose and sinuses.
Method: Grass pollen counts have been locally monitored since 2005 and all surgical procedures at the district general hospital were recorded with Medicode software since 2002. Annual peak pollen counts and number of days with high pollen counts (>50 grains/m^3) were compared with annual numbers of surgical procedures on turbinates and sinuses of the nose. Spearman's rank tests were used for statistical analysis.
Results: Findings indicated statistically significant correlation between peak grass pollen counts and number of sinus operations (Spearman's $r = 0.85$; $P < 0.004$) and also combined procedures on turbinates and sinuses (Spearman's $r = 0.84$; $P < 0.004$). Also a positive and significant correlation was seen between number of days with high pollen counts and turbinate procedures, sinus procedures and combined procedures on turbinates and sinuses (Spearman's $r = 0.80$, 0.78 and 0.93 resp.).
Conclusion: The results suggest a link between pollen level severity and the number of nasal surgeries performed during the corresponding year. This could possibly indicate that we may not have optimized the medical treatment on AR before surgery was considered.

Poster Discussion Session PDS 2

Effector cells

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IL-10 up-regulates IL-3-mediated production of Granzyme B in human basophils: enhancement of a potential anti-bacterial function?

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Background: Our previous work demonstrates that IL-3 induced de novo expression of the granule-associated serine protease Granzyme B (GzmB) in human basophils, which are known for their effector and immunoregulatory role in allergic inflammation. IgER-dependent and IgER-independent stimulation triggered exocytosis of GzmB but did not alter its level of formation or kinetic of release. This is in contrast to other mediators (e.g. LTC_4, histamine), which need IL-3 in combination with a second basophil trigger for optimal production and/or release. In this study we investigate whether and how other cytokines apart from IL-3 affect the level and kinetic of GzmB formation and release on their own and in combination with IL-3.
Method: To study the plasticity of GzmB production and release we cultured purified human basophils with different cytokines in the presence or absence of IL-3. Release of LTC_4, histamine and GzmB was triggered by C5a treatment while CD63 expression was induced by FcεRIα crosslinking. Cellular content of GzmB was quantified using flow cytometry and ELISA. In order to investigate underlying pathways we performed western blotting and PhosFlow. In search of a function of basophil-derived GzmB we incubated *E. coli* with supernatants of stimulated basophils.
Results: The present study demonstrates that among all tested cytokines IL-3 is unique in inducing GzmB production. Interestingly, although the class II cytokine family members IL-10, IFN-α and IFN-γ do not affect GzmB production on their own, they most strongly modulate the expression levels of IL-3-mediated GzmB expression. IL-10 selectively increases the amount of IL-3-mediated GzmB production, whereas IFNs decrease its expression. IL-10-dependent increase of GzmB expression is accompanied by a predominant phosphorylation of STAT3. Neither IL-10 nor IFNs do however affect the kinetic of GzmB formation, which peaks after 24 h. Furthermore, IL-10, IFN-α and IFN-γ do not alter the kinetics and levels of release of LTC_4, histamine and GzmB and expression of CD63 in IL-3-stimulated basophils. Preliminary experiments on the function of basophil-derived GzmB provide evidence that a basophil-derived mediator hinders growth of *E.coli*.
Conclusion: IL-10 and IFNs quantitatively increase and decrease, respectively the expression levels of IL-3-mediated GzmB, but do not affect its exocytosis. The elucidation of the function of basophil-derived GzmB needs further investigations.

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Number and affinity of IgE clones determines human mast cell activation

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Background: Allergen specific IgE consists of individual clones with unique affinity, concentration and complexity, comprising both the number of clones binding the same allergen (clonality) and their relative ratio. This study explores how these characteristics of the IgE repertoire direct mast cell activation.
Method: Human mast cells (MC) were generated from stem cells and sensitized with combinations of well-characterised recombinant human IgE (rhIgE) clones specific for *Dermatophagoides pteronyssinus 2* (Der p 2) or *Phleum Pratense 5* (Phl p 5). Activation of mast cells was measured as upregulation of CD63 by flow cytometry. Mast cell reactivity (fraction of mast cells activated, $\%CD63^+$ MC) and sensitivity (allergen concentration triggering a half-maximal response, EC_{50}) were estimated by non-parametric curve fitting. Statistical significance was analyzed using Kruskal-Wallis test.
Results: Increasing fraction of total rhIgE specific for Der p 2 significantly increased reactivity ($P = 0.0006$) and sensitivity ($P = 0.0008$). Optimising the ratio of Der p 2-specific rhIgE clones from 1:99 to 1:1 increased reactivity ($P = 0.038$) but not sensitivity ($P = 0.13$). Linear correlations with parallel slopes were obtained when plotting total concentration of Der p 2-specific rhIgE and net concentration of the rhIgE clone expressed in the smallest fraction, respectively, against mast cell reactivity. Increasing rhIgE affinity increased reactivity ($P = 0.0068$) and sensitivity ($P = 0.0005$). A linear correlation exists between product of affinities (K_D) and mast cell reactivity. A 10-fold decrease in product of affinites increased reactivity with 9.5%. Increasing Der p 2-specific rhIgE clonality increased reactivity ($P = 0.0039$) but not sensitivity ($P = 0.49$). Increasing clonality of rhIgE antibodies specific for Phl p 5 equally only increased mast cell reactivity ($P = 0.0286$) but not sensitivity ($P = 0.6571$).
Conclusion: Composition of allergen specific IgE directs the human mast cell response. The number of productive IgE pairs crosslinking the allergen is more important than the total amount of one clone. When increasing affinity of the IgE pairs both reactivity and sensitivity increase in proportion to product of the affinities, and additional IgE clones binding the same allergen increase reactivity independent of IgE specificity. Knowledge of IgE epitope number, specificity and affinity may contribute significantly when predicting the development of individual patient's allergy.

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Expression of inhibitory receptors on human basophils rapidly increases following anti-IgE activation but does not discriminate between non-allergic and peanut allergic subjects

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Background: Human basophils express a wide range of surface receptors allowing them to modify their cellular response. CD172a, CD200R and CD300a are described as inhibitory receptors and act by activating and recruiting phosphatases that counteract phosphorylation in the

intracellular signaling cascade. This study aimed at investigating the expression level of inhibitory receptors on resting and anti-IgE stimulated human basophils from non-allergic and peanut allergic subjects.

Method: Blood samples were drawn from non-allergic ($n = 6$) and peanut allergic ($n = 9$) subjects following informed consent. Whole blood was incubated with buffer or anti-IgE (1 μg/ml) for 30 min at 37°C and concurrently stained with fluorescently labeled antibodies targeting CD3, CD14, CD32, CD123, CD172a, CD193, CD200R, CD203c and CD300a. Erythrocytes were lysed and cells stained with fluorescently labeled anti-FcεRIα for 30 min at 4°C. Cells were fixed and acquired on a BD Fortessa flow cytometer. Human basophils were gated on singlets as $CD3^{-}CD14^{-}CD123^{+}CD193^{+}$ cells. Statistical analysis was performed using paired and unpaired t-test with Bonferroni correction for multiple comparisons in Prism 6 software. P-values≤0.05 were considered statistical significant.

Results: The mean net median fluorescence intensity (mean net MFI) of the inhibitory receptors CD172a, CD200R and CD300a were significantly augmented after anti-IgE stimulation for non-allergic subjects, 117 to 388, 2073 to 3608 and 603 to 969 as well as peanut allergic subjects, 107 to 306, 2288 to 3787 and 799 to 1223, respectively. No significant difference between surface expression of CD172a, CD200R and CD300a were detected on non-allergic vs peanut allergic individuals.

Conclusion: Expression of CD172a, CD200R and CD300a significantly increases on human basophils following anti-IgE activation, indicating that human basophils might rapidly become more sensitive to inhibitory factors after IgE-mediated stimulation. However the expression levels of CD172a, CD200R and CD300a on resting and stimulated basophils are comparable between non-allergic and peanut allergic subjects.

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Apolipoprotein A-IV negatively regulates eosinophil trafficking

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Background: Enhanced eosinophil migration from the blood into the tissue is a hallmark of allergic diseases such as bronchial asthma. Apolipoproteins are known to modulate normal lung development and homeostasis, as well as adaptive immune responses and host defense in the lungs. Several studies support the concept that apolipoprotein A-I (apoA-I) and apoA-I mimetic peptides have a protective effect in allergic asthma. Apolipoprotein A-IV (apoA-IV) is a 46-kDa glycoprotein that is produced mainly in the small intestine and liver. Although the precise function of apoA-IV has not been completely elucidated, several functions have been proposed, such as lipid transport and metabolism, control of food intake and antiatherogenic effects. In contrast to apoA-I, little is known about the anti-inflammatory properties of apoA-IV. Thus, we set out to characterize the effect of apoA-IV on human eosinophil trafficking.

Method: *In vitro* studies including adhesion and migratory responsiveness as well as Ca(2+) mobilization, were conducted in human peripheral blood eosinophils.

Results: Both apoA-I and apoA-IV attenuated the chemotaxis of human peripheral blood eosinophils and the upregulation of the adhesion molecule CD11b, whereby apoA-IV seems to be more potent. These effects were accompanied by the inhibition of cytoskeletal rearrangement and Ca(2+) mobilization. ApoA-IV-induced inhibition of eosinophil migration was PI3K-dependent whereas the effect of apoA-I was not altered by specific inhibitors of intracellular signaling pathways relevant to the chemotactic response.

Conclusion: These data show that ApoA-IV potently inhibits eosinophil trafficking and might hence be a useful therapeutic option in eosinophilic asthma and other eosinophil-driven diseases.

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D-type prostanoid receptor signaling promotes survival of eosinophils by inhibition of the intrinsic apoptosis pathway and activates related gene regulation elements

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Background: This study addresses the potential pro-survival effect of the Prostaglandin D_2 receptor DP on eosinophils. The specific role of DP in the functionality of eosinophils in the setting of allergic diseases remains unclear. Characterizing the detailed signaling and function of DP will help to understand the mechanism of enhanced survival and eosinophil persistence in tissues of atopic patients.

Method: Human peripheral blood eosinophils and HEK293 cells overexpressing DP and/or CRTH2 were used as a two-way approach optimized for functional assays with primary cells and a system to screen for signaling pathways. Survival of eosinophils and HEK293 cells was determined by AnnexinV/PI co-staining, MTS testing and JC1 staining. Proliferation of HEK293 cells was monitored constantly by Electrical Cell Substrate Sensing (ECIS). Serum response element induction was determined by reporter gene assays.

Results: The DP-mediated pro-survival effect of PGD_2 on human peripheral blood eosinophils (shown by AnnexinV/PI co-staining) was reflected by protection of the mitochondrial membrane potential ($\Delta\psi m$) and inhibition of Caspase 3/7. Further, PGD_2 enhanced the viability of serum-starved HEK293 cells overexpressing DP (HEK-DP and HEK-CRTH2+DP) but not HEK-CRTH2 cells. Besides viability enhancement, DP receptor expression increased the proliferation of HEK293 cells upon treatment with PGD_2. DP but not CRTH2 receptor stimulation induced SRE (serum response element) activation (luciferase assay), indicating the potential of DP to regulate eosinophil homeostasis by modulation of gene expression. BCL-XL, CCR3 and VLA-4 mRNA expression was induced by the DP agonist BW245c in human eosinophils, reflecting the potential of the DP receptor to regulate gene transcription and to activate anti-apoptotic pathways.

Conclusion: The DP receptor profoundly complements the immediate chemotactic stimulus of CRTH2 by, first, maximizing the response to PGD_2 and second, activating gene regulation which leads to inhibition of the intrinsic apoptosis pathway and hence promotes eosinophil survival. In this way the distinct effects of DP and CRTH2 complement each other and might contribute to the early and late phases of an allergic response.

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Direct infection and rhinovirus replication in human mast cells results in activation but not degranulation

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Background: Allergic rhinitis and asthma are both diseases characterised, in part, by increased numbers of mast cells in the respiratory mucosa and increased susceptibility to viral infection. Human rhinovirus (RV) is the most common microorganism

triggering acute exacerbations of asthma. Both frequency and severity of exacerbations are increased in patients with aeroallergen and/or food sensitisation; a factor also associated with increased RV specific wheeze. This indicates an interaction between HRV and mast cells which may accentuate susceptibility and symptomatology in asthma and rhinitis.

Method: The human mast cell line, LAD2, and respiratory epithelial cells lines, Calu-3 & Beas-2B were infected *in vitro* with RVA1B, RV14 & RV16. Peripheral blood derived primary human mast cells (hMC) were infected with RVA1B. Cells and supernatants were collected at intervals 2–72 h. Viral replication and cellular gene expression were assessed by real-time PCR. Cytokine release was determined by ELISA.

Results: An increase in viral copy number was detected in cell pellets and supernatants of LAD2 and hMC indicating viral replication in these cells and release of viral particles. Viral replication in culture corresponded with induction of pro-inflammatory and anti-viral genes similar to that seen in epithelial cells. Supernatants from RV infected epithelial cells had little effect on mast cells suggesting that mast cell activation was due to direct infection.

Conclusion: Human mast cells are susceptible to infection by different RVA1B, RV14 and RV16 which directly induce pro-inflammatory responses.

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ICOS-ligand expression on basophils membrane

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Background: ICOS is related to the CD28 superfamily and is crucial for the survival and function of T cells, Th cell differentiation and lung inflammatory responses. ICOS ligand (ICOS-L), one of the five B7 family members, is expressed on professional antigen-presenting cells as well as on several types of non immune cells. Binding ICOS to ICOS-ligand activates a cascade of intracellular signaling molecules that prevents T cell apoptosis and induces production of several patterns of cytokines depending on the microenvironmental cytokine milieu. Very recently, it has been demonstrated that innate lymphocytes type 2 (ILC2) express ICOS and ICOS-L and the ICOS:ICOS-L interaction is required for efficient ILC2 function and provides a survival signal for ILC2. Whether basophils, cells of innate immunity, express ICOS or ICOS-L is not known.

Method: The presence of ICOS and ICOS-L on basophils membrane was investigated by the Basophils Activation Test (BAT) in 5 subjects with allergic rhinitis and 6 healthy controls, before and after IgE, fMLP and ICOS stimulation.

Results: ICOS-L was equally expressed on basophils membrane of allergic and healthy subjects in baseline conditions (ICOS-L positive cells 4.75 ± 6.21 vs $6.74 \pm 5.9\%$, respectively), while ICOS was not expressed.

IgE and fMLP stimulations were able to increase ICOS-L expression, but not CD63 expression, on basophils of allergic and healthy subjects: from 4.75 ± 6.21 to $17.92 \pm 12.7\%$ ($P = 0.05$) and to 8.92 ± 7.27 ($P = 0.04$) following IgE and fMLP stimulation respectively in allergic patients and from 6.74 ± 5.9 to $48.76 \pm 44.16\%$ ($P = 0.03$) and to 17.2 ± 17 ($P = 0.05$) following IgE and fMLP stimulation respectively in healthy controls, with no significant differences between the two groups.

ICOS-L triggering with a Fab2-like soluble construct of ICOS obtained by fusing the extracellular portion of ICOS with the CH3 domain of IgG1 at different incubation times (20, 40 and 60 min) did not affect ICOS-L expression and did not induce CD63 up-regulation on basophils.

Conclusion: Basophils from healthy controls and allergic patients express ICOS-L on their membrane and the expression is upregulated by IgE stimulation. Binding ICOS to ICOS-L of basophils membrane does not induce basophils activation. Whether binding ICOS to ICOS -L of basophils membrane promotes cytokine production (i.e. IL-4) from basophils needs to be investigated.

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IL-17A induces FGF-2 and VEGF secretion in cultured human mast cells

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Background: Mast cells (MCs) are important immune cells implicated to several respiratory diseases such as chronic obstructive pulmonary disease (COPD). Alarmingly, the WHO has predicted that COPD will become the third leading cause of death worldwide by 2030. Therefore, novel therapeutic agents are needed to prevent the severity of COPD. Patients with advanced COPD exhibit an accumulation of MCs in the peripheral lung, though; the functional role of MCs in COPD is unknown. The proinflammatory cytokine interleukin-17A (IL-17A) plays a key role in COPD pathogenesis and disease progression, yet the response of MCs to the inflammatory milieu involving increased IL-17 remains undetermined. Thus, we characterized the expression of IL-17 Receptor (R)A and IL-17RC on MCs in COPD, and investigated the response of MCs to stimulation by IL-17A.

Aim: To investigate the effect of IL-17A/F on the MC secretion of mediators associated with COPD.

Method: IL-17RA/F expression was confirmed by qPCR in PBMCs and by immunohistochemistry of normal MCs in human lung tissue. Human peripheral blood derived mast cells (PBMCs) were cultured for 8 weeks and then stimulated for 8 h with IL-17A and/or IL-17F. The secretion of 41 mediators was analyzed by multiplex analysis.

Results: Both PBMCs and normal MCs in human lung tissue express IL-17RA and IL-17RC. None of the chemokines (IL-8) and cytokines (IL-1α, IFNγ, G-SCF) associated with COPD were induced by IL-17A and/or IL-17F stimulation in cultured PBMCs. IL-17A significantly induced the secretion of both FGF-2 and VEGF in human PBMC lines ($n = 5$).

Conclusion: Our data suggest that MCs secrete FGF-2 and VEGF in response to IL-17A stimulation. As these growth factors promote vascular remodeling, our data indicate a novel role of MCs in COPD. Therefore, modulation of MC mediator release in the peripheral lung can be a novel target to control the severity of COPD.

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Reference range of peripheral blood eosinophils at 12 months of age

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Background: Eosinophil counts in healthy adults range between 0 and 500/l. In the current pediatric literature the accepted eosinophil reference range for children is between 50–250/l or 1–3% of the total

leukocyte count. Values for infants have not been reported. Most infants in Israel undergo a blood count at age 10–14 months to screen for iron deficiency anemia. Incidental eosinophilia classified as an absolute eosinophil count of >500/l is frequently encountered.

Method: A retrospective analysis of the Maccabi Health Care Services′ database was carried out. Blood count results of infants aged 10–14 months born from 2007 to 2014 were analyzed.

Results: Of 279 025 blood count results fulfilled the inclusion criteria and were included in the analysis. The mean absolute eosinophil count was 277/L. The 95% percentile was 800/L. The relative eosinophil values ranged between 0 and 41% of total leukocyte count with a 95% of 6% of total WBC.

Conclusion: The range of eosinophil absolute counts in one year old infants is similar to the range in adults. Further work is in process to determine if incidental eosinophilia at 1 year is correlated with atopy.

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Basophil reactivity and sensitivity are not affected by diurnal variation and may therefore be stable metrics of the allergen response

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Background: Manifestation of allergic symptoms appear to show diurnal variation, with exacerbation in the night and early morning. These findings are based on clinical parameters like peak expiatory flow (PEF) and questionnaires. We would like to explore whether diurnal variation is based on cellular mechanisms in allergic effector cells. The Basophil Activation Test (BAT) is a very useful tool to investigate cellular differences in basophil reactivity and sensitivity when stimulated with allergen.

A study with allergic subjects sensitised to cedar pollen suggest that there is diurnal variation in the activation marker CD203c, where basophil reactivity was increased at 07H compared with 19H in allergic subjects. In a pilot study using BAT we found that non-allergic subjects had no difference in the basophil sensitivity when assessing MFI of CD63+ basophils.

Aim: Further investigation of diurnal variation in allergic subjects at six time points.

Method: To study diurnal variation in basophils six blood samples were drawn outside the pollen season in the late autumn of 2015 from 10 allergic subjects. The samples were collected at 13H, 16H, 19H and 01H, 04H and 07H. After each sample a BAT was performed with CD63, CD203c and relevant seasonal allergen to determine whether any diurnal fluctuations are present in basophil reactivity and sensitivity. PEF was measured before each blood sample was taken to confirm diurnal variation in lung function. Each participant answered a standardised questionnaire including reports of nightly exacerbation.

Results: There is no significant variation when 1wayANOVA is performed on reactivity and sensitivity of BAT with CD63 or with CD203c ($P > 0.05$). PEF results showed a significant variation between 07H/19H ($P = 0.0234$), but not at other time points and 7/10 participants reported exacerbations at night.

Conclusion: PEF measurements showed an expected, significant diurnal variation. There were no diurnal changes in basophil reactivity and sensitivity when assessing % CD63+ basophils or %CD203c+ basophils. The diurnal variation in lung function is not reflected at the level of the basophil granulocyte, and may reside in tissue response instead. Basophil activation is a reproducible method not affected by diurnal variation.

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Pathological proliferation of mast cells resulting from either an extracellular domain mutation or stem cell factor autocrine/paracrine

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Background: Abnormal proliferation of mast cells involves in serious diseases including mastocytosis, mast cell tumors, and allergic inflammation. It is well known that gain of function mutations in the juxtamembrane or tyrosine kinase domain of KIT have been reported in neoplastic mast cells derived from 10–30% of human cutaneous mastocytosis, as well as 20% of mast cell tumors in dogs. However, the extracellular domain mutations of KIT as well as KIT mutation-independent mechanism in tumorigenesis have been found recently. In this study, we examined the impact of an extracellular domain mutation of KIT on mast cell tumorigenesis and attempted to find out KIT-independent mechanisms that induce tumorigenic proliferation of mast cells.

Method: For the analysis of the mutation in the extracellular domain, a dye-exclusion test and western blot was conducted using IC-2^{N508I} cells, which express KIT with an extracellular domain mutation (N508I) derived from a canine mast cell tumor. For the analysis of the KIT mutation-independent mechanism, a wild-type KIT-expressing canine mast cell line (HRMC cells) was used. Stem cell factor (SCF) expression was detected by both RT-PCR and flow cytometry, and dye-exclusion test was carried out to evaluate the effect of RNAi as well as neutralizing antibody against SCF.

Results: IC-2^{N508I} cells proliferated in a cytokine-independent manner, and the mutant KIT was aberrantly phosphorylated due to ligand-independent dimerization. SCF was highly expressed in the HRMC cells, resulting in the spontaneous phosphorylation of KIT. Because neutralization of SCF as well as SCF gene silencing inhibited the growth of the cells, it is suggested that SCF autocrine/paracrine contributed the cell proliferation. Production of SCF was also observed in several mast cell lines originated from humans and rodents, which was enhanced after PMA/ionomycin stimulation.

Conclusion: These results indicate that both the extracellular domain mutation and SCF autocrine/paracrine contribute to the neoplastic proliferation of mast cells. It suggests that these KIT activation mechanisms may become an effective strategy for the treatment of mast cell malignancies.

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Src-homology 2 domain-containing tyrosine phosphatase (SHP-2) controls aryl hydrocarbon receptor-mediated mitochondrial and ER stress response in mast cells

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Background: Exposure of mast cells to AhR ligands resulted in activation of mast cells *in vitro* and *in vivo*. However, the underlying mechanisms remain to be fully elucidated.

We tested a hypothesis that SHP-2 may integrate AhR-mediated Ca^{2+} and ROS signals and control mast cell's functions.

Method: Mitochondrial membrane potential was measured with MitoHealth staining, and ROS production was determined with mitoSOX. Mitochondrial Ca^{2+} was measured with genetically encoding probe and cytosolic Ca^{2+} with Fluo-4/fura-red staining. ER stress response, including eIF2α phosphorylation, was assessed with Western blotting analysis. Physical

interaction between adenylate kinase 2 (AK2) and SHP-2 was examined by co-immunoprecipitation and Western blotting analysis.

Results: We found that an AhR ligand, FICZ, induced a transient increase in mitochondrial SHP-2 activity and significant functional alteration in mitochondria, including decreased ATP synthesis, enhanced membrane potential loss and ROS generation, concomitant with a reduction of intracellular GSH. Significantly, we showed that in FICZ-treated mast cells, SHP-2 promoted, in a ROS-dependent manner, mitochondrial Ca^{2+} uptake through Ca^{2+} mobilization from the ER. This resulted in ER stress response involving primarily the PERK signaling pathway, ATF4 activation and eIF2α phosphorylation, which could be reversed by the addition of an anti-oxidant, NAC, and was inhibited in cells with SHP-2 knockdown. Moreover, SHP-2 was found to target AK2 in mitochondria and regulate AK2-dependent Ca^{2+} entry into mitochondria.

Conclusion: Our findings suggested that SHP-2 is critical in controlling ER and mitochondrial stress signals in response to AhR activation, providing a new regulatory pathway in mast cells.

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Btk targeting drugs and their effects on high-affinity IgE receptor-mediated signal transduction and activation of mast cells and basophils

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Background: Mast cells (MC) and basophils (BA) are key effector players in allergic inflammation. Both types of cells express high-affinity receptors for IgE (IgERI). Activation of MC and BA through IgERI is associated with activation of downstream signalling pathways and enhanced expression of cell-surface antigens such as CD63 or CD203c. Recently, the Bruton′s tyrosine kinase (BTK) has been identified as a new potential downstream-target in IgERI -cross-linked MC and BA. The aim of this study was to explore the effects of various BTK blockers on IgE-mediated histamine release, phosphorylation of IgERI downstream signalling targets and upregulation of cell-surface antigens.

Method: We examined human blood BA from 3 healthy donors and 8 patients allergic to Bet v 1, Der p 2, and/or Phl p 5 as well as the human mast cell line HMC-1 by flow cytometry. Furthermore, histamine release experiments were performed with BA and $ROSA^{KIT\ WT}$ cells.

Results: We found that the BTK blocker Ibrutinib counteracts anti-IgE-induced and allergen-induced upregulation of CD63 and CD203c ($IC50 < 0.5\ \mu M$) and histamine release ($IC50 < 0.025\ \mu M$) in human BA. The other two BTK inhibitors tested, AVL-292 and CNX-774, were also found to suppress IgE-mediated histamine release ($IC50 < 0.05\ \mu M$). Moreover, as determined by flow cytometry, all BTK blockers tested were found to inhibit phosphorylation of BTK in HMC-1 cells as well as in IgERI-cross-linked BA.

Conclusion: All in all, our data show that Ibrutinib and other BTK inhibitors suppress anti-IgE-induced upregulation of CD63 and CD203c as well as IgE-mediated histamine release in BA at reasonable drug concentrations. These results confirm the important role of BTK as one of the major players in IgERI mediated activation.

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Poster Discussion Session PDS 3

Autoimmune Disorders

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E-type prostanoid receptor 4 (EP4) agonist treatment has dose-dependent beneficial and harmful effects in nephrotoxic serum nephritis

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The lipid molecule prostaglandin E2 (PGE2) acts on four different prostaglandin E (EP) 1–4 receptors. The EP4 receptor is expressed on immune cells, resident kidney cells and endothelial cells, which are key players in the nephrotoxic serum nephritis (NTS) model, a murine model of rapid progressive glomerulonephritis (RPGN).

NTS was induced in C57BL/6 mice. *In vivo* treatment with two different doses of an EP4 receptor agonist L-902688 [1000 μg/kg and 280 μg/KG BW] or vehicle was performed for 14 days. Furthermore, murine tubular epithelial cells were treated with an EP4 receptor agonist ONO AE1-329 [1000 nM–30 nM] *in vitro*.

In vivo, high dosage EP4 receptor agonism led to significantly increased acute tubular injury, as depicted by increased tubular casts, possibly due to recurrent hypotensive episodes immediately after injection of the agonist. In contrast, low dose EP4 receptor agonist treatment improved the kidney phenotype after NTS. Decreased numbers of CD4+ and CD8+ T cells infiltrated the kidneys of both EP4 receptor low- and high-dose agonist treated mice on day 14. Furthermore, agonist treated mice displayed significantly increased numbers of proliferating tubular epithelial cells. *In vitro*, EP4 receptor agonist treatment augmented the survival and proliferation of distal convoluted tubular cells deprived of serum compared to vehicle.

In summary, treatment with high-doses of the EP4 receptor agonist leads to acute kidney injury because of hemodynamic effects of the drug. In contrast, low-dose EP4 receptor agonism improves the NTS phenotype mainly because of increase tubular proliferation, but also due to anti-inflammatory effects.

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IgE autoreactivity in bullous pemphigoid

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Bullous pemphigoid (BP) is an autoimmune disease typically associated with old age. It is characterized by bullae at the dermal-epidermal junction (DEJ) that are thought to be induced by the binding of auto-antibodies. These antibodies can recruit inflammatory cells through complement activation, culminating in the proteolytic destruction of cell adhesion structures. While IgG has been the class consistently associated with the disease, more recent studies point to a potential involvement of IgE. In line with previous literature, we have detected significantly higher levels of NC16a- and BP230-specific IgE in the sera of BP patients comparing with healthy controls, via ELISA. Consistently, using whole skin lysates for immunoblotting, we have also demonstrated peripheral BP IgE reactivity against antigens with approximately 60, 120, 180 and 230 kD. These likely represent intra- and extra-cellular domains of BP180 and the full-length BP180 and BP230 proteins, respectively. Furthermore, we have found IgE in perilesional skin of 21 out of 32 (66%) BP patients. This IgE was not found at the DEJ, but instead on the surface of mast cells and eosinophils, most likely bound as an immune complex. We have evidence that the high-affinity receptor for IgE is the primary molecule involved in this interaction and that eosinophils are expressing FcεRI in BP patients. Given that the clinical picture of BP consists of erythema and bullae, appearing alone or concomitantly, an association between self-reactive IgE and urticarial-like lesions is therefore plausible and suggests an alternative pathway of disease pathogenesis. Uncovering the dominant epitopes for both IgG and IgE in different presentations of the disease could further clarify this question and additionally argue for the development of new IgE-based therapeutic approaches.

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Modulation of different peripheral B cell sub-populations in rheumatoid arthritis patients during IL-6R inhibition

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Background: Enhanced B cell activity has been proposed in the pathogenesis of rheumatoid arthritis. With the advent of B cell targeted therapies the modulation of memory B cells seems to be a prime target. Human peripheral memory B cells can be distinguished by the phenotypic expression of CD27 and IgD defining three major B cell subpopulations: CD27+IgD+ pre-switch, CD27+IgD- post-switch and CD27-IgD- double negative memory B cells. We analyzed these different memory populations in RA and under IL6R blockade.
Method: B cells were phenotypically analyzed from RA patients at baseline, week 12 and week 24 under tocilizumab (TCZ) treatment. Memory B cell subsets were defined by CD27 and isotype surface expression. Mutational frequencies were analyzed by single B cell RT-PCR and were further analyzed by 8 color flow cytometry. B cell activation was identified by surface staining with CD95 and intracellular ki-67 staining. Mann Whitney U test was used for statistical analysis by using GraphPad Prism 5.
Results: The Ig receptor mutational frequency was highest in class switched memory with 6.2±0.3% compared to double negative (3.9±0.2%) and pre-switch memory (4.2±0.2%). The phenotypically analyzed isotype profile in RA patients ($n = 80$) and healthy donors ($n = 40$) revealed that the memory B cell pool was a heterogeneous mixture of IgA, IgG and IgM expressing cells. The double negative B cell memory population showed a clear dominance of IgG followed by IgA and IgM (~70%, 20% and 10% respectively), whereas CD27+IgD- B cell had an equal distribution of IgA and IgG. Under IL-6R

inhibition by TCZ, the isotypic profile remained stable at week 12 and 24. Surface and intracellular staining of B cells showed a significantly higher percentage of CD95 ($P = 0.01$) and Ki-67 ($P = 0.03$) expression in RA, which was highest in post-switched memory B cells.

Conclusion: Peripheral memory B cell populations are activated in RA with enhanced CD95 and Ki-67 expression compared to healthy individuals and which can be reduced by IL-6R inhibition *in vivo*. The double negative B cell pool displays a significantly higher proportion of IgG bearing cells compared to post-switch B cells which is not changed by IL-6R inhibition.

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Dendritic nanoarchitectures for the treatment of psoriasis

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Background: Psoriasis is a multigenic, cutaneous inflammatory disorder, involving a variety of pathological changes in skin. The study aimed to evaluate the potential of dendrimers for safe and efficient topical delivery of antipsoriatic agent Dithranol, one of the most promising anti-psoriatic agents via topical route whose application is inconvenient and troublesome.

Method: The Polypropylene Imine dendrimers (PPID) were synthesized by divergent synthesis method. The ethylene diamine was used as core material and acrylonitrile to form branching units. Dendrimers were then characterized by IR & NMR spectroscopy and transmission electron microscopy. Dithranol loaded PPID were evaluated for *in-vitro* drug release, haemolytic toxicity, skin irritation, tape stripping studies and drug penetration studies.

Results: Loading of Dithranol was found to be pH dependent. PPID showed significantly enhanced permeation rate constant and lesser skin irritation when compared with the plain drug solution. Skin separation studies and confocal laser scanning microscope images showed that the dye-loaded dendrimers exhibits deposition of dye in pilosebaceous compartment. The entrapment of drug in dendritic system reduced skin irritation, haemolytic toxicity, enhanced permeation rate constant and penetration and accumulation in the skin. The Dithranol loaded dendrimers extended drug retention time in the skin *vis-a-vis* reducing the associated disadvantages and improving the topical bioavailability of the molecules in a controlled pattern.

Conclusion: The enhanced accumulation of drug via dendrimer carrier within the skin might help optimize targeting of this drug to the epidermal and dermal sites, thus creating new opportunities for well-controlled, modern topical application of Dithranol for the treatment of psoriasis.

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Folate conjugated nanoparticulate drug delivery system for the effective management of rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disease of unknown etiology, characterized by synovial inflammation, progressive destruction of cartilage and bone resulting in gradual immobility. The aim of the study was to develop anti-arthritic drug loaded albumin nanoparticulate system having drug targeting potential for the management of rheumatoid arthritis.

Method: Thus, in the present investigation, it was proposed to prepare etoricoxib loaded bovine serum albumin (BSA) nanoparticles and compare the biodistribution of nanoparticles with that of plain etoricoxib after intravenous administration in arthritic rats. The nanoparticles were made by desolvation method and activated folic acid was conjugated. The in-vitro characterization parameters included ftir analysis, transmission electron microscopy (TEM), particle size, zeta potential and stability studies. The in-vivo studies included biodistribution of drugs in various organs, pharmacodynamic study by carrageenan induced paw edema method.

Results: Optimized nanoparticles were spherical shape as shown by TEM images. Reduction in the amount of drug present in blood indicated the sustained release behavior of nanoparticulate formulations. Folic acid conjugation retards drug release resulting in slower drug release as compared to plain nanoparticles. Significantly higher % inhibition of edema was observed. Higher concentration of drug in inflammatory knee joint was found in case of f- etx-nps as compare to the free drug.

Conclusion: Thus, development of folate-targeted therapeutic agents for guided intervention into arthritis enhance its site specific drug delivery at inflamed joints in RA and can be used as sustained drug delivery system in rheumatoid arthritis.

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Solid lipid nanoparticles for effective management of psoriasis

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Background: Psoriasis is characterized as chronic, recurring, genetically determined, immune-mediated inflammatory skin disease characterised by scaly patches due to excessive skin production. Skin rapidly accumulates at the site of onset and takes a silvery white appearance which erodes with excessive itching. To design and develop Novel particulate Carrier loaded with Antipsoriatic drug (Dithranol) for safe, efficient and constant delivery for radical cure of psoriatic plague. Solid lipid nanoparticles(SLN) have emerged as an alternative to liposomes due to various advantages such as improved physical stability, low cost compared to phospholipids and ease of scale-up and manufacturing.

Method: Solid lipid nanoparticles are special lipid aggregates that can penetrate efficiently and retain in to the skin. The SLN were prepared by solvent injection method. Drug loaded SLN's were characterized *in-vitro* for their shape, size, percent antigen entrapment and stability. The mean particle size was determined by photon correlation spectroscopy(PCS) using a Malvern Zetasizer. Scanning electron microscopy & Transmission electron microscopy was performed.

Results: The amount of entrapped antigen was determined after removal of unentrapped antigen. Recovered fractions were challenged with tritonx-100 (0.2%, v/v) and amount of drug was determined using BCA method. *In-vivo* studies constituted of Quantitative estimation of drugs in different skin layers by Tape stripping method, Skin irritation studies by Draize patch test & Fluorescence Microscopy was carried out to confirm the uptake of SLN's. SLN's formed were multilamellar and were found to be stable in gastric and intestinal fluids.

Conclusion: Fluorescence microscopy suggested that SLN's were taken up by gut associated lymphoid tissues. Encapsulation of dithranol in SLN resulted in dramatic improvement in its stability. SLN based gel resulted in remarkably less erythematic episodes as compared to plain drug based gel. Enhanced accumulation of dithranol via SLN within the skin might help to optimize targeting of this drug to the epidermal and dermal sites.

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Interaction between human dental pulp-derived mesenchymal stem cells (hDP-MSCs) and CD4+ T lymphocyte subsets

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Background: Our aim is to investigate the interaction between human dental pulp-derived mesenchymal stem cells (hDP-MSCs) and CD4+T-lymphocyte subsets, which are effector cells of adaptive immune system.
Method: Dental and peripheral blood samples were taken from volunteers (n = 5 and 18–24 year old) for the isolation of Mesenchymal stem cells (MSCs) and CD4 + T lymphocytes. Isolated MSC from the dental pulp analysed by the flow-cytometry. These cells were positive for markers of CD13, CD44, CD90, CD166, CD73, CD29, CD105 and negative for markers of CD34, CD117, CD14, CD15, CD5, HLA-DR, CD106, CD45, CD19. In addition, these cells were differentiated into osteogenic, adipogenic and chondrogenic line. Isolation of peripheral blood CD4 + T Lymphocyte was made with Immuno-selection (negative selection), used density gradient separation technique. MSCs and CD4 + T lymphocytes were co-cultured for 4 days in the indirect co-culture system. WST-1 assay was performed to demonstrate the suppression of T-cell proliferation. We also performed IFN-g, IL-4 and IL-17a ELISA assays and CD4, CD25, Tbet, Gata3, and Stat3 flow cytometry assays which are specific markers for CD4 T cell subsets.
Results: The T cell proliferation was significantly suppressed by the hDP-MSCs at 4th day of indirect co-culture (P = 0.0201). IFG-g, IL-4 and IL-17 levels were detected in a statistically significant decrease ($P < 0.0001$, $P < 0.0001$ and $P = 0.0025$) in co-culture group compared to the control group but IL-10 ($P < 0.0001$). Tbet+, Gata3+ T cells significantly suppressed and Stat3+ and FoxP3+ T cells significantly increased in co-culture group compared to the control group.
Conclusion: Our study suggested us, hDP-MSCs showed similar immunomodulatory activity to cells derived from other sources. We believe that, the hDP-MSCs could use in autoimmune and chronic inflammatory diseases as an alternative to other sources derived MSCs. Therefore, in the future, waste dental tissues especially the exfoliated deciduous teeth will be an important source for cell and tissue banking.

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Leptin effect on cytokine gene expression in childhood idiopathic thrombocytopenic purpura (ITP): an anti-inflammatory agent?

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Background: Leptin is a hormone secreted mainly by adipocytes that participates in the regulation of food intake and energy homeostasis, reproduction and other peripheral functions. Several studies have also indicated that leptin plays a significant role in the pathogenesis of autoimmune diseases.
Methods: To investigate the effect of leptin in childhood ITP, an autoimmune disorder with clear Th1 polarization, we measured serum leptin levels in 23 children with acute ITP, before and after treatment, and in remission, and in 23 healthy age- and body mass index (BMI)-matched controls. We also cultured ITP and control peripheral blood mononuclear cells (PBMC) ± mitogens ± recombinant leptin (rleptin) in different doses, to assess leptin's direct effect on pro- and anti-inflammatory cytokine gene expression.
Results: A 6-fold increase in plasma leptin was observed in children with active disease compared to controls. Intravenous immunoglobulin (IVIg) treatment resulted in a slight decrease in plasma leptin, while steroid treatment reduced leptin to below control levels. In remission, leptin levels were within control range. Leptin levels were negatively correlated with platelet count and plasma TGF-β1 levels. On the contrary, leptin levels followed the patterns of IL-2, IFN-γ, IL-4 and IL-10 gene expression. Addition of rleptin to PBMC cultures resulted mainly in a dose-dependent enhancement of IL-10 gene expression. Further experiments with purified T-cells (CD3) and monocytes (CD14) identified monocytes as the exclusive source of leptin-induced IL-10.
Conclusion: We suggest that in childhood ITP leptin acts as an active anti-inflammatory agent by promoting IL-10 secretion by monocytes.

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Topical delivery of aceclofenac by novel nanostructured carrier

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Background: Rheumatism is any painful disorder of the supporting body bone, ligaments, tendons or muscles i.e. not caused by infections or injury.The primary aim of treatment of rheumatic disease is reduction of pain and inflammation, maintenance of joint mobility and prevention of deformity. The aim of the study was to develop and characterize Aceclofenac loaded solid lipid nanoparticles (SLN) and nanostructure lipid carriers (NLC)to study the skin permeation profile and deposition kinetics.
Method: The SLN/NLC were prepared using glyceryl monostearate(GMS)by ultrasonication method and optimized. The *in-vitro* characterization parameters included Optical microscopy, Particle size, shape, polydispersity, Zeta potential and surface morphology, Differential scanning calorimetry, X-ray diffraction and Entrapment efficiency. The in-vivo studies included carrageenan induced paw edema method and CLSM studies.
Results: Compared to SLN, NLC showed improved drug loading capacity and a goodability to reduce the drug expulsion during storage.The data indicates prolonged and higher magnitude of edema inhibition exerted by SLN than NLC formulations. GMS based SLN or NLC hydrogel can be proposed for topical use for providing sustained effect to inflammatory disease like arthritis and ankylosing spondylits.
Conclusion: Thus, SLN or NLC based hydrogel of anti-inflammatory agent could be designed for treatment of rheumatic diseases. Permeation studies through human cadaver skin and clinical implication of data are required to develop topical drug delivery system. Nevertheless, further specialized studies are required to confirm the present hypothesis and to better investigate the role of nanoparticulate carriers for controlling and sustaining the release of the drug.

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Serum IL-18 as biomarker in predicting pediatric-onset systemic lupus erythematosus Nephritis treatment response

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Background: Pediatric-onset systemic lupus erythematosus (pSLE) has a high

prevalence of lupus nephritis (LN). Interleukin-18 has been shown to associate SLE disease activity and LN status in patient serum and urine. To pinpoint its act of causation and for clinical applications, a thorough investigation of IL-18 in pSLE patients was performed.

Method: Clinical data and laboratory workups from 40 pSLE patients were collected at time of disease onset, when disease activity changed, and 6 month after treatment. Serum IL-18 was determined by sandwich ELISA and urine IL-18 was measured by Luminex's XMAP Technology based multi-analyte suspension array. Renal biopsy was performed on patients with evidence of renal involvement.

Results: Average age of all cases was 12.80 ± 4.61 years old and 32 of them underwent renal biopsy. Serum IL-18 associated SLEDAI ($P = 0.0001$), and was significantly higher during active LN, especially at time of acute flares ($P = 0.012$; $P = 0.0005$). Urinary IL-18 has no correlation with disease activity but renal histopathological presentations ($P = 0.05$). IL-18 level was significantly higher in cases with CNS lupus, dermatologic manifestations and vasculitis ($P = 0.001$, 0.013 and 0.002). Interestingly, cases with high IL-18 responded better under current LN treatment recommendation

($P = 0.019$) and IL-18 level genuinely reflected treatment response.

Conclusion: Though urinary IL-18 was not associated with SLE disease activity, serum IL-18 as biomarker in pSLE patient representing global disease activity and renal flares was assured. IL-18 level may be used to guide LN treatment for its level represented the likelihood of responses to current LN guideline and reflected treatment responses.

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Kikuchi-Fujimoto disease

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Background: Kikuchi-Fujimoto disease or hystiocytic necrotizing lyhmphadenitis is a rare, benign and self-limiting condition. It is mainly described in Eastern countries and it usually affects young women. The most common presentation is cervical lymphadenopathy with sistemic aspecific manifestations like fever, asthenia, weight loss, arthromyalgia.

Case presentation: A 24 years old male presented to our Internal Medicine Department referring a one month history of high fever, chest pain, dyspnea and polyarthralgia. Distended jugular veins, malar rash, non painful axillary, inguinal and retronucal lymphadenopathy and moderate pleural and perdicardial effusion were detected on physical exam. Laboratory investigations revealed anemia, leucopenia, high levels of inflammatory indexes. Further examinations were performed to verify a possible viral, haematological, autoimmune, neoplastic, parasitologic or bacteriological involvement. Lymphocyte subpopulations showed low $CD4^+$ lymphocyte cells; viral markers (*HIV, CMV, EBV, HBV, HCV*) were negative; *ANA, ENA, nDNA, C3,* C4 autoimmune pattern were found at high levels (*ANA* 1:1280; *ENA Sm* 281.2 EU/ml and *ENA* Sm-*RNP* 110.2 EU/ml; *nDNA* negative; C3 50 mg/dl, C4 13 mg/dl); neoplasm indexes were negative; parasitological and bacteriological values (*Toxoplasma, Leishmania, Leptospira, M.peumoniae, C. pneumoniae)* were negative.Total body computed tomography confirmed a widespread lymphadenopathy involving cervical, supraclavicolar, axillary, paraortic, inguinal regions. Finally the patient underwent to lymphnode biopsy. Histological exam demonstrated a picture of necrotizing lymphadenitis without neutrophilic granulocytic infiltration and massive necrosis. The patient confirmed to have Kikuchi-Fujimoto disease. Clinical aspects and laboratory investigations also suggested a diagnosis of Systemic Lupus Erythematous. For this reason steroid systemic therapy was administrated, until clinical remission.

Conclusion: We affirm how Kikuchi-Fujimoto disease and Systemic Lupus erythematous are linked as seen in previous studies. Still today it is unknown if these two disease are two different aspect of the same illness (Kikuchi-Fujimoto as a localized manifestation of Systemic lupus erythematous or Kikuchi- Fujimoto as a early onset of Systemic lupus erythematous), or two different pathologies with common etiopathogenesis. Further studies are required to explain the pathophysiological connection between these two diseases.

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Arthus reaction in a psoriatic patient treated with adalimumab

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Background: Arthus reaction is a type III hypersensitivity reaction which involves formation of antigen-antibody complexes after intradermal injection of an antigen into a previously immunized patient. The deposition of the antigen-antibody complexes in the blood vessels leads to acute inflammation and sometimes even necrosis. Adalimumab is a fully human anti-tumor necrosis factor-α monoclonal antibody which has been approved for the treatment of moderate to severe plaque-type psoriasis. Even though it is fully human, anti-adalimumab antibodies have been detected.

Method: We report the case of a 73 year old female patient who had been diagnosed with psoriasis for 12 years and had stopped responding to all conventional anti-psoriatic treatments. The patient started treatment with adalimumab in 2012, with very good clinical response and complete clearance of the lesions after approximately nine months of therapy. Between 2014 and 2015 the patient decided to stop adalimumab treatment for personal reasons, unrelated to the medication. In 2015 however, the patient returned with extensive disease and we decided to restart adalimumab.

Results: After the first intradermal injection, the patient developed erythema and edema which rapidly progressed to ulceration. The patient was admitted to our clinic, where the second injection was performed, with the same result. After two adalimumab injections, we did not notice any clinical improvement. The treatment was switched to etanercept, with no adverse reactions and good clinical response.

Conclusion: Anti-adalimumab antibodies have been detected and are mainly associated with lower serum adalimumab concentrations and non-response to adalimumab treatment. In the case we are presenting, the ulceration was probably secondary to interaction between the previously formed anti-adalimumab antibodies and the newly injected adalimumab. We therefore report a rare case of Arthus reaction occurring after adalimumab treatment.

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Bladder dysfunction as first manifestation of systemic lupus erythematosus

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Background: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by numerous immunological abnormalities and multiple organs involvement. Although any organ can be affected, in general, urinary bladder involvement is

consider as unusual. Lupus cystitis is known to be related to bladder dysfunction in patient with SLE. It has also been suggested that intestinal tract is frequently associated but pathogenesis has not be fully explained.

Method: Case report of patient with bladder dysfunction and associated gastrointestinal symptoms as initial presentation of SLE. Bladder dysfunction was diagnosed on the basis of clinical presentation and findings of radiological, urodynamics and cystoscopy examinations.

Results: Previously health female patient referred to urologist due to suddenly appearance urinary tract symptoms with disuria, microscopic hematuria, lumbal pain, and subfebrile temperature. There were no other symptoms. The patient was treated as urinary tract infection but without improvement. Prominent gastrointestinal symptoms (vomiting, abdominal pain, diarrhea, ascites) were the reasons to hospital admission after one moth of initial symptoms and detailed diagnostics was done. Laboratory studies revealed elevated inflammatory markers, lymphopenia, proteinuria, as well as positive ANA, anti dsDNA and low complement levels. Abdominal ultrasound and abdominal CT showed bilateral urethral dilatation, hydronephrosis, bladder dilatation and thickened bladder wall, as well as bowel dilatation and atony. Bowel examination revealed ulcerous changes, intestinal biopsy was performed, but evidence for vasculitis was no found. Urodynamic evaluation revealed low detrusor contractility, decreased bladder capacity and sensation. Cystoscopic evaluation showed diffuse inflammation, erythema and hemorrhage at the trigone. Renal biopsy confirmed lupus nephritis IIb. Diagnosis of SLE with lupus cystitis was made. Therapy with high dose glucocorticoids and immunosuppressive drug was effective with clinic and laboratory improving.

Conclusion: We present a case SLE characterized by bladder dysfunction and prominent gastrointestinal manifestation. These unusual clinical presentation may be misleading and postpone SLE diagnosis. Lupus cystitis is an uncommon but definite manifestation of SLE. Early diagnosis and initiation of glucocorticoid therapy is crucial to prevent further bladder damage.

Poster Discussion Session PDS 4

Diagnosis of food allergy

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Characteristics of Pru p 7-sensitized patients allergic to fruits: a clinical investigation of Japanese adults

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Background: Pru p 7 is a recently described allergenic protein in peach. Its amino acid sequence is highly conserved through different botanical species, thus raising the possibility of cross-reactivity between various plant foods. The clinical relevance of sensitization to Pru p 7 among patients allergic to fruits has not been fully described previously.
Method: Thirty-three fruit allergic adult patients whose clinical presentation could not be explained by sensitization to PR-10 or profilin were recruited from a food-allergic population visiting Sagamihara National Hospital (Sagamihara City, Kanagawa, Japan) between 2006 and 2015. The levels of IgE binding to the nPru p 7 allergen were measured using an experimental ImmunoCAP assay

(Thermo Fisher Scientific). Clinical characteristics of the patients sensitized to Pru p 7 ($\geq$0.35 kU_A/l) were observed and recorded.
Results: Among 33 patients studied, 24 showed IgE sensitization to nPru p 7; 1 patient was positive to rPru p 3-IgE. Pru p 7-sensitized patients experienced allergic reactions after eating peach

(n = 20 patients), umeboshi (Japanese salt-preserved plums) (n = 10), apple (n = 8), pear (n = 1), citruses (n = 14), and fig (n = 2). They also showed positive results in prick to prick testing using these fruits. Most food-induced symptoms noted in patients were eyelid/face swelling and nasal symptoms rather than oral symptoms. In some patients, the reproducibility of the reaction after ingestion of offending foods was low. Fifty-four percent of the patients experienced food allergic episodes induced by a combination of fruit consumption and exercise or ingestion of NSAIDs.
Conclusion: Pru p 7 may be related to the allergic reaction to Rosaceae fruits, not limited to peach and citruses. Clinicians may have to consider Pru p 7 as a possible causative allergen when treating patients with Rosaceae fruit or citrus allergy whose symptoms are not explained by sensitization to PR-10, profilin, or LTP.

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Better management of peanut allergy by low dose oral food challenge

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Background: Most patients with a history of an allergic reaction after eating peanuts are advised to avoid peanut completely. However, some individuals can eat a small amount of peanuts without symptoms. Confirming this may result in an improvement in their quality of life, e.g., a decrease in the fear of anaphylaxis following accidental ingestion. We aimed to evaluate whether individuals with known peanut allergy could eat a small amount ofpeanut by setting less target dose of oral food challenge (OFC).
Method: Of the 118 subjects on whom we performed an OFC with 125 mg of peanuts protein between April 2014 and September 2015 at our hospital, we further assessed 77 subjects who had previously experienced a definite allergic reaction after eating peanuts. We retrospectively analyzed results of the OFC and their background.
Results: The median age at OFC was 6.8 years, and the median peanut and Ara h 2-specific IgE level were 19.2, and 11.4 kUA/l, respectively. Among 77 subjects 27 (35%) had a history of anaphylaxis to peanuts. OFC was negative in 30/77 (39%) subjects. Among 47 failed subjects, oral mucosal symptoms were most common, occurring in 23 (72%), followed by gastrointestinal symptoms in 30 (64%), respiratory symptoms in 20 (43%), and cutaneous symptoms in 13 (28%). Twelve (26%) of them developed anaphylaxis and only 3 (6.4%) subjects needed adrenaline intramuscular injection. On the other hand, 15 (32%) relieved their symptoms without medication. In logistic regression analysis, Ara h 2-specific IgE level was the only variable associated with a positive OFC (OR 13.7; 95% CI 1.6–120.2). The 50% and 80% positive predictive value of Ara h 2-specific IgE level estimated from the probability curve was 4.95 and 35.8 kUA/l, respectively.
Conclusion: Approximately 40 percent of subjects who have a definitive past history of allergic reaction to peanuts could consume 125 mg of peanut by undergoing OFCs and Ara h 2 specific IgE level seems to be a good marker to predict the result. Low dose peanut OFC has significance to perform positively on the basis of Arah2-specific IgE level.

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Prawn sensitised infants elicit differential IgE binding compared to adults – Finding early indicators for paediatric prawn allergy

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Background: Prawns are a major cause of IgE-mediated food allergic reactions. Correct diagnosis and the substantial risk of progression of prawn allergy among paediatric patients remains a challenge for clinicians. Known prawn allergens include tropomyosin, arginine kinase, myosin light chain, sarcoplasmic calcium binding protein and hemocyanin, with tropomyosin being the major allergen involved in allergic reaction to prawns in adults.
Aim: To compare and characterise prawn allergen recognition profiles of young children with that of adults with confirmed prawn allergy.
Methods: A total of 45 subjects were recruited for this study; 27 adults with positive clinical history of shrimp allergy and shrimp-specific IgE and/or positive skin

prick test (SPT), and 18 children with positive shrimp SPT. Atopic subjects with no history to shrimps were used as controls. Tropomyosin specific-IgE was detected using a novel ImmunoPCR method. IgE recognition of shrimp allergens were analysed using immunoblotting against black tiger prawn (*Penaeus monodon*) raw extract. Major and minor IgE binding proteins were identified using MALDI-TOF mass spectrometry.

Results: IgE recognition and binding to prawn allergens were observed in 74% adults and 66% children. Tropomyosin-specific IgE was detected by immunoblotting in 33% adults and 22% children. In contrast IgE recognition to sarcoplasmic calcium binding protein was observed in 7% adults and 28% children. None of the children elicited IgE recognition to arginine kinase, but 27% had specific IgE to hemocyanin. IgE recognition to tropomyosin was observed in most prawn-tolerant children. High prawn IgE titres in adults was associated with strong IgE recognition to tropomyosin and arginine kinase.

Conclusion: Tropomyosin and Arginine kinase specific IgE may be good predictors of prawn allergy in adults, and sarcoplasmic calcium binding protein and hemocyanin are possible early indicators of onset of prawn allergy in young children. Specific component resolved diagnostics for paediatric patients may assist in the early diagnosis of prawn allergy.

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Specific IgG4/IgE ratios to Ara h 1, 2, 3 and whole peanut extract serve as markers for clinical reactivity to peanut

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Background: Specific IgE to Ara h 2 is the best marker for peanut allergy. During tolerance development, specific IgG4 increases whereas specific IgE decreases or remains at the same level. Our aim was to examine whether specific IgG4/IgE ratio to peanut and its' allergen components can serve as a marker for reactivity to peanut and whether it predicts the reaction severity.

Method: We performed double-blind placebo-controlled peanut challenges for 97 children and adolescents (6–18 years) who were sensitized to peanut (SPT > 8 mm or S-peanut-IgE > 15 kU/l) or had always avoided peanuts. Peanut protein was administered at 30 min intervals (5, 50, 200, 1000 mg; cumulative dose 1255 mg). The reaction severity was scored according to Astier et al. as no symptoms (grade 0), localized symptoms (grade 1), one organ involved, nonlaryngeal angioedema, mild asthma (grade 2), two organs involved (grade 3), three organs involved or asthma requiring treatment or laryngeal oedema or hypotension (grade 4) and pronounced dyspnoea or hypotensive symptoms requiring intensive care (grade 5). We measured serum specific IgE and IgG4 to whole peanut extract and to Ara h 1, 2, 3, 8 and 9 (ImmunoCAP, Thermo Fisher Scientific Phadia, Sweden). IgE values were converted to μg/L by a factor of 1 kU/l = 2.4μg/l.

Results: Of 97 patients, 32 (33%) had no symptoms at peanut challenge (grade 0), 8 (8%) had grade 1, 14 (14%) grade 2, 20 (21%) grade 3, 23 (24%) grade 4 and 0 grade 5. The IgG4/IgE ratio to Ara h 1, 2, 3 and whole peanut extract were higher ($P < 0.001$) in patients who passed the challenge. Ara h 2-IgG4/IgE had the best discriminative ability with area under curve 0.946 (95% CI 0.902 to 0.991). With cut-off value 244 the sensitivity was 84% and specificity 95%. The IgG4/IgE ratios to Ara h 1, 2, 3 and whole peanut extract associated with reaction severity: with lower IgG4/IgE ratios, reactions were more severe. In Jonckheere-Terpstra test, Ara h 2-IgG4/IgE showed the strongest trend with reaction severity ($T_{JT} = 493.00$, $z = -3.156$, $P = 0.002$).

Conclusion: IgG4/IgE ratios to Ara h 1, 2, 3 and whole peanut extract serve as markers for clinical peanut allergy and lower ratios predict more severe reactions.

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How egg specific IgE can predict oral food challenge outcome?

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Background: Oral food challenge (OFC) is the gold standard for diagnosis in food allergy. Parameters to predict OFC risk of reaction are useful to optimize patient care. We evaluated the relationship between Ovalbumin and Ovomucoid IgE levels and egg OFC outcome with egg at different degree of cooking.

Method: Of 96 patients with egg allergy underwent the OFC at the Food Allergy Centre of Padua. Patients were selected including also those with severe asthma, previous anaphylaxis, or high egg IgE level. OFC was designed using foods at three different degrees of cooking: food containing baked egg, boiled and lastly raw egg. Differently from other studies the selected baked products were packaged and therefore standardized in ingredients and degree of cooking. Patients who tolerated at least 2.08 g of egg protein from baked food continued the OFC with boiled egg (white and yolk) served in increasing amounts up to 5.28 g of protein. The last part was conducted with raw egg reaching at least 1.23 g of egg protein. OFC was considered positive and stopped at objective symptoms or persistent subjective symptoms.

If patients did not present any subjective or objective symptoms, the OFC was considered negative.

Results: Of 45 patients (46.8%) did not tolerate the whole baked product administration; 39 (40%) tolerated baked products, but reacted to boiled egg; 4 children (4%) tolerated boiled egg, but reacted with raw egg; for 8 patients the OFC outcome was negative. As far as IgE levels are concerned, a significant correlation was found between low IgE Ovalbumin level and the probability to tolerate baked products; no correlation was found between Ovalbumin and boiled and raw egg tolerance. No significant results were found for Ovomucoid values. Age at the time of challenge, gender or previous anaphylactic reaction to egg did not affect the OFC outcome. Other allergic diseases (asthma, atopic dermatitis, rhino-conjunctivitis or other food allergies) did not prejudice significantly the OFC outcome.

Conclusion: The detection of Ovalbumin IgE level could be useful before egg OFC, in order to predict the risk and to choose the most suitable degree of cooking of egg to start with. Our results suggest to assess the baked product tolerance during OFC with standardized baked products, in order to introduce them in the diet (if they are completely tolerated) for better daily management or to start a desensitization protocol (if partially tolerated).

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The value of monitoring respiratory and vital signs during food challenges

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Background: A double blind placebo controlled food challenge (DBPCFC) is usually terminated and considered positive when objective symptoms occur. Subjective symptoms are difficult to interpret. Less obvious objective symptoms (as mild dyspnoea) are easily missed. Our aim was to investigate if monitoring of respiratory and

vital signs contributes to the accuracy of DBPCFC.

Method: A prospective study in 83 children with suspected peanut allergy that underwent a DBPCFC and subsequent open challenge was performed. The following parameters were monitored during challenge: oxygen saturation, heart rate, blood pressure, respiratory function and respiratory sounds. Differences in the course of the parameters over time between active and placebo for allergic and tolerant children were investigated using linear mixed effects modelling.

Results: Allergic children had a significant mean decrease in FEV1%pred on active compared to placebo (−0.59% (SE 0.17) per 30 min, $P < 0.001$). However, tolerant children also had relevant changes in FEV1 on both challenge days. Only a drop in FEV1 > 20% on the active day was distinctive for peanut allergy. No significant differences between active and placebo for allergic or tolerant children were seen for the course of other respiratory and vital parameters during challenge.

Conclusion: A decrease in FEV1 > 20% is indicative for an allergic reaction during DBPCFC. Other respiratory and vital signs can not be used to discriminate children with and without peanut allergy.

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Independent seed and peel lipid transfer proteins (LTPs) are involved in tomato allergy without cross-reactivity associated

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Background: Several reports of cases of food allergy without a positive *in vitro* diagnosis test with standard extracts and the identification of new allergens, located in specific tissues poorly represented in the whole extract, have initiated new studies leading to clarify the diagnosis of certain food allergic-patients. Two different non-specific lipid transfer proteins (nsLTPs) have been specifically identified in tomato seeds: Sola l 6 and Sola l 7, not present in the pulp or peel of this fruit, and presenting a low identity sequence degree with Sola l 3, the peel nsLTP. Both proteins are associated as an only molecule of 30 kDa and their presence at so low levels in the whole tomato extract rendered negative SPT when the full extract is used as a diagnostic tool.

The main objective of this study is to clarify the clinical profile of the tomato allergic patients trying to understand the relevance of Sola l 3, Sola l 6 and Sola l 7 and to know if a cross-reactivity could be involved in the sensitizations mediated by these allergens.

Method: Peel and seed tomato extracts were available and LTP proteins have been isolated with chromatographic steps, as HPLC. Different sera of patients allergic to tomato, patients allergic to tomato seeds and finally, patients allergic to peach LTP are included in the study. IgE recognition assays with these sera (immunoblotting and ELISA techniques) and inhibition experiments with the whole food extracts and the purified LTPs have been done.

Results: *In vitro* IgE recognition to Sola l 6/7 and Sola l 3 separately have been observed in several sera. Patient allergic to tomato only, is able to recognize Sola l 3 but not Sola l 6 or 7. In the contrary, patients sensitized to seeds can sometimes recognized only Sola l 7 but also there are IgEs directed to both Sola l 3 and 6/7 allergens. No cross-reactivity is observed when any of the three LTPs are used as inhibitors, although all tomato LTP allergens are recognized with the sera allergic to the peach LTP, showing a great cross-reactivity.

Conclusion: The results of this study lead us to believe that the presence of two different proteins of the same family located in different tissue of the same fruit could be responsible of an independent sensitization of the patients with allergic symptoms to this vegetable. The IgE recognition to any of these proteins does not appear to be a consequence of a cross-reactivity process by the presence of IgE common epitopes.

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Co- and cross sensitivity and clinical reactivity amongst Danish tree nut allergic children adolecents and young adults (TACAYA)

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Tree nut- and peanut allergens show sequence homology of IgE-binding epitopes in various degrees. Patients with primary allergy towards tree nuts (PTNA) and peanuts (Pe) are often co- or cross sensitized to nuts and plant foods. A pilot study from a Danish pediatric ward have found that the most common tree nuts causing allergies are hazelnuts (HN), walnuts (W), cashew nuts (C), pistachio nuts (Pi) and almonds (Al), and often the patients are co- or cross sensitized to Pe.

Aim: To investigate the co- or cross sensitivity and clinical reactivity in children and young adults suspected of having PTNA.

Method: 31 patients aged 3–20 with a previous allergic reaction to one or more tree nuts, together with sIgE HN > sIgE birch pollen or sIgE to any other tree nut, could enter the study. Oral food challenges (OFCs) either open challenges or double-blind placebo controlled food challenges were performed with HN, W, C, Pi, Al and Pe. A convincing medical history of recent tolerance or an immediate allergic response upon ingesting the nuts was also accepted. Data was not included in cases where information on allergy towards nuts was unavailable, due to patients refusing- or due to a history of anaphylaxis where OFCs was contraindicated. Data on sIgE was not included if information was missing. sIgE ≥0.35 KU/l was regarded as positive.

Results: 8/22 was allergic (A) to ≥3 tree nuts

1/30 patients	only allergic to HN	–
1/31	only allergic to W	–
12/25	HN-A	and W-A
11/26	C-A	and Pi-A
2/14	C-A	but tolerant to Pi
12/21	HN-A and/or W-A	and Pe-A
7/16	C-A and/or Pi-A	and Pe-A
9/27	HN-A and/or W-A	and C-A and/or Pi-A
4/18	HN-A and/ or W-A	Al-A
2/17	C-A and/or Pi-A	Al-A

[Co- or cross allergies towards nuts]
17/18 HN Allergic patients (AP) had sIgE to Cor a 14
9/11 HN-AP had sIgE to Cor a 9
14/31 AP had sIgE ≤ 0.35 KU/l to birch
5/5 W-AP had sIgE to Jug r 1
15/16 C-AP had sIgE to Ana o 3
11/12 Pe-AP had sIgE to Ara h 2

Conclusion: This study found that Danish patients with PTNA have a high degree of co- or cross allergy to other tree nuts and Pe. Allergy towards the pairs HN and W, and C and Pi was common. PTNA - patients are often co- or cross allergic to ≥3 tree nuts and/or Pe. sIgE to allergen components are important, but not always sufficient as a positive predictive measure for allergy.

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Jug r 1 is the best discriminating allergen in the diagnosis of walnut allergy

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Background: To date, several walnut (WN) allergens have been identified even though, in some cases, their clinical significance has yet to be determined. Our aim was to study the importance of component resolved diagnosis in WN allergic patients and compare allergen microarray (ISAC) determination vs ImmunoCAP.
Method: Forty children suspected of having allergy to fruit, nuts and/or legumes were selected. Patients were classified as allergic or tolerant if they had suffered at least 2 reactions unequivocally related to WN ingestion in the last 2 years or if they consumed WN on a regular basis, respectively. Clinical questionnaire, skin prick test (SPT), serum total and specific IgE and MIA-ISAC IgE

(Thermo Fisher Diagnostics, Uppsala, Sweden) were performed.
Results: Nineteen patients (11 males) were defined as allergic and 21 (11 males) as tolerant. WN-SPT wheal size (diameter mm) (median; IQR: 5; 3.75–7.75 vs 0; 0.00–2.50; $P = 0.000$) and WN-sIgE (kU/L)(median; IQR: 5.5; 3.63–8.13 vs 0; 0–2.75, $P = 0.000$) were significantly higher in allergic than in tolerant children. Allergic patients had positive Jug r1-sIgE significantly more frequently (84.20% vs 4.70%, $P = 0.000$). Jug r1-sIgE (ISU) values measured by MIA-ISAC were also significantly higher in allergic children (median, IQR: 5.5; 0.7–14 vs 0; 0–0, $P = 0.000$) as well as measured by ImmunoCAP (kU/L) (median 7.73; IQR:1.65–23.90 vs 0.0; IQR:0.00–0.05, $P = 0.000$). This was not found for Jug r2 or Jug r3. ROC curves were constructed with Jug r1 showing the best diagnostic performance measured by ImmunoCAP (AUC: 0.925, 95%CI:0.828–1, $P = 0.000$) and by ISAC (AUC:0.857, 95% CI:0.729–0.985).
Conclusion: Jug r1-sIgE measured by ImmunoCAP or ISAC has the best diagnostic performance in WN allergy.

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Specific IgE to Jug r 1 is a marker for walnut allergy

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Background: Several walnut allergens have been identified and are available for specific IgE (sIgE) testing. Literature on their use in diagnosing walnut allergy is scarce.
Objective: To prospectively assess the diagnostic value of sIgE to walnut components Jug r 1, 2 and 3 in subjects suspected of walnut allergy and compare this with the routinely used skin prick test (SPT) and sIgE to walnut extract. In addition, to analyse their ability to predict a moderate to severe walnut allergy, defined as symptoms more than only oral allergy symptoms.
Methods: Adult subjects with a suspected walnut allergy were recruited in our tertiary allergology clinic between October 2012 and August 2015. Diagnostic evaluation included three different SPT extracts and sIgE against walnut extract and walnut components Jug r 1 and 3 on ImmunoCAP and Jug r 2 on ImmunoCAP ISAC. A double blind placebo controlled food challenge with walnut was performed as reference test in all subjects.
Results: A total of 57 subjects were challenged. Walnut allergy was confirmed in 33 subjects (58%). A positive sIgE to Jug r 1 and walnut extract, as well as SPT with the ALK extract, were observed significantly more often in the walnut allergic subjects ($P < 0.01$). Of all components, sIgE to Jug r 1 had the best diagnostic accuracy (AUC 0.78, vs 0.63 for sIgE to Jug r 2 and 0.44 for sIgE to Jug r 3). This value was slightly lower compared to sIgE to walnut extract (AUC 0.79) and SPT with the ALK extract (AUC 0.82). When applying the clinically used cut-off values of $\geq$0.35 kU/l for ImmunoCAP and $\geq$3 mm for SPT, sensitivity and specificity values were 55 and 91% (sIgE to Jug r 1), 70 and 86% (sIgE to walnut extract) and 84 and 67% (SPT ALK), respectively. A 100% positive predictive value could be calculated for all 3 tests using different cut-off values: 1.49 and 2.02 kU/l for sIgE to Jug r 1 and walnut extract respectively, and 5 mm for SPT, correctly classifying 23%, 21%, and 32% of subjects as walnut allergic, respectively. All three tests showed a strong ability to identify subjects with a moderate to severe walnut allergy (all three AUC 0.83).
Conclusions and clinical relevance: The best diagnostic value of all walnut components was found for sIgE to Jug r 1, which was slightly lower compared to the routinely used sIgE to walnut extract and SPT. All 3 tests could equally identify patients at risk for a potentially severe reaction to walnut.

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Fish hypersensitivity: clinical manifestations and fish-specific IgE-sensitization

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Background: Patients with fish hypersensitivity may present a wide range of symptoms: from mild skin or GI manifestations to severe anaphylactic reactions. Various immunological and non-immunological mechanisms may be involved in the pathogenesis of fish-induced reactions. The goal of the study was to analyze a clinical profile of patients with fish-induced hypersensitive reactions and to refer clinical profile to IgE sensitization to fish allergens.
Method: The study included 40 patients aged 7–80 years who reported hypersensitivity reactions after eating fish. Patients had an allergy interview and skin prick tests (SPT) with a panel of fish allergens (11 species: Cod, Tuna, Sea bream, Anchovy, John Dory, Sole, Hake, Anglerfish, Salmon, Sardine, Trout) and with the allergen fish parasite, the nematode *Anisakis simplex* (Bial Aristegui company, Spain).
Results: Almost half of the patients (19 subjects) presented oral allergy syndrome (burning, itching, swelling of lips and tongue) following eating fish-containing food. Symptoms of asthma and dyspnea were reported by 16 patients, urticaria by 14 patients and angioedema by 4 patients. Anaphylactic shock after eating a fish occurred in 9 patients. Milder symptoms of skin erythema, itching or burning were reported by 16 patients and gastrointestinal symptoms occurred in 18 patients. In addition, individual patients reported conjunctivitis, eyelid edema, burning and watering of the eyes, dizziness, rhinorrhea, weakness, disorientation, tremors, dryness of the mouth. Positive SPT to at least one fish allergen were found in 24 patients; 14 patients reacted to all 11 species of fish while only 4 patients reacted only to 1 species of fish. Among 24 patients who were allergic to fish 5 subjects had also positive SPTs to nematode allergen *Anisakis simplex*.

Conclusion: Fish-specific IgE can be detected in nearly 2/3 of patients with symptoms of fish hypersensitivity. Majority of fish-allergic subjects are sensitized to more than one fish allergen.

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Cross-reactivity and tolerance in fish allergic patients: a randomized double-blind placebo-controlled food challenge tria

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Background: Cross-reactivity among fish species is a common feature of fish allergy. Anecdotely, fish-allergic patients react to multiple species, and others seem to tolerate single or several species. Diagnostic data on discrimination between these different phenotypes of fish allergy during the clinical work-up are scarce.
Objective: To study the clinical reactivity to commonly consumed fish species in allergic patients by diagnostic oral challenges and to correlate clinical reactivity with IgE-sensitization patterns.
Method: Children and adolescents (n = 35) with verified fish allergy were included in a randomized double-blind placebo-controlled food challenge (DBPCFC) trial with cod, salmon and mackerel, using a fish powder in standardized EuroPrevall-recipes. Open food challenges (OFC) with a higher fish dose (fish burgers) were performed with all DBPCFC-negative patients. Specific IgE were measured to cod, salmon and mackerel extracts (ImmunoCAP), parvalbumins, aldolases and enolases (ELISA).
Results: Rates of reaction with objective symptoms after DBPCFC/OFC were 82%, 58% and 41% respectively for cod, salmon and mackerel, with 27% reacting to all three species. Rates of reaction with objective and/or subjective symptoms were for cod 91%, salmon 85% and mackerel 79%. Only 3 patients experienced mild, transient, subjective placebo reactions. The majority (91%) of patients were sensitised to parvalbumins from all 3 fish species. In contrast 66% were sensitised to enolases and aldolases from cod, whilst 14% and 34% were sensitised respectively to enolases and aldolases from salmon. For patients reacting to all three fish species, clinical reactivity correlated to sIgE to parvalbumins. For patients with negative challenges to one or two fish species clinical reactivity correlated to sIgE to cod/salmon enolase and aldolase in 68% and 88% of the patients with objective and subjectice symptoms respectively.
Conclusion: Multiple reactivity to several fish species was common, but almost 30% of patients were either tolerant or reacted only with subjective symptoms to one or more fish species. While food challenges confirmed allergy to specific fish species, IgE to fish extracts lacked such specificity. IgE to parvalbumins was only correlated with clinical reactivity to multiple species but was not indicative of tolerance to one or more species. Instead IgE to fish enolases or aldolases may be more relevelvant to consider in such patients.

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Cetuximab specific IgE in the diagnosis of red meat allergy

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Background: Red meat allergy (RMA) is an emergent food allergy, increasingly prevalent in tick-endemic areas of United States. Delayed onset hypersensitivity reactions 3–6 h after ingestion of mammalian food products in patients with IgE to galactose-α-1,3-galactose (α-Gal) have been revealed. Detection of α-Gal specific IgE (sIgE) is important for diagnosis of RMA. Moreover bovine thyroglobulin (BTG) and cetuximab shared antigenic epitope with α-Gal.
Method: Six patients having suspected symptoms of RMA, three patients with immediate hypersensitivity reactions after ingestion red meat and twelve asymptomatic sensitizers were enrolled (positive beef and pork sIgE by ImmunCAP®). Sera of 10 non-atopic patients were also enrolled by negative control. IgE antibodies against beef, pork, bovine thyroglobulin, and cetuximab using ImmunoCAP® assay. In our study, over 0.35 kUA/L of sIgE level was considered as positive.
Results: Six patients presented with anaphylactic reactions after 3 h of red meat consumption, and they tested positive for sIgE with beef, pork, BTG and cetuximab. And 10 non-atopic patients all have been negative for four categories of sIgE. Both BTG and cetuximab sIgE showed 100% of sensitivity to diagnose RMA. In terms of specificity, Cetuximab sIgE (83.3%) was higher than BTG sIgE (40.0%).
Conclusion: Measurement of sIgE against both BTG and cetuximab will be helpful to distinguish RMA from the others. Furthermore, cetuximab sIgE positivity showed higher specificity rather than BTG sIgE, cetuximab sIgE measurement will be more useful to discriminate true RMA patients from asymptomatic sensitizers.

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Differences in the oral food challenge results of 3 tree nuts (almond, cashew nut, and walnut)

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Background: Since tree nuts are known to cause severe allergic reactions, performing an oral food challenge (OFC) is often avoided, in spite of the fact that the OFC is the gold standard for diagnosis of food allergy. Alternatively, the diagnosis of tree nut allergy is made based on confirmation of sensitization by positive skin prick test or elevated specific IgE levels, which may however lead to overdiagnosis. Determining the risk factors of a positive tree nut OFC may help identify patients in which OFCs can be performed relatively safely, enabling them to avoid unnecessary elimination when the OFC is negative. The objective of the study was to compare the differences in the results of OFCs performed on 3 tree nuts (almond, cashew nut, and walnut) and to determine risk factors of a positive OFC.
Method: Subjects were 284 patients who received an OFC of 3 g or more of almond, walnut, or cashew nut from March, 2006 to April, 2014 at Sagamihara National Hospital. All OFCs were open OFCs administered in 2 or 3 doses. During each challenge, the patient was observed for at least 2 h after the last dose. At any sign of subjective or objective symptoms deemed clinically significant, the challenge was terminated and necessary treatment was provided. Patient characteristics, positivity rate and symptoms induced by OFCs were retrospectively analyzed.
Results: 145 almond, 71 cashew nut, and 68 walnut OFCs were performed. Median age was 7 years (range: 1–22 years). OFC was positive in 4.8% (n = 7) of almond, 18.3% (n = 13) of cashew nut, and 50% (n = 34) of walnut OFCs. Anaphylaxis was seen in 38.4% (n = 5) of positive cashew

nut OFCs and 14.7% ($n = 5$) of positive walnut OFCs, more frequently in patients with a higher specific IgE level. Symptoms in positive almond OFCs were relatively mild with no case of anaphylaxis. Four patients required treatment with oral antihistamines but the remaining 3 patients required no treatment for symptoms. In cashew nut and walnut, specific IgE level was significantly higher in the patients with a positive OFC (both $P < 0.01$). As for almond, however, no significant difference in specific IgE level was seen.

Conclusion: The results suggest that the decision to perform OFCs should be based on the characteristics of each individual tree nut. Almond OFCs can be performed relatively safely, but in the case of cashew nut and walnut, caution is needed for severe reactions, especially if the specific IgE level is high.

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Concerns and expectations of pediatric patients and parents with regards to oral food challenges

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Background: Pediatric patients undergoing oral food challenges (OFCs), and their parents, have unmet expectations and a variety of unresolved concerns upon completion of examinations.

Objective: The present study aims to investigate concerns and expectations of pediatric patients and their parents before and after OFCs, as well as explore issues in patient supervision.

Method: Approval was obtained from the Ethics Committee of the medical facility of the pediatric patients. The participants of the study were 60 parents of pediatric patients who underwent OFCs in the same department from July 2014 to January 2015, as well as 34 pediatric patients of elementary school age (mean age, 9.29 years). A questionnaire was completed by pediatric patients and their parents immediately prior, directly after, and during the first outpatient visit following OFCs. The questionnaire covered concerns on the onset of food allergy symptoms, concerns about OFC, and expectations for the future.

Results: The level of concern regarding the effects of food allergies to daily life was highest immediately prior to OFCs in both patients and parents. This level significantly dropped directly after OFCs, and was even lower at outpatient examination. Concern was alleviated because the induced symptoms could be specifically understood, because patients gained actual experience in handling the onset of symptoms, and because symptoms did not appear to be induced during OFCs. Regarding OFC expectations, 56% of pediatric patients expressed feeling comfortable with eating out, and 47% stated that they began to stop feeling different from their peers. For parents, these responses were given by 27% and 20%, respectively ($P < 0.01$), indicating a gap between the expectations of parents and children.

Conclusions: OFCs significantly alleviated concerns about daily life in pediatric patients and their parents. The use of likely effective approaches in patient supervision, as well as consideration and empathy for the emotional burden of pediatric patients and their parents, may be linked to improved quality of life.

Poster Discussion Session PDS 5

Ocular allergy

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A novel grading scale score to assess ocular surface epithelial damage in patients with vernal keratoconjunctivitis

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Background: Vernal keratoconjunctivitis (VKC) is a severe inflammatory disease that appears in children and adolescents with seasonal recurrences. The assessment of ocular surface epithelial damage is considered an essential tool to evaluate disease severity and treatment approach. Oxford (Ox) and Van Bijsterveld (VB) scoring systems are the most diffused objective clinical measures to quantify corneal and conjunctival staining. Although these systems have been developed for dry eye disease (DED), they are also used in VKC clinical trials. The aim of the present study is to evaluate the effectiveness of a new scoring system (NC) in the assessment of the corneal epithelial damage in VKC patients.
Method: 25 VKC patients were included and evaluated by symptomatology questionnaires (QUICK) and objective clinical measures: fluorescein and lissamine green staining, and corneal-confocal microscopy (CFM) (Heidelberg Retina Tomograph 3). Ox, VB and NC scoring systems were applied to evaluate epithelial damage after corneal and conjunctival staining. We also performed an inter-observer evaluation using Ox, VB and NC scores assigned by an experienced ophthalmologist and an assistant.
Results: Mean Ox and NC(F) scores were statistically significant different after fluorescein staining ($P < 0.001$), and values were significant directed correlated ($P = 0.001$). The same data was obtained comparing VB and NC(L) after lissamine green staining ($P < 0.001$) ($P = 0.001$). In limbal VCK patient a statistically significant difference was found comparing new (NC) and standard scoring systems (Ox, VB) values ($P < 0.001$). On the contrary no statistically significant difference was found considering tarsal VKC patients. A statistically superior concordance was present between Quick and NC scores compared to standard staining scores values ($P < 0.001$). The inter-observer study showed a significant correlation of scores given by ophthalmologist and assistant ($P < 0.001$).
Conclusion: Oxford Van Bijsterveld tests do not seem to be adequate for evaluation of the surface damage in limbal VKC patient because the staining patterns proposed by these tests do not correspond to the staining patterns in VKC patients. We propose a new scoring system to better evaluate corneal epithelial damage in both limbal and tarsal VKC patients.

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IL-9 upregulation in experimetal allergic conjunctivitis: IL-9R blockade downregulates mast cell responses

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Background: IL-9 is a well-known growth factor, mediating mast cell and T cell production of proinflammatory cytokines. We have previously demonstrated enhanced human conjunctival IL-9 expression localised to mast cells in post-challenge allergic conjunctivitis (AC)[1] relative to unchallenged controls.
Aim: We investigated the role of IL-9 in mouse models of mild and severe AC and its immunomodulatory effect(s) on mouse bone marrow-derived mast cells (BMMC) *in vitro*.
Method: Balb/c and C57Bl/6 mouse models were used to mimic clinically mild and severe AC, respectively. Allergens were administered intraperitoneally followed by ocular challenge, using short ragweed (SRW) for mild AC and ovalbumin (OVA) for severe disease. Immunophenotyping of mouse conjunctival cells and draining lymph nodes (dLN) was performed to investigate the localisation of IL-9 expression in AC, and to identify the cellular source and contribution of IL-9 during disease *in vivo*. Bone marrow mouse mast cell cultures (BMMC) were established *in vitro* to study the functions of IL-9 using neutralising IL-9 and IL-9 receptor (IL-9R) mAbs.
Results: Increased levels of conjunctival c-kit$^+$ cells were detected by flow cytometry in both SRW and OVA challenged mice as compared to controls ($P < 0.05$). Levels of c-kit$^+$IL-9$^+$ cells were significantly increased, but only in the severe OVA model ($P < 0.03$). In contrast, in the dLN, significant increases were observed in the levels of CD4$^+$GATA3$^+$ cells in both SRW and OVA challenged mice ($P < 0.05$). There was also a significant increase in CD4$^+$T cells expressing IL-9 in the SRW model ($P < 0.05$), but not in the OVA model. Levels of CD4$^+$GATA3$^+$IL-9$^+$ cells were also significantly upregulated in both models post challenge. *In vitro* blocking IL-9R, but not IL-9, on mast cells significantly downregulated expression of FcER1 (<72 h), histamine (<24 h), IL-4, IL-5, IL-9 and IL-13 secretion by PMA/ionomycin stimulated mast cells ($P < 0.05$). A similar effect on histamine and cytokine secretion was also observed when *Il9r* gene function was silenced.
Conclusion: IL-9 is upregulated on mast cells in the severe model of AC and blocking IL-9 at the level of its receptor significantly decreased mast cell degranulation.

[1]Mohd Zaki et al (2013) EAACI poster.

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Exhaled nitric oxide levels in children with vernal keratoconjunctivitis

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Background: Nitric oxide (NO) is endogenously released in the airways and measurement of the fractional concentration of NO (FeNO) in exhaled breath is a non-invasive test for the detection of eosinophilic airway inflammation. The aim of the study is to evaluate FeNO levels in children with vernal keratoconjunctivitis (VKC) compared to non-atopic healthy children.
Method: FeNO levels of age- and sex-matched 29 healthy children (15 male, 51.7%, mean age 11.79±2.7 years) and 17 VKC children (11 male, 64.7%, mean age 12.65±3.7 years) were measured by NIOX-MINO Airway Inflammation Monitor ($P = 0.082$ and 0.54, respectively). FeNO levels were also analyzed according to

severity of VKC, the presence of atopy (prick and specific IgE tests), eosinophilia and IgE levels in VKC group.
Results: Mean FeNO levels of VKC children was significantly higher compared to the control group (23.53 ± 11.9 and 13.66±5, respectively) ($P = 0.003$). Eosinophil numbers, percentage and eosinophil/lymphocyte ratio was higher in VKC group compared to healthy children ($P < 0.05$). The clinical findings were mild-moderate in 7 patients and severe in 10 patients and there were no differences in FeNO levels according to severity of VKC ($P = 0.81$). Although mean FeNO level was higher in VKC children with atopy (30.57±13.9) than without atopy (18.60±7.6), the difference was not statistically significant ($P = 0.16$). Except serum IgA levels, remaining antibody levels, eosinophil, neutrophil, leucocyte numbers, eosinophil %, eosinophil/lymphocyte and neutrophil/lymphocyte ratios were similar between mild-moderate VKC group and severe VKC group.
Conclusion: FeNO levels were found to be higher in VKC children. These results suggest that VKC subjects may have the elevated risk for airway inflammation. However, large number of subjects is needed to evaluate the association of FeNO levels with clinical findings and co-associated systemic allergies.

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Identification of N-glycan profiles in tears of vernal and atopic keratoconjunctivitis patients

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Background: Vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC) are chronic ocular allergic diseases characterized by severe inflammation and complex physiopathology. Recent studies showed alterations of the protein-linked glycans in other pathology of the ocular surface. The purpose is to investigate the N-glycome tear profile differences between healthy subjects and patient affected by VKC and AKC; to find possible glyco-biomarkers; and to study the role of N-glycans in the pathogenesis of such diseases.
Method: Tear samples from 27 VKC patients, 7 AKC patients and 11 control subjects were diluted in a denaturant solution with 1/10 ratio, reduced, alkylated and treated with N-glycosidase F (PNGase F). This is an enzyme which specifically deglycosylates N-glycoproteins. The relased N-glycans were purified, permethilated and analyzed by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS and MALDI-TOF/TOF MS/MS).
Results: More than 150 complex N-glycans, including highly fucosylated biantennary, triantennary, tetraantennary and bisecting species were observed in the measured spectra. A subset of these structures was verified by MS/MS analysis. The data analysis highlighted three different patterns for VKC patients, AKC patients and control subjects, respectively, in terms of relative intensities of some N-glycans structures. Statistical tests showed that there are remarkable differences in the quantity of some N-glycans among the three groups.
Conclusion: The MALDI-TOF-MS technique allows for biomarker detection and for the characterization of glycan structures. The lacrimal N-glycome analysis can provide relevant information for a better understanding of VKC and AKC. The identification of given peaks may be used as potential biomarker for these diseases.

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Perennial allergic conjunctivitis and vernal keratoconjunctivitis: quality of life of children treated by sublingual immunotherapy to mites

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Background: In perennial allergic conjunctivitis without allergic rhinitis (PAC) and vernal keratoconjunctivitis (VKC) ocular symptoms are often triggered by allergen exposure. We evaluated the short-and middle-term evolution of quality of life (QoL) in patients receiving immunotherapy to house dust mite (HDM) for ocular allergy.
Methods: Retrospective study involving patients affected by PAC and VKC and prescribed sublingual immunotherapy (SLIT) to HDM in 2012–2014. All patients were sensitized to HDM: Skin Prick-tests wheal diameter 3 mm greater than the negative control and specific IgE to dermatophagoïdes pteronyssinus or farinae over 0.10 IU/ml (ImmunoCAP, Thermo Fischer ®). Conjunctival allergen challenge to HDM was positive in all the patients. SLIT was scheduled for 3 years with OSIRIS HDM mix ALK® (DP/DF), from 0.1 IR to 300 IR. Quality of life was assessed daily by the patient and his/her parents and graded from 0 (good) to 10 (worse). The rates were computerized and a mean score was calculated for each trimester. Every year a clinical score was recorded, ranging from −100 (considerably worse) to +100 (considerably better) by the parents and the physician on a visual analogic scale.
Results: 13 patients (3 females, 10 males) were included, aged 8.49±2.54 years, 7 with VKC and 6 with PAC. The protocol was followed unchanged by 7 patients (2 VKC/5 PAC) but had to be modified for the remaining 6 (3 times for 2 VKC, twice for 2 VKC, and once for 1 VKC and 1 PAC). We recorded QoL grading during the first 2 years in all but 1 patient who stopped at 18 months. The 3-monthly assessment of QoL decreased in the 13 patients from 2.36±1.24 in trimester 1 to 1.75±1.74 in trimester 8. In PAC patients QoL decreased from 2.52±1.20 to 0.90±0.78 ($P < 0.001$) and in VKC patients fluctuated from 2.23±1.36 to 2.49±2.05. The average clinical score in the first year was 21±20% for the entire population (18±16% in VKC patients and 17±18% in PAC patients) and 54±25% in the second year (55±19% in VKC patients and 53±33% in PAC patients).
Conclusion: SLIT to HMD showed a positive impact on daily QoL, in both PAC and VKC patients. Additional criteria such as the number of ophthalmologist rescue consultations and the use of topic steroids are needed to evaluate the impact of SLIT in allergic conjunctivitis. Even in PAC without nasal involvement, allergic conjunctivitis is a good indication for SLIT.

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Long term experience with omalizumab in severe refractory vernal keratoconjunctivitis in children

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Background: Vernal keratoconjunctivis (VKC) is a severe form of pediatric ocular allergy, characterized by acute and chronic corneoconjunctival inflammation that may lead to visual sequelae. Although topical immunosuppressive drugs such as cyclosporine are usually effective, some severe forms may be refractory and require prolonged steroid therapy. Very few papers report the use of omalizumab in VKC in the literature. In the present study, we describe our clinical experience with omalizumab in severe VKC children.
Method: We retrospectively reviewed the files of 4 boys treated with omalizumab because of severe VKC, defined as

persistent corneal inflammation despite continuous topical 2% cyclosporine and steroid eye drops.

Results: Four boys, aged 7 to 13 years old, were treated. All children had asthma and 1 had severe lid eczema. Two patients had required intrapalpebral depot-steroid injections. Omalizumab was administered every 2 weeks by subcutaneous injections, at doses varying from 450 to 600 mg per injection. Three patients out of 4 responded to the treatment, with a decrease in global symptoms (median symptom rating decreasing from 89 to 29 on a 100 mm visual analogic scale), frequency and in duration of the inflammatory flares, and also a decreased need for topical steroid. Their median clinical grade decrased from 4 to 3 (Bonini grading). However, the response was incomplete and they still had inflammatory corneoconjunctival flares despite continuous topical cyclosporine. On the other hand, asthma and lid eczema were completely controlled in these 3 patients. The fourth child did not respond to omalizumab and needed oral steroids for his VKC and his asthma. Noticeably, this latter patient did not have detectable sensitization to any allergen, contrary to the other cases. The treatment was stopped in this refractory case, but is still ongoing in all other cases, with a median duration of 33 months (range, 16–42 months).

Conclusion: Omalizumab is an interesting treatment in severe refractory forms of VKC, but its efficacy is incomplete in these very severe cases.

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Long-term experience of the concurrent co-operation allergist/ophthalmologist in an ocular allergy tertiary referral center

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Background: The partnership between allergist and ophthalmologist in the field of ocular allergy, due to the increasing frequency of this quality-of-life involving disease, becomes more and more essential. It helps the correct diagnosis and early therapeutical management of this condition which in a high percentage of subjects is associated with systemic diseases such as asthma, rhinitis and dermatitis.

Method: Our North-Western Italian region (Liguria) and neighbouring areas are at high-increasing prevalence of ocular allergies in the main severe forms (vernal and atopic kerato-conjunctivitis). For this reason, it became relevant to organize our ophthalmological referral center together with (at the same time) the allergist to shorten the waiting lists and make easier for the patient to be treated and followed.

Results: From Jan 2000 until now, our referral center visited more than 2500 subjects suffering from ocular allergy; many of them (more than 60% were affected with seasonal or perennial rhino-congiuntivitis, but the remaining part more than 30% suffered from vernal or atopic kerato-conjunctivitis).

In our work we describe the organization of our outpatients ocular allergy department showing the improvements and the changes adopted during this 15 years period of co-operation allergist/ophthalmologist which permitted us to correctly diagnose in most cases the kind of ocular allergy thanks to local and systemic specific tests (such as CPT) and to prescribe drugs or specific immuno-therapy if required.

Conclusion: Our long-term experience showed the importance of the concomitant presence of the allergist and the ophthalmologist in an allergic conjunctivitis department for the follow-up and management of the affected patients avoiding self- or uncorrect treatment measures.

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Scoring conjunctival provocation test: chemosis among other positivity criteria

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Background: The conjunctival provocation test (CPT) is validated in daily practice to confirm the involvement of respiratory allergens in allergic ocular diseases. We investigated the value of maintaining chemosis in the scoring scale of CPT in addition to ocular itching, redness, and tearing.

Method: Retrospective study of all CPTs performed between 2013 and 2015 in the Ophthalmology and Allergy outpatient department. 52 patients (17 females, 35 males), aged 41.9±11.5 years, were included after undergoing 65 CPTs for respiratory allergens: dermatophagoïdes (38), grass pollen (16), cat dander (4) and alternaria (7). Usual recommendations were followed: signed informed consent, evidencing sensitization by SPT or specific IgE levels greater than 0.10 IU/ml (ImmunoCap® Thermo Fischer®), drug discontinuation and medical environment able to deal with any event occurring during the challenge, practice outside exposure period, and no ongoing ocular symptoms. Stallergenes® lyophilised extracts were diluted into concentrations of 3IR, 6IR, 12IR, 25IR, 50IR, and 100IR. Positivity criteria were graded at 15 min 0–3 for redness and tearing, and 0–4 for ocular itching. Chemosis was rated as follows: 1 detectable with slit lamp, conjunctiva raised from sclera; 2 ballooning of conjunctiva; 3 visually evident, raised conjunctiva, especially at the limbal area. CPT was discontinued and declared positive when the cumulated score reached 5.

Results: CPT was declared positive in 41 cases and negative in 24. During the 65 challenges, 452 quotations were performed, in 11.6% of which chemosis was observed. The overall chemosis score was 0.77±0.65. A score of 1 was quoted from a dilution of 6IR up to a dilution of 100IR. In the last score of each CPT, chemosis was observed in 29 cases (45%). To be associated with positive CPT, the sensitivity of chemosis is 68.3% and specificity 91.7%. Thus, the ROC curve of chemosis was lower than of the three other criteria (AUC = 0.80).

Conclusion: Diagnosing and grading chemosis requires a slit lamp examination and a trained health professional in ophthalmology. Chemosis is generally rated as positive during CPT when other symptoms are also positive, and the challenge is positive. Chemosis should be considered as a severity symptom rather than a positivity criterion during CPT management. It does not appear mandatory during the increments of a conventional CPT in non-severe forms of allergic conjunctivitis.

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Usefulness of ocular pruritus score system for assessing conjunctival provocation test in daily practice

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Background: Conjunctival provocation test (CPT) is considered the confirming diagnostic test of ocular allergy. The aim of this study was to compare the diagnostic accuracy of the ocular pruritus score system (OPSS) and the 4 total ocular symptoms score (4TOSS), two different symptoms' score systems recommended by the EAACI Interest Group on Ocular Allergy, for assessing the response to CPT. Secondarily the presence of allergic nasal

and/or bronchial response to CPT was also evaluated.

Method: CPT with mixed grass pollen extract (LETI SL, Madrid, Spain) was performed in 19 subjects (11 patients with moderate-severe allergic rhinoconjunctivitis to grass and 8 controls (4 healthy and 4 allergic controls with allergic rhinitis to *D. pteronyssinus*)). The positive CPT criterion was OPSS ≥ 2, or 4TOSS (pruritus, hyperemia, tearing, and chemosis) ≥ 5. The OPSS and 4TOSS results were compared with SPT results, the gold standard. The presence of nasal and bronchial symptoms was also evaluated. The local ethic committee approved the study. All participants were informed and signed the corresponding informed consent.

Results: OPSS had a higher sensitivity, negative predictive value (NPV) and concordance with SPT than 4TOSS, and the same specificity and positive predictive value (PPV). OPSS had 100% sensitivity, specificity, PPV, NPV, and concordance with SPT (kappa index 1, $P < 0.001$). No cases with false positive or negative responses were observed. 4TOSS also showed a high sensitivity (90.9%), specificity (100%), PPV (100%), NPV (88.9%) and concordance with SPT (kappa index 0.894, $P < 0.001$). Thirty six per cent of cases with a positive CPT had a positive nasal response. No bronchial responses were detected. A significant negative correlation between serum *Phleum*-sIgE levels and grass pollen threshold CPT concentration was detected (Rho -0.918, $P > 0.001$).

Conclusion: In this study the OPSS has demonstrated to be a very useful parameter to assess the response to CPT in patients with moderate-severe allergic rhinoconjunctivitis. The presence of nasal symptoms is a common feature in CPT that should always be evaluated.

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Allergic and nonallergic rhinitis and skin sensitization to metals: is there a link?

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Background: Chromium, Cobalt and Nickel are responsible for contact dermatitis, that is largely prevalent in the general population. They can act also as irritants in the upper and lower respiratory airways. Also rhinitis (allergic and nonallergic) is a high prevalence disorder. Both diseases probably share some common inflammatory mechanisms, but the clinical association between skin sensitization to metals and rhinitis was never studied. We assessed the presence of skin sensitization to metals in subjects with different forms of rhinitis.

Method: Consecutive patients suffering from rhinitis underwent a standard diagnostic procedure, including skin testing, nasal endoscopy and nasal cytology. Control healthy subjects were also included. None of the patients had skin diseases. All subjects underwent patch test with Chromium, Cobalt and Nickel. Rhinitis subjects were subdivided into allergic, nonallergic (vasomotor) and overlapping.

Results: None of the 26 controls had positive skin prick test or nasal cytology. The 82 rhinitis patients were subdivided into allergic (group A = 27), nonallergic (group B = 31) and overlapping (group C = 24). The prevalence of positive patch test to metals was 26% in group A, 45% in group B, 42% in group C and 31% in controls. The percentage of patch-positive subjects was significantly different between Group A and B ($P = 0.0045$; OR: 0.43), Group A and C ($P = 0.0186$; OR: 0.49), and Group B and controls ($P = 0.0360$; OR: 1.85). There was a significant difference between groups A+controls and B+C.

Conclusion: Even in the absence of skin diseases, the prevalence of sensitization to metals (patch test) is greater in nonallergic and overlapping rhinitis as compared to allergic rhinitis and healthy controls.

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Evaluation of allergic sensitization by specific IgE or prick skin tests in patients with allergic conjonctivitis

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Background: Allergic conjunctivitis (AC) is a common allergic condition that strongly affects patients' quality of life. Proper diagnosis and determination of allergic sensitization in patients with conjunctivitis is of great importance for proper control of the disease.

Method: Cross-sectional study conducted at the Hôtel-Dieu de France hospital (Beirut, Lebanon) during a period of 18 months. Patients with seasonal or perennial AC presenting for ophthalmic consultation had measurement of total and specific IgE. A matching group of patients with AC seen at the allergist office during the same period underwent prick skin tests.

Results: Fourty-four patients were enrolled for bloodwork by their ophthalmologists. Seasonal and perennial forms were almost equivalent. Twenty-five patients (56.8%) had presence of positive specific IgE, with higher prevalence in patients with seasonal allergic conjunctivitis ($P = 0.002$), other associated allergies in particular allergic rhinitis ($P = 0.002$) or a family history of allergy ($P = 0.005$). Ocular surface severity scales have not been found as predictors. High levels of total IgE were commonly detected in those with positive specific IgE ($r = 0.345$; $P = 0.022$). Thirty-eight patients were assessed with prick skin testing and all had a positive result for at least one allergen. Dust mites are found to be the most frequent allergens based upon both specific IgE (72%) and prick tests (92%), followed by Parietaria and other pollens.

Conclusion: In our study, Dust mites mono- or co-sensitization is present in the majority of patients with AC, with odds of positivity being higher using prick skin testing than specific IgE. The latter are found more readily in the seasonal form and in the presence of personal and family history of allergy.

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Two cases of severe vernal kerato-conjunctivitis successfully treated with omalizumab and monitored by conjunctival cytology

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Background: Vernal Kerato-Conjunctivitis (VKC) is a severe ocular chronic relapsing disease which can produce loss of visual acuity and blindness. Often treatments with topical antihistamines or corticosteroids, or topical immunosuppressors (i.e.: cyclosporine) are not enough to improve VKC symptoms. Omalizumab is a monoclonal anti-IgE agent approved for treating severe allergic asthma and severe chronic idiopathic urticaria.

Method: A 9 y.o. female child and a 21 y.o. man with severe VKC unresponsive to treatment with topical antihistamines, mast cells stabilizers, corticosteroids and cyclosporine, and only partially and temporary responsive to oral corticosteroids, were respectively treated with s.c. omalizumab 300 mg/months and 600 mg/months for 6

consecutive months from April to September 2015. Conjunctival physical examination was performed at every visit. Each ocular symptom (burning/itching, redness, lachrymation and photophobia)

was evaluated by a visual analogue scale (VAS) and conjunctival scrape smear for cytology was obtained at each visit.

Results: Both patients dramaticaly improved VKD during omalizumab treatment in terms of symptoms (reduction in VAS for each symptom and in need of using topical antihistamines as rescue drugs), physical examination (eye redness and cobble stone papillae were abolished) and conjunctival cytology (eosinophils: from basal 69% in the child and 51% in the young man to 3% and 0% respectively after 6 months of treatment). All these improvements were kept at least for 4 months after discontinuation of treatment (time of submission of this abstract).

Conclusion: Only 3 cases of patients with VKC (and concomitant severe asthma) treated with omalizumab are described, so far, in literature. This is the first report of 2 cases of severe VKC not associated with asthma successfully treated with omalizumab, and for the first time symptoms and physical examination improvements were documented also using cytological examination of conjunctival smear.

Poster Discussion Session PDS 6

Diagnosis and management of drug allergy

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Evaluating beta-lactam allergy with skin testing and drug provocation in the elderly

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Background: Elderly patients with suspected beta-lactam allergy are frequently referred to the allergist when beta-lactam therapy is required. In this study, we analyzed the outcomes of skin testing using skin prick test (SPT) and intradermal test (IDT); and drug provocation test (DPT) in the elderly with history of beta-lactam allergy.
Method: Elderly patients with suspected beta-lactam allergy are frequently referred to the allergist when beta-lactam therapy is required. In this study, we analyzed the outcomes of skin testing using skin prick test (SPT) and intradermal test (IDT); and drug provocation test (DPT) in the elderly with history of beta-lactam allergy.
Results: There were 30 patients, mean age 68.83 ± 7.11 years; 73% female. The suspected culprit drugs were penicillin in 10 patients, aminopenicillin in 16 and cephalosporin in 4. Ten patients were deemed to have probable/possible, 12 indeterminate and 8 unlikely beta-lactam allergy based on history. Nine patients reported urticaria and/or angioedema, 5 anaphylaxis, 1 maculopapular rash, 7 non-specific rashes and 8 unknown reactions. The majority (17/30) were referred because beta-lactam therapy was required. All skin prick tests were negative (mean wheal diameter for histamine was 5.68 ± 1.27 mm). Only one patient, who had anaphylaxis to cefazolin, had positive IDT to minor determinant, ampicillin and cefazolin. Delayed intradermal reading was not done. DPT to amoxicillin or the beta-lactam antibiotic require for treatment of infection was performed in 29 patients with negative skin tests. There were no immediate reactions. Sixteen patients received the beta-lactam antibiotic of choice. One patient developed maculopapular rash after 7 days of amoxicillin.
Conclusion: The majority of the elderly patients referred were found not to have beta-lactam allergy and were able to receive this form of treatment successfully. Skin testing and drug provocation tests are safe and valuable in the evaluation of beta-lactam allergy in the elderly.

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Patients with a history of betalactam hypersensitivity: what's the value of skin tests?

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Background: Betalactams hypersensitivity remains overestimated, mainly among patients with imprecise clinical history, leading to unjustified therapeutic alternatives.

This study has been performed in order to evaluate the skin tests value among patients with a history of betalactam hypersensitivity.
Methods: We included all patients with a history of betalactam hypersensitivity. Reports have been notified to the pharmacovigilance unit of Monastir during 11 years. The drug imputability was established according to betalactam hypersensitivity Naranjo algorithm. Skin tests were performed as recommended by ENDA.
Results: Among 138 cases with a history of betalactam hypersensitivity, skin tests were performed in 123 (89%) of them. For the remaining cases, betalactam's implication was chronologically excluded in 7 cases and 8 patients were lost to follow-up. Skin tests revealed negative results in 97 cases (78%) (in 93 cases, symptoms were not related to drug use and in 4 cases, the culprit drug wasn't a betalactam). Thus, skin tests were positive in 26 cases (21%) and cross reactivity was evaluated for all of them. The hypersensitivity was selective to one betalactam in 5 cases. Cross-reactivity was objectified, between penicillins and cephalosporins in more than half of cases, among penicillins in one third of cases and among cephalosporins in one case.
Conclusion: Through the present study, only 18% of cases with history of beta-lactam hypersensitivity reactions were confirmed after testing. Thus, skin tests are a reliable tool in the evaluation of imprecised history of betalactam hypersensitivity since it help in the identification of the culprit betalactam and the evaluation of cross reactivity.

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Skin tests and drug provocation test utility for the study of betalactams hypersensitivity among chilean patients at an allergy centre of Clinical Hospital University of Chile

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Background: Betalactams hypersensitivity is the most common drug allergy, including immediate reactions and non-immediate reactions to betalactams, both reactions can be studied by *in vivo* or *in vitro* tests. *In vivo* tests include skin prick test (SPT), Intradermal Reaction (IR) and Patch Test (PT). SPT and IR are sensitive in evaluating immediate reactions on betalactams allergy and PT can be useful in the evaluation of non-immediate reactions to betalactams. Allergollogic approach of hypersensitivity reactions to betalactams is difficult, long lasting and not always complete. It is well known that drug provocation test (DPT) is necessary for doing correct diagnosis of this condition, but skin tests are the initial approach.
Objective: To study the diagnostic modalities used in betalactams allergy/ hypersensitivity among patients studied at allergy centre of Clinical Hospital University of Chile between 2012 and middle 2015.
Method: Retrospective review of Skin Prick Test, Intradermal reaction, Patch Test and oral challenge made between 2012 and middle 2015 for diagnosis betalactams allergy in our centre.
Results: 170 patients were studied in the selected period, 35 of them were categorized as allergic to betalactams, 41 not allergic, 15 like inconclusive and in 79 patients the allergologic study was not completed. The 35 patients with positive result for allergy to betalactams, 18 women and 17 men, median age 17 years old. Diagnosis include: urticaria/angioedema (6), anaphylactic shock (7) and maculopapular exanthems (8). Up to 20 of them were categorized as allergic only because

positive PT, 9 positive IR, 3 positive SPT and only 3 for oral challenge. In contrast, 41 patients not allergic to betalactams, 26 women and 15 men, median age 28 years old. Diagnosis include urticaria and/or angioedema (16) and maculopapular exanthems (7). All patients categorized like not allergic had negative results in SPT, IR and PT, and received the culprit drug (DPT).

Conclusion: This study shows the diagnostic modalities perfomance in a chilean centre of allergy. Up to 24% patients were categorized as not allergic to betalactams, this number is very low compared to past datas. Although a lot of patients did not finish the study, is our duty to increase drug provocation tests in patients with suspected betalactams allergy and perform better diagnostic modalities for these patients.

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Intravenus route in beta-lactam challenges

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Background: Controlled drug challenges are the gold-standard procedure for diagnosing drug allergy. Current guidelines recommend using the oral route.

Objective: The aim of this study was to evaluate the performance, in terms of safety and convenience (time-saving), of intravenous drug-challenges.

Method: Retrospective review of beta-lactam challenges performed from January 2013 to December 2015. Intravenous challenges were performed in a hospital setting, using infusion pumps with up-dosing rates every 15 min (average duration 60 min), under strict medical surveillance, using the culprit drug or an alternative, depending on severity and likely-hood of causality (history, skin tests).

Results: Out of 425 beta-lactam challenges, 148 were intravenous: 56% patients were female, with a median age of 57.6 (18–100) years. A median of 7.5 years (IQR 0.0–35.7) had elapsed since the primary reaction. Frequent co-morbidities were: hypertension (31.1%), type 2 diabetes (18%), asthma (7.4%) and ischemic heart disease (3%). 23 (15.5%) patients were on ACE-inhibitors, 15 (10%) on beta-blockers, and 12 (8.1%) on angiotensin receptor blockers. Penicillin was the most frequent drug involved in the primary reaction (42%) followed by amoxicillin with (28%) or without clavulanic acid (20%). In 133 cases (89%) the initial reaction was immediate; severity was mild in 52%, moderate in 24.5% and severe in 14% (in 9.5% the severity was unknown). Skin tests were performed in 87 patients, with a positivity rate of 27 (31%). 103/148 (69.6%) were challenged with the culprit drug with a posivity rate of 0.67% (1/148). The only reaction consisted of a mild non-immediate reaction (pruritus, erythema and dyspnea without objective bronchospasm or desaturation) rapidly resolved after systemic antihistamine and corticosteroid treatment.

Conclusion: In this study, intravenous controlled challenges with beta-lactams proved to be a safe procedure, even in patients with co-morbidities on pharmacological treatments such as beta-blockers or ACEi. Convenience due to the time-saving protocol and to the possibility to control the exact dose administered at each time-point and interrupting the administration of the drug in case of an adverse event, are an added value.

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Positive skin test or positive specific IgE to penicillin does not predict penicillin allergy

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Background: Diagnosis of penicillin allergy is based on case history, skin testing (ST, prick and intracutaneous tests) and measurement of specific IgE (s-IgE) to a penicillin and challenge with penicillin. If ST or s-IgE is positive, the patient is classified as allergic to penicillin according to the European Network of Drug Allergy guidelines and challenge is omitted. Therefore, the true sensitivity and specificity of ST and s-IgE are presently not known.

The aim of this study was to investigate the clinical relevance of a positive skin test and s-IgE to penicillin.

Method: Forty-four patients with a positive ST and/or s-IgE were included; ST with penicillin was done and s-IgE was measured in all 44 patients at the time point designated $\mathbf{T_0}$. Challenge with the culprit penicillin was performed immediately hereafter although abstained in patients with recent anaphylaxis to penicillin ($n = 8$), systemic reactions to ST ($n = 3$) or development of delayed positive ST ($n = 8$). These were classified as allergic to penicillin; thus, 25 patients were challenged.

18 of the patients had been evaluated previously ($\mathbf{T_{-1}}$) and reproducibility of the results were compared to $\mathbf{T_0}$ and again four weeks post challenge ($\mathbf{T_1}$).

Results: Nine of the 25 challenged patients were positive. There was a significantly increased probability of being allergic to penicillin if both skin test and s-IgE were positive at $\mathbf{T_0}$ ($P = 0.007$). Positive ST or s-IgE alone did not predict penicillin allergy ($P = 0.313/P = 0.051$).

Among the 18 patients repeatedly tested, only 12/26 (46.2%) of positive skin tests at $\mathbf{T_{-1}}$ were reproducibly positive at $\mathbf{T_0}$, and only one further ST became positive at $\mathbf{T_1}$. For s-IgE, 14/24 (58.3%) of positive measurements were still positive at $\mathbf{T_0}$ and seven further became positive at $\mathbf{T_1}$, although s-IgE levels at $\mathbf{T_0}$ and $\mathbf{T_1}$ did not differ significantly ($P = 0.599$).

Conclusion: In this study, the best predictor for a positive penicillin challenge was history combined with both positive ST and s-IgE. There was a relatively low reproducibility of a previously positive ST and s-IgE.

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Desensitization to chemotherapy: our experience

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Background: Desensitization is a procedure that allows the administration of a necessary drug to a patient allergic to it. These procedures should be performed in a hospital setting in accordance to very specific protocols and must be done by highly qualified and trained personnel in the management of severe hypersensitivity reactions that may occur during the procedure. We expose our experience with patients undergoing a chemotherapy desensitization process.

Method: Demographic, clinical and safety variables were collected in all patients included in the process of desensitization to chemotherapy conducted by the Allergology Service of our Hospital during 2010–2015.

Results: 102 desensitization procedures were administered to 33 patients (3.09 procedures pp) with a median age of 59.5 years, of whom 29 (87.8%) were women and 4 (12.1%) were men.

The patients, all oncology patients, were being treated for Breast Ca 9 (27.27%); Ovary Ca 8 (24.24%); Colon Ca 6 (18.18%): Rectal Ca 3 (9.09%); Lung 2 (6.06%); Endometrial 2 (6.06%); Cervix 1 (3.03%), Bladder 1 (3.03%); Prostate 1 (3.03%).

The drugs used in desensitization procedures were: Docetaxel in 9 patients (27.27%) and in 27 cycles; Oxaliplatin in 9 patients (27.27%) and in 22 cycles;

Paclitaxel in 7 patients (21.21%) and in 19 cycles; Carboplatin in 7 patients (21.21%) and 31 cycles and Cisplatin in 1 patient (3.03%) and in 3 cycles.

Patients were diagnosed with hypersensitivity to chemotherapeutic drugs by clinical history and by an allergology study with skin tests that were positive for the drugs responsible for the reaction in 22 patients (66%) and negative in 11 (33%).

Adverse reactions occurred during cycles of desensitization in 14 (42.42%) patients requiring treatment, but all patients were able to complete the scheduled cancer treatments.

Conclusion: Desensitization with chemotherapeutic drugs is a feasible procedure that allows the administration of drugs of first choice in oncology patients allergic to these and where avoidance is not a choice. It can be considered safe when performed by highly qualified personnel and done with the appropriate protocols.

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Desensitization to anti-neoplastic drugs: experience of 225 procedures in 51 patients

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Background: Anti-neoplastic drugs are able to elicit allergic and/ or infusion reactions in some patients. Nowadays, desensitization is an option to continue with first line treatment. Here we present a prospective observational study of three years experience with desensitization in a third level hospital.

Method: Patients referred from Oncology Department for chemotherapy suspected allergic reaction during the study period (January 2013 – December 2015) were recorded. Severity of reactions was graded in three levels according to Brown's classification.

Skin test were performed in patients with suspected allergy to platinum.

Desensitization was offered to all patients who experienced an immediate hypersensitivity reaction and in whom Oncologist advised to continue with the culprit drug.

Patients were premedicated with aspirin 200 mg, montelukast and/ or antihistamines. A one solution protocol was used for desensitization. The drug was prepared at the usual concentration and administration started at 5 ml/ h and then stepped up to 10, 25, 50, 75 and 100 ml/h at 15 min intervals. Reactions were managed by giving treatment and return to a previous step and then restart the protocol with one intermediate step.

Results: 114 patients were referred during the study period and 74 (54 women) presented immediate reactions.

Implicated drugs were oxaliplatin ($n = 35$, 47.29%), carboplatin ($n = 15$, 20%), paclitaxel ($n = 9$, 12.16%), docetaxel ($n = 4$, 5.4%), monoclonal antibodies ($n = 5$) doxorubicin ($n = 3$) and others.

Skin tests were positive to oxaliplatin ($n = 30$, 85%) and to carboplatin ($n = 11$, 73%).

Treatment was stopped in 20 patients because tumoral progression or they were in the last cycle of adjuvance. Three patients refused desensitization.

51 patients (32 women, mean age 57.07, range 32–79) were desensitized to 52 drugs and received 225 cycles. All but three procedures were completed. There were 15 mild or grade I (6.66% of cycles), 7 moderate or II (3.11%) and one severe (0.44%) reactions. Most reactions (70%) occurred in 50 and 75 ml/h steps. There were no fatalities or hospital admissions due to desensitization.

Conclusion: Platines and taxanes are the agents most frequently implied in immediate reactions to anti-neoplastic drugs.

Skin test are useful for diagnostic in reactions due to platinum derivatives.

Desensitization in an Allergy Unit proved to be effective in our study population.

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Risk stratification for penicillin desensitization in allergic pregnant women with syphilis

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Background: Allergy to penicillin is a major cause of drug anaphylaxis worldwide and may preclude the only effective treatment of pregnant women with syphilis. Rapid Drug Desensitization (RDD) is an alternative approach and has become a cornerstone in the management of anaphylaxis to drugs, but biomarkers to predict breakthrough reactions during the procedure are needed.

Method: The study assessed the safety and efficacy of risk stratification for guiding penicillin re-introduction in pregnant women with syphilis and history of immediate hypersensitivity reaction (HSR) to penicillin. According to the risk stratification, which was based on the initial HSR and skin testing, patients were re-exposed to penicillin either through desensitization, provocation or regular administration. Patients with a clinical history suggestive of penicillin-anaphylaxis and/or positive immediate skin test were considered at high risk and were desensitized.

Results: We evaluated 15 pregnant women with latent syphilis and history of penicillin allergy. Clinical history was suggestive of immediate HSR in 8 out of these 15 (53.3%) patients, who were desensitized. Intradermal tests were positive in 3/8 (37.5%) and all of them reacted during RDD. The remaining 5/8 (62.5%) patients had negative skin tests and underwent uneventfully RDD. There was a statistically significant association between positive intradermal tests and breakthrough reactions ($P = 0.03$). Seven patients (87.5%) completed the RDD. One patient had a severe breakthrough reaction with uterine contraction and did not finish the procedure. The other 7/15 (46.7%) patients without a history suggestive of HSR and with negative skin tests were subdivided based on clinical history. History was uncertain in 3/7 (42.8%) and these patients were submitted to drug provocation. The remaining 4/7 (57.2%), with an inconsistent history of HSR, received regular administration. These 7/15 patients did not present any adverse reactions until penicillin full treatment.

Conclusion: Risk stratification based on the initial clinical reaction and skin testing to guide penicillin re-introduction, including RDD, was safe and effective. Skin testing identified allergic patients to penicillin with increased risk of reactions during RDD.

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Choosing an alternative drug in analgesic allergy

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Introduction: Although drug allergies represent a small percentage of adverse drug reactions, it continues to be a considerable health problem both for patients and physicians. The most common drug reactions are due to antibiotics and analgesics. In this report we aimed to present our clinical experience about management of analgesic allergy.

Method: The documents of the patients who applied to GATA Immunology and

Allergy clinic between January 2010 and June 2014 related to analgesic allergy were retrospectively evaluated.

Results: In the range of between 18 and 78 years, a total of 100 patients including 48 women and 52 men were suspected to have hypersensitivity reactions caused by analgesics. Of these patients, eighty-nine defined early, 8 defined late and 3 defined both late and early reactions after analgesic use. Also, while fifty patients indicated a single analgesic reaction, the other fifty patients expressed multiple drug reactions with analgesics. The most commonly incriminated analgesics were flurbiprofen ($n = 30$), naproxen ($n = 19$), metamizol ($n = 19$), diclofenac ($n = 18$), paracetamol ($n = 17$), acetylsalicylic acid ($n = 17$) and dexketoprofen ($n = 13$). Remained 11 patients did not remember the suspicious analgesic. To evaluate a possible analgesic allergy, skin tests were performed in 72 patients, oral provocation tests were performed in 22 patients and patch tests were performed in 9 patients. A total of 47% of the patients had positive test results. These results were with paracetamol in 4 of the 63 patients, with meloxicam in 15 of the 74 patients, with dexketoprofen in 6 of the 59 patients, with tenoxicam in 2 of the 25 patients, with diclofenac in 6 of the 19 patients, with metamizole in 6 of the 15 patients, with acetyl salicylic acid in 6 of the 7 patients and with naproxen in 2 of the 2 patients.

Conclusion: As a result of analgesic tests performed in our clinic the most negative results were obtained with paracetamol, meloxicam, tenoxicam and dexketoprofen. On the basis of test results these drugs may be recommended as an alternative analgesic for the patients previously suffered from analgesic allergy.

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The value of the clinical history for the diagnosis of immediate NSAIDs hypersensitivity and safe alternative drugs in children

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Background: Diagnosing hypersensitivity reactions that develop as a result of NSAIDs with history is mostly misleading, and skin tests and/or provocation tests are needed for definitive diagnosis. We aimed to determine the frequency of actual NSAIDs hypersensitivity and whether there were any parameters in the history to predict NSAIDs hypersensitivity. In addition, to determine safe alternative drugs for children who are diagnosed with actual NSAID hypersensitivity.

Methods: Children with a history suggesting NSAIDs hypersensitivity were evaluated by an allergist. Safe alternatives in children with a confirmed NSAID hypersensitivity were found by oral provocation tests. The Inonu University Hospital ethics committee approved the study, and written informed consent was obtained from all patients and/or their parents.

Results: Sixty-four patients who were admitted with a suspicion of immediate-type reaction to NSAIDs were included in the study. The median age of the patients was 6 (1–17) years old, and 37 (57.8%) of the patients were male. We performed skin tests with the suspected NSAID in 35 (54.7%) patients. Of these, two had positive results. Provocation tests were performed on 62 patients whose skin tests were negative or skin tests were not available. During the provocation tests, 16 patients (25.8%) developed reactions. Low- and high-dose acetaminophen, nimesulide, and tolmetin sodium were used to find safe alternative drugs. Two patients developed reactions to high-dose acetaminophen but no reaction to nimesulide and tolmetin sodium. When statistically significant parameters were analyzed in a logistic regression model, the presence of multiple NSAIDs hypersensitivity in the patient history (OR: 26.6, 95%CI: 1.47–481.63, $P = 0.026$) and the emergence of a reaction within an hour (OR:26.4, 95%CI: 1.73–403.11, $P = 0.019$) were found as the independent factors to predicted actual NSAIDs hypersensitivity.

Conclusion: The emergence of a reaction within an hour of taking the drug and the presence of multiple NSAIDs hypersensitivity history increases the possibility of actual NSAIDs hypersensitivity. Nimesulide, low-dose acetaminophen, and tolmetin sodium can be used as safe alternative drugs in patients with multiple NSAIDs hypersensitivity.

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Who admitted for testing with local anesthetics and who should be tested?

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Background: Although majority of the reactions related to Local Anesthetics (LA) are either toxic or autonomic in origin, debate still continues about management of hypersensitivity reactions with LA agents as many patients are refferred to an allergist because of assumption that the reaction is allergic. Other than this, the patients with other allergies such as asthma, allergic rhinitis are also referred for these tests despite no history of hypersensitivity to LAs. In this study, we aimed to documente the indications and test results of the patients referred to our clinic for testing with LAs in real life conditions and provide data related to necessaty of these tests.

Method: All consecutive subjects who were referred for evaluation of LA testings were included into the analysis. Ethical committie approved the study. Demographics as well as disease characteristics of the patients recorded. All subjects were underwent skin prick/intradermal tests with LAs which was followed by subcutaneous challenge in case of negativity of the formers. The patients were not underwent testing with LA for diagnostic purpose. Instead, they were tested for the LA recommended by their physician. If the patient had a history of a allergy to a certain LA, another amide group was selected for testing. Unless a specific LA was stated by their physician; lidocain was tested if no history of hypersensitivity existed. Otherwise, requested LA was tested.

Results: A total of 228 subjects were included. The main referral reason (56%) was presence of history of drug hypersensitivity to other drug classes which was followed by asthma in 21.4% of the cases. History of LA allergy was existed in 64 cases (28.1%). The most common LA used in the tests were prilocain (46.8%) and mepivacain (31.1%). Ten out of 228 cases (4.3%) had positivity in skin testing/challenges. Eight out of 10 cases who underwent to skin testing/challenge with an alternative LA were tolerated the alternative LAs.

Conclusion: The results of the study reflect the real life profile of the patient's admission for LA testing and indicates that the majority of the patients who referred to our clinic because of requesting tests for LAs are able to tolerate the tested LAs. It is seems worth of doing these tests in patients with LA allergy. Although the risk is low, the patients with history of drug hypersensitivity to other drugs can also be considered for testing with LAs.

Poster Discussion Session PDS 7

Innate Immunology

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Synergistic collaboration of Fc-gamma receptor III and TLR-4 define the pro-inflammatory responses of nasal epithelium during re-infection with *P. aeruginosa*

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Introduction: The bacterial flora of the nasal cavity comprises of many different bacterial species, including potential opportunistic pathogens, such as *Pseudomonas aeruginosa*. It is a Gram-negative bacterial pathogen capable of causing a broad range of infections of the lower and upper airway. LPS, a major component of bacterial cell wall is a potent inducer of the innate immune responses. The innate immunity may not be sufficient for a complete eradication of an invading pathogen, therefore it is followed by the adaptive immune responses that lead to a production of antibodies and consequently elimination of the microbe. Epithelial cells play an important role within the innate responses since they are the first line of defense against microbes residing in the nasal cavity. They are able to recognize potential immunogens by pattern recognition receptors (PRRs), including Toll-like receptors. Stimulation of an individual PRR is known to induce cell responses, however the cross-talk between multiple receptors may define the ultimate amount and the profile of cytokine production. Here, we seek to investigate how a development of the adaptive immunity and, as a consequence, a subsequent presence of specific IgGs against *P. aeruginosa* contributes to responses of the nasal epithelial cells to *P. aeruginosa* challenge.
Methods: We exposed nasal epithelium to IgG-opsonized *P. aeruginosa* or to LPS co-stimulated with IgG and measured the production of cytokines and their gene expression. Selective Fc-gamma receptors were blocked to determine their contribution to epithelium responses to LPS+IgG.
Results: Despite the presence of the LPS-binding complex, nasal epithelium does not respond to challenges with *P. aeruginosa* and consequently to its major cell wall component LPS. However, when *P. aeruginosa* is opsonized with IgG or LPS stimulation is accompanied by IgG, the tolerance of the nasal epithelium is broken and a massive production of IL-6 and IL-8 can be measured. Blocking of receptors revealed that the pro-inflammatory responses to *P. aeruginosa* are mediated by the low-affinity Fc-gamma receptor III and TLR-4.
Conclusion: During a re-infection, IgG rapidly opsonize *P. aeruginosa* and only then the local pro-inflammatory responses of nasal epithelium can be triggered. The data indicate the complexity of cell responses to this pathogen and that the involvement of the adaptive immunity may be crucial for proper cell responses to *P. aeruginosa*.

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Short chain fatty (SCFA) acids induce apoptosis in peripheral blood eosinophils and promote endothelial barrier function

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Background: The increasing incidence of allergic inflammatory diseases points out the growing necessity for eosinophil-targeting therapeutics. Accumulation of eosinophils in the lung tissue is a hallmark of asthma and it is believed that eosinophils play a crucial pathogenic role in allergic inflammation. Short chain fatty acids, e.g. acetate, propionate and butyrate are produced in high concentration in the gastrointestinal tract by commensal bacteria and are readily secreted into the blood stream and thereby show various biological functions. Prompted by the observation that propionate hampers lung eosinophilia in models of allergic inflammatory diseases we hypothesize that SCFA modulate the survival and the recruitment of eosinophils.
Method: Ca^{2+} flux and respiratory burst in human eosinophils was measured with flow cytometry. Induction of apoptosis was detected using annexin V/propidium iodide (PI) double staining and caspase 3/7 activation assay. Additionally, expression of adhesion molecules on pulmonary microvascular endothelial cells was analyzed by flow cytometry. Endothelial resistance was detected via cell substrate impedance sensing (ECIS).
Results: We found that propionate concentration-dependently induces Ca^{2+} flux and respiratory burst in isolated human eosinophils. Both, propionate and butyrate significantly reduce the survival of human eosinophils starting 18 h after treatment. Similarly, activation of caspase 3/7 was induced by propionate and butyrate. Interestingly, this observation was restricted to eosinophils from allergic donors. Moreover, propionate strengthens the barrier function of human pulmonary endothelial monolayers and concentration dependently decreases the TNF-α induced expression of VCAM and E-selectin.
Conclusion: Our results suggest that propionate and butyrate induce apoptosis in human eosinophils from allergic donors, reduce the expression of adhesion molecules on pulmonary microvascular endothelial cells and, furthermore, strengthen the endothelial barrier function. We therefore propose that propionate and butyrate could serve as potential therapeutic agents in allergic inflammatory diseases.

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Human olfactory mucosa-derived mesenchymal stem cells induce a tolerogenic profile in monocyte-derived dendritic cells

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Background: Immunosuppressive activity of mesenchymal stem cells (MSC) may interact with dendritic cells (DC). This study assesses the effects of olfactory mucosa-derived mesenchymal stem cells (hOM-MSCs) on the antigenic profile of dendritic cells (DC).
Methods: Nasal mucosa samples were taken from 8 patients with non-inflammatory diseases of nasal cavity. To obtain hOM-MSCs cells explant culture methods were applied. The obtained hOM-MSCs

were $CD90^+CD105^+CD73^+/CD31^-CD45^-$. Monocyte-derived dendritic cells (mdDC) were obtained from peripheral blood monocytes cultured for 7 d in the serum-free media containing GM-CSF and IL-4. mdDC and hOM-MSCs were co-cultured in a ratio of 1:1 providing direct cell-to-cell contact (MSC-DC) and using culture insert to avoid direct cell contact (Ins-DC). Negative (DC cultured without stimuli – iDC) and positive controls (mdDC cultured with LPS – LPS-DC) were also used. mdDC were assayed for expression of CD32, CD80, CD85k, CD86, CD273 and HLA-DR antigens.

Results: LPS-DC were characterized by increased expression of CD32, CD80, CD86, HLA-DR and significantly reduced expression of CD85k and CD273. mdDC cultured in the cell insert over the monolayer of hOM-MSCs phenotypically were similar to the iDC. Only the direct cell contact led to DC differentiation towards the tolerogenic profile. Increased expression of CD85k and CD273 and reduced expression of HLA-DR, CD80 and CD86 was shown.

Conclusion: Direct cell-to-cell contact of hOM-MSCs and mdDC led to the induction of the tolerogenic profile of the DC. hOM-MSCs are a promising tool for generation of tolerogenic DC ex vivo.

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Higher expression of complement receptor 1 on monocytes and granulocytes during bacterial infection than during viral infection in children

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Background: Clinical differentiation between bacterial and viral infection is very difficult. Unfortunately, there is still lack of quick and accurate diagnostic test, which would help clinicians in establishing the diagnosis and taking a decision on a treatment. Complement receptor 1 (CD35) is involved in phagocytosis of IgG- and complement-opsonized pathogens and plays an important role in inflammatory processes. The aim of the study was to compare the expression of CD35 antigen on phagocytes during bacterial and viral infection in children.

Method: The expression of CD35-FITC, CD14-APC, CD15-V450, CD45-AmCyan in 40 blood samples from children with high fever and infection suspicion was assessed by flow cytometry (FACSCantoII). Only 100 µl of residue blood collected on EDTA (tube for CBC test) was used for analysis. 27 children were diagnosed with bacterial and 13 with viral infection. Expression of CD35 was analyzed according to mean fluorescence intensity (MFI) and antibody binding cites (ABC). Statistical analysis was performed using nonparametric Mann-Whitney test for independent samples.

Results: CD35 antigen had significantly higher MFI on granulocytes (median (25 percentile; 75 percentile): 3437 (2081; 5666), 2140 (1453; 3033), respectively, $P = 0.0104$) and monocytes (median (25 percentile; 75 percentile): 5486 (3684; 8717), 3519 (2277; 3977), respectively, $P = 0.0041$) during bacterial infection in children in comparison to viral infection. Significantly higher ABC for CD35 antigen on granulocytes (median (25 percentile; 75 percentile): 82178 (50006; 135187), 51414 (34993; 72676), respectively, $P = 0.0136$) and monocytes (median (25 percentile; 75 percentile): 130922 (88167; 207346), 84246 (54669; 95116), respectively, $P = 0.0041$) was also observed during bacterial infection in comparison to viral infection.

Conclusion: Measurement of expression of complement receptor 1 on peripheral blood phagocytes could help in distinguishing between bacterial or viral origins of infection and facilitate speedy decision on a treatment. It may help to avoid unnecessary use of antibiotics.

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Maturation of innate immune responses of the respiratory epithelium

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Background: The respiratory epithelium is critical both for the clearance of infections and the development of adaptive responses. Very little is known on the maturation of epithelial responses. The aim of the present study was to assess the maturation of epithelial responses to viral infection in relation to age.

Method: Primary nasal epithelial cells (NECs) were obtained from healthy ($n = 26$) donors of a wide age range (0–55 years). NECs were cultured and infected with Human Rhinovirus 1B (RV1B). Expression of IFNB1 mRNA was measured with RTQPCR. A large array of epithelial cytokines ($n = 25$) were measured in cell culture supernatants at 48 h with Luminex. Virus replication was titrated. Cytotoxicity levels were evaluated at 24 h, 48 h, 72 h with crystal violet staining. Age-related differences were evaluated by regression analysis.

Results: RV1B-induced IFNB1 mRNA expression, linearly increased with age ($P < 0.05$). Also, IL28A protein increases with age in healthy NECs after RV1B infection ($P < 0.05$). CCL5 protein, a downstream effect, increases with age in healthy NECs after RV1B infection ($P < 0.05$). Virus load was higher in NECs from children than adults at 8 h post infection ($P < 0.05$). Cytotoxicity levels of healthy NECs increase with age infection at 48 h and 72 h after RV1B ($P < 0.05$).

Conclusion: This is the first study investigating the maturation process of airway epithelial responses to viral infection, showing age-related evolution of antiviral responses. IFN responses upon RV1B infection increased with age.

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Specific induction of TSLP by the viral RNA analogue poly(I:C) in primary epithelial cells derived from nasal polyps

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Introduction: Chronic rhinosinusitis with nasal polyposis is an inflammatory disease that, although not directly linked to allergy, often displays a Th2-skewed inflammation characterized by elevated local IgE and IL-5 levels. The nasal cavity is constantly exposed to bacteria and viruses that may trigger epithelial inflammatory responses. To gain more insight into mechanisms by which such a biased inflammation might arise, we have investigated the epithelial expression of the Th2 skewing mediators (TSLP, IL-25, and IL-33) in relationship to disease and microbial triggers.

Methods: Epithelial cells were obtained from polyp tissues of nasal polyposis patients and from inferior turbinates of non-diseased controls. Cells were exposed to various TLR-specific triggers to study the effect on mRNA and protein expression level of TSLP, IL-25, and IL-33 and the potential regulatory mechanisms through the expression profile the transcription factors ATF-3, DUSP-1, EGR-1, and NFKB-1.

Results: The TLR3 agonist and viral analogue poly(I:C) induced TSLP mRNA 13.0

± 3.1 fold ($P < 0.05$) and protein expression by 12.1 ± 2.3-fold ($P < 0.05$) higher in epithelium isolated from nasal polyposis patients than in epithelium form healthy controls. No statistically significant differences between responses of polyp or control epithelium to TLR1, TLR2, TLR4, TLR5, TLR7/8, and TLR9 agonist could be measured. Gene expression analysis revealed a significant down-regulation of the DUSP-1 gene in polyp epithelium. The enhanced induction of TSLP may be a consequence of a down-regulated expression of DUSP-1, since knocking-down of DUSP-1 led to an increased production of TSLP upon poly(I:C) challenge.

Conclusion: The TLR3 induced expression of TSLP introduces a mechanism by which the Th2-skewed tissue environment might arise in nasal polyps and invites a further evaluation of the potential contribution of current or past viral infections to polyposis pathogenesis.

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Functional assessment of virus-recognizing receptors in human Langerhans cells

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Background: Keratinocytes and Langerhans cells(LCs) ascertain a potent skin barrier against environmental threats. To better understand whether LCs respond functionally to viral antigens, we investigated whether virus-recognizing receptors such as TLR3, RIG-1, MDA5 and PKR can be activated/regulated in LCs upon recognition of poly(I:C).

Method: To efficiently disrupt the physical epidermal barrier which mainly consists of the stratum corneum, normal human skin obtained from plastic surgery, was stripped sequentially 50 times to remove this layer. Punch biopsies were then placed in 24 well culture plates and PBS (control) or poly(I:C), a potent inducer of a strong inflammatory response in several cell types, was epicutaneously applied. Samples were harvested after 24 and 48 h of incubation. Cryosections and epidermal sheets were prepared and analyzed for virus-recognizing receptor expression in skin cells using immunofluorescence. In addition, migratory LCs were isolated from skin explants upon 48 h of culture. After $CD1a^+$ magnetic bead sorting, LCs were incubated with poly(I:C) for 2 h and analyzed as describe above.

Results: We found that poly(I:C) upregulated TLR3 but not PKR in keratinocytes and failed to upregulate/induce TLR3 and PKR in LCs. In contrast, in the presence of poly(I:C), MDA5 was strongly upregulated in some resident and emigrating LCs compared to controls. Keratinocytes failed to express MDA5.

Conclusion: Our data suggest that not TLR3 but MDA5 may play a key role in the innate immune response of LCs to viral infection. This model will now allow us to further define the key signaling pathways involved in LCs as well as in keratinocytes upon viral infection.

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CAPS and TRAPS in Russian patients with manifestation of systemic juvenile idiopathic arthritis

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Background: The main clinical manifestations of monogenic autoinflammatory syndromes and polygenic Systemic juvenile idiopathic arthritis (sJIA) are similar and presented with fever, myalgia, migratory erythematous rash, arthritis or arthralgia, eye manifestations, and abdominal pain. The right diagnosis is reached using gene analysis and prognosis depends on correct personified therapy.

Method: 130 pts (41 boys, 89 girls) at the age from 1 to 17 years. (7.0 (3.9;9.5) followed with a diagnosis of sJIA were selected according to the clinical manifestations, with subsequent obligatory genetic counseling in the Department of Rheumatology of the Scientific Center for Children's Health. The median age of disease onset was 2.9 (1.2;6.1)y., disease duration – 4.1 (2.4;5.6)y. The commonest features were fever (100%), arthritis or arthragia (100%), rash (125 pts/96%), hepato- and splenomegaly (122/94%), lymphadenopathy (120/80%), headache (67/52%), abdominal pain (75/58%) and eye manifestations (31/24%). Pts' DNA was sequenced in all coding exons and intronic flanks of the TNFRSF1A and NLRP3 genes.

Results: In 14/130 (10.8%) pts genetic autoinflammatory syndrome was established. In 11 pts we found mutations in TNFRSF1A: In 9/11 pts – the most frequent mutations c. 362G > A (p.R92Q) located in exon 4 and associated with the mild progression of TRAPS. One TRAPS pt. had a frameshift mutation c. 792delT (p.Lys265Serfs*87) in exon 9 of TNFRSF1A gene. Another pt revealed a TNFRSF1A mutation c. 374G > A (C96Y). The median age of disease onset was 5.3 years. Pts had a median 85 symptomatic days per year, attacks were recurrent in 79%. A family history was present in 3 pts: 2 girls with R92Q and one – with C96Y mutation.Three pts identified mutations in NLRP3 gene. None of the mutations were previously described in the databases for mutations. One pt had a mutation c. 796C > T (p.Leu266Phe) in exon 04 of NLRP3 gene. Two other pts had mutations c. 2861C > T and c. 2173C > A, respectively. Pts with c. 2173C > A and c. 796C > T mutations have CINCA/NOMID phenotype and dramatic effect of canacinumab. In 8 (6.2%) pts we identified NLRP3 gene polymorphism Q705K associated with elevated levels of IL1.

Conclusion: Our results suggests for a relatively frequent incidence of CAPS and TRAPS in Russian systemic JIA pts. The number of genetically confirmed pts with periodic fever syndromes in Russia is very low. It is important to establish a network for genetic testing of periodic fever syndromes.

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The relationship between chronic hepatitis C virus infection and TNFα-308 gene polymorphism

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Background: Tumor Necrosis Factor Alpha (TNFα) plays a significant role in the pathogenesis of Chronic Hepatitis C Virus Infection (CHC). In this study, the relationship between KHC infection and TNFα-308 (rs. 3091256) polymorphism was investigated.

Methods: The study was carried out with 95 anti-HCV positive patients (70 HCVRNA positive – Chronic Active Hepatitis C [CHC]), 25 HCVRNA negative (Spontaneous Clarence [SC]) and 97 healthy control group admitted to Gaziosmanpasa University, Faculty of Medicine, Department of Infection Disease clinic between the dates January 2011 – February 2012. All of the patients in the CHC group infected with HCV genotype 1 and received treatment for 48 weeks (Pegilated INFalpha + Ribavirin). They were monitored for

6 months at the end of treatment. AST/platelet ratio index (APRI) scoring system was used for determining the stage of liver fibrosis. TNFα-308 (rs 3091256) genotyping were evaluated with PCR-RFLP method. PCR was performed by using DNA samples obtained from the blood of patients and control individuals. Genotyping was done by PCR products being cut by the use of Nco 1 Cutting Enzyme and being separated with 2.5% Agarose Gel Electrophoresis.

Results: There was no significant difference when CHC patients (62 female, 33 male) and healthy control group (57 female, 40 male) were compared in terms of TNFα-308 (rs3091256) polymorphism genotype distribution ($P = 0.362$). While AA genotype was detected in the study group with the rate of 16.3%, but not in the control group. This can be relatively due to lack of sample, a significant difference couldn't be found ($P = 0.119$).

Results of the comparison between CHC and SC groups are given in the table. ALT values were found higher in patients with TNFα-308 GG polymorphism compared to patients with GA + AA polymorphism ($P = 0.036$) among CHC patients. APRI score was significantly found high in CHC patients with TNFα-308 GG polymorphism compared to the patients with GA+AA polymorphism ($P = 0.0006$).

Conclusion: TNFα-308 (rs3091256) polymorphism was not related to the recovery of Chronic Hepatitis C. TNFα-308 GG polymorphism in KKC patients was found related to high ALT and high fibrosis stage.

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The relationship between Crimean-Congo hemorrhagic fever virus and IL-28B gene polymorphism

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Background: Crimean-Congo Hemorrhagic Fever Virus is one of the subgroups of Nairovirus group′s which involved to Bunyaviridae family, leads to acute zoonotic viral infection associated with findings as fever, ecchymosis, bleeding, thrombocytopenia and liver dysfunction. In this study, fatality and severe clinical course factors investigated on CCHF patients and relationship with severe prognosis and IL 28-B gene polymorphism was evaluated.

Materials and methods: In this study 107 patients were included which hospitalized and monitored with preliminary diagnosis of CCHF that CCHFV RNA were positive in the service of Infectious Diseases and Clinical Microbiology, Gaziosmanpaşa University Faculty of Medicine Research and Application Hospital. IL 28-B rs12979860 gene polymorphism was determined by PCR-RFLP method with the blood samples taken from patients. IL 28-B gene study results and the results of various data which recorded and laboratory values of patients' were used in the statistical analysis.

Result: In 9 of 107 patients (8.4%) resulted in death and the others were discharged uneventfully by the course of the disease. In 24 cases (22.43%) CC genotype, 64 patients (59.81%) CT genotype, 19 patients (17.76%) TT genotype was detected. In the 3 patients (33.33%) CC genotype, 6 patients (66.67%) CT genotype was detected of group presenting with death. The TT genotype was not detected in any of the ex patients.

found between fatality and IL28B rs12979860 polymorphism. In the patients who died, abdominal pain ($P = 0.001$), diarrhea ($P < 0.001$), bleeding ($P < 0.001$), rash ($P < 0.001$), PT prolongation ($P = 0.023$), aPTT prolongation ($P = 0.007$), INR prolongation ($P = 0.004$), CK elevation ($P < 0.001$), LDH levels ($P < 0.001$), AST ($P < 0.001$), ALT elevation ($P < 0.001$), platelet decrease ($P < 0.001$), leukocytosis ($P = 0.040$) found statistically significant.

Conclusion: In conclusion, although none of the patients showing fatal course had TT genotype, fatality or serious clinical course pointing findings were not have statistically significant relationship between IL 28-B rs12979860 gene polymorphism and Crimean-Congo haemorrhagic fever disease. Previous studies based on fatality associated with a known PT prolongation, aPTT prolongation, CK, LDH levels, AST, ALT, platelet impairment and bleeding symptoms, in addition to our study it is found that leukocytosis, abdominal pain and diarrhea were more often in fatal cases than others.

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A possible role of galectin-9 in the lung inflammation and fibrosis of patients with interstitial pneumonia

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Background: Galectin-9 (Gal-9) is a β-galactoside-binding protein that exhibits various biological reactions, such as chemoattraction, cell aggregation, and apoptosis. Recent studies demonstrated that Gal-9 has a role as an immunomodulator in excessive immunological reactions by expanded regulatory T cells (Tregs). We examined the role of Gal-9 in the pathogenesis of one of major idiopathic interstitial pneumonia, cryptogenic organizing pneumonia (COP) as compared with idiopathic pulmonary fibrosis (IPF).

Method: Gal-9, transforming growth factor-β1, and interleukin (IL)-10 levels in the bronchoalveolar lavage fluid (BALF) of patients with COP and IPF were estimated by enzyme-linked immunosorbent assay. Forkhead box protein 3 (Foxp3) expressing Tregs were evaluated by flow cytometry. The effect of Gal-9 on the growth and apoptosis of human lung fibroblast cells was assessed *in vitro*. The effect of Gal-9 on interactions between human lung fibroblast cells and hyarulonan was also assessed *in vitro*.

Results: Gal-9 and IL-10 levels in the BALF were significantly higher in patients with COP than in patients with IPF. The number of CD4+Foxp3high+ cells was significantly higher in the BALF of patients with COP than in those with IPF. Gal-9 levels significantly correlated with the absolute number of CD4+CD25+Foxp3+ cells or CD4+Foxp3high+ cells, but not with the absolute number of CD4+CD25+Foxp3− cells, in the BALF of patients with COP. Gal-9 suppressed the growth of human lung fibroblast cells and induced apoptosis of them in a dose-dependent manner. Further, Gal-9 suppressed the CD44-dependent interaction of human lung fibroblast cells with hyarulonan in a dose-dependent manner.

Conclusion: Our findings suggest that increased Gal-9 levels in the lung have a protective role against lung inflammation and fibrosis in patients with COP through the induction of Tregs in the lung and CD44-dependent inhibitory effects on lung fibroblast cells.

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Immunological mechanisms activated by a polyvalent bacterial preparation used for the treatment of recurrent urinary tract infections (RUTIs)

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Background: MV140 is a defined mixture of entire inactivated bacteria (*K.*

pneumoniae, *E. coli*, *P. vulgaris* and *S. faecalis*, 25% each) used as a novel sublingual vaccine that has been shown to prevent recurrent urinary tract infections (RUTIs). Although the mucosal immune system at both induction and effector sites are likely to be involved, the underlying immunological mechanisms induced by MV140 remain unknown.

Objective: To study the capacity of MV140 and its individual bacterial components to immunomodulate the phenotype and function of human dendritic cells at the molecular level.

Method: MV140 and the individual bacteria were from Inmunotek S.L. NF-κB activation in THP1 cells and human monocyte-derived dendritic cells (hmoDCs) induced by MV140 or individual bacteria was quantified by ELISA or immunobloting. The expression of activation surface markers and cytokine signature were determined by flow cytometry or ELISA. Allogeneic co-cultures of MV140-activated hmoDCs and naïve $CD4^+$ T cells, CFSE-dilution assays, flow cytometry, real-time quantitative PCR, ELISA, blocking and pharmacological inhibition experiments were performed.

Results: MV140 induces NF-κB/AP-1 activation and IL-8 production in THP1 cells. MV140-activated hmoDCs acquire a mature phenotype as determined by the increased expression of CD86 and CD83 and produce significant levels of pro-inflammatory cytokines (IL-1β, IL-6, IL-23 or IL-12) as well as high levels of the anti-inflammatory IL-10. The individual contribution of each bacteria was also determined and significant differences between the Gram negative and positive bacteria demonstrated. MV140-activated hmoDCs promote the generation of T_H1 and T_H17 as well as IL-10-producing T cells. Blocking experiments demonstrated that Toll-like receptors via MyD88 and C-type lectin receptors via Syk contribute at different extend to the immunological mechanisms of action of MV140 in hmoDCs. Initial experiments demonstrate that downstream signaling pathways involving Akt, MAPK and NF-κB might well be also downstream signalling molecules contributing to the observed effects.

Conclusions: The findings reported in this study provide novel insights into the immunological mechanisms by which MV140 might exert its clinical efficacy in patients suffering from RUTIs.

Poster Discussion Session PDS 8

Immunological tests in allergy diagnosis

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Diagnosis of cow's milk allergy in children under 1 year

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Background: Cow's milk allergy (CMA) is a complex disorder, arising early in life. Skin prick tests and serum specific IgE (slgE) can help determine a diagnosis. Usually, slgE is negative in patients with gastrointestinal (GI) symptoms; but CMA shouldn't be excluded in patients who have an atopic dermatitis (AD).There isn't noninvasive marker of GI symptoms of CMA.The gold standard is an orally induced double-blind placebo-controlled food challenge, but it's time-consuming and difficult to perform in daily clinical practice.
Method: Data about clinical picture, effect of elimination diet, open provocation test, determination of immunoglobulin free light chains (FLCs) using ELISA, total and specific IgE in serum; reaction of mast cell degranulation in rats (RMCD), prick skin test; basophil activation test (BAT)(the activation marker anti-CD203c was used), density of expression of lgE on the surface of basophils, relative number of basophils expressing lgE by flow cytometry; fecal calprotectin (FC) using immunohistochemistry were used for the diagnosis. Results were examined in 41 children under 1 year with suspected of CMA before and during 6 months of an elimination diet (ED).
Results: We found positive basophil activation in 31% of sIgE negativity and in 30% of RMCD negativity.FLC's levels (kappa $P = 0.018$, lambda $P = 0.046$) and BAT ($P = 0.026$) in serum were higher in children with GI manifestations of CMA prior to the prescription of an ED. During ED there were a decrease of degranulating forms (%) in the RMCD test (with GI $P = 0.003$, without GI manifestations $P = 0.0001$),relative number of basophils expressing lgE ($P = 0.022$, $P = 0.013$ respectively) in all children. There were a significant decrease in the levels of FC during an ED in children with GI manifestations of CMA ($P < 0.05$). In children with unsuccessful introduction of cow's milk after 6 months of ED had an increased levels of relative number of basophils expressing lgE ($P < 0.05$).
Conclusion: The determination of kappa and lambda chains in serum could be as a candidate for immunological marker of GI manifestations of CMA in children under 1 year. Determination of relative number of basophils expressing lgE during ED could be as a marker of successful introduction of cow's milk. Our data confirm, that dynamic determination the level of FC during an ED may be recommended as a noninvasive marker for GI manifestations of CMA in children under 1 year.

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Novel transcriptomic and immunoproteotomic approaches in identifying cross-reactive allergens between crustacean and molluscs

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Background: Utilizing crude protein extracts is currently inadequate to identify allergenic proteins in shellfish. Currently, only three proteins – tropomyosin, arginine kinase, and paramyosin – have been fully identified and characterized as potential cross-reactive allergens between crustacean and mollusks. Bioinformatics analysis could provide a powerful and versatile tool for the in-depth molecular characterization. Therefore this study was aimed to identify putative and cross-reactive allergens using *in-silico* analysis of the transcriptome from the mollusk Pacific oyster (*Crassostrea gigas*).
Methods: Several allergen sequences related to shellfish allergy including from Black tiger prawn, lobster and abalone were documented from different databases. TBLASTN analysis was performed on these sequences against the genomic database of Pacific oyster to retrieve putative and cross-reactive allergen sequence. Whole protein extracts where analysed by mass spectroscopy as well as IgE binding of shellfish allergic patients.
Result: Based on amino acid sequence similarity, cross-reactive allergens and several putative allergen genes were identified after detailed *in silico* analysis of the genomic data of Pacific oyster. Some of the putative oyster allergens demonstrated the potential to cross-react to crustacean allergens, based on conserved domains and similar structural features. This method also revealed the presence of various isoforms of the oyster allergens. The phylogenetic trees of six different allergens demonstrate that the Pacific oyster clusters with the corresponding allergens from other mollusk, however is grouped distinct separately from crustacean allergens.
Conclusion: Bioinformatics approaches reveal several putative and cross reactive allergens that have not been fully elucidated using less sensitive immuno-chemical IgE-based methods. *In-silico* approaches, in combination with recombinant allergen generation, will be an eminent method for the comprehensive assesment of allergenicity and opens an additional path for efficient allergy diagnostics and immunotherapeutics.

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IgE cross-reactivity between the major peanut allergens Ara h 2 and Ara h 6

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Background: 2S-albumins Ara h 2 and Ara h 6 are the most potent peanut allergens and IgE responses toward these proteins are now considered to be good predictors of clinical reactivity in sensitized patients. According to a sequence identity of 59% and to similar tertiary structures, IgE-binding capacity of Ara h 6 has been suggested to be partially due to cross-reactivity with Ara h 2. Here, we investigated the cross-reactivity between the 2S-albumins and its potential impact on component-resolved diagnostics (CRD).
Method: Highly purified Ara h 2 and Ara h 6 proteins were obtained from roasted peanuts. Recombinant allergens were also

produced in order to ensure the absence of any cross-contamination between Ara h 2 and Ara h 6 preparations. Cross-reactivity between Ara h 2 and Ara h 6 was then evaluated by competitive inhibition of IgE-binding with sera from 17 peanut-allergic patients.

Results: Competitive inhibition assay using native or recombinant allergens provided comparable evaluations of the different populations of IgE antibodies recognizing either one or two 2S-albumins. The level of cross-reactivity observed between Ara h 2 and Ara h 6 was variable among peanut-allergic patients. In two patients, the IgE response to Ara h 6 was only due to cross-reactivity with Ara h 2. A cross-reactivity higher than 5% was observed in only four other patients. In contrast, no or low-affinity cross-reactivity (lower than 0.5%) was observed in the other sera.

Conclusion: Although cross-reactivity occurred between Ara h 2 and Ara h 6, IgE binding to both 2S-albumins was mostly mediated by non-cross-reactive epitopes, which is indicative of sensitization of the tested patients to both allergens. Our results thus confirmed the importance of measuring specific IgE to the two peanut components Ara h 2 and Ara h 6 in order to get the best predictive value from CRD.

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Interest of 2D Immunoblot for the diagnosis of allergy to wheat

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Background: Wheat allergy is relatively common and clinical manifestations depend on the involved allergen and the way of exposure: it could be wheat dependent exercise induced anaphylaxis (WDEIA), baker's asthma (BA), atopic dermatitis (AD), pollen rhinitis (PR) and urticaria. Many wheat allergens have been described. Nevertheless, due to the wheat protein complexity, only a small number of allergens has been documented in an allergic manifestation (ω5-gliadin is responsible of WDEIA, for example). For the same reason, sensitizations cannot be detected by traditional diagnostic tests because the molecular allergens are not commercialized. Our study proposes to use the protein separation capacity of 2D Immunoblot to contribute to wheat allergy diagnosis.

Method: Standardized protein extracts obtained from wheat seeds were separated on the basis of their isoelectric point and their size. We selected 169 patients sensitized to wheat on the basis of their positive specific IgE (sIgE) to wheat. Amongst the 169 sera, we studied first 25 sera of patients that we could distinguished into 4 groups (WDEIA, AD, PR, and mixed AD/PR). All of them were analyzed on 2D Immunoblot with these standardized extracts in the aim to evaluate a sIgE reactivity. Then we compared their sIgE sensitization profiles.

Results: The method identified specific sensitization profiles for each group. For WDEIA, we observed a protein profile around 37 kDa (pH 6–9) and 37–50 kDa (pH 5–6). For AD, the profile is observed around 50 kDa (pH 9), 10 kDa (pH 9) and 20–75 kDa (pH3). For PR, the profile is found around 90 kDa (pH 9). Finally, for mixed AD/PR, we did not find any specific protein profile.

Conclusion: At this stage of the experiment, we obtained different sensitization profiles as we identified different specific proteins areas for the 3 studied groups. These areas recognized by sIgE concern one or more allergens. It would be interesting to identify them in order to associate a symptomatology to a responsible allergen but, due to the wheat matrix complexity, we will need to use mass spectrometry to establish their identification.

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AllergoOncology: acrolein suppress the immune system preventing allergic sensitization and promoting tumor-growth

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Background: Acrolein is a compound generated in large amounts during smoking and has been correlated with increased prevalences of allergies and cancer. Here we intended to investigate the immunomodulatory effects of acrolein in a BALB/c mouse model of allergy using the model antigen KLH. By our results we were unexpectedly prompted to investigations using tumor grafts in an allergooncology approach.

Method: BALB/c mice were nasally sensitized 5 times in biweekly intervals with KLH alone or in conjunction with acrolein. Control groups were sham-treated with PBS or with acrolein alone. Airway hyperreactivity was measured by whole body plesmytography and systemic reaction upon oral challenge. Specific antibodies as well as cytokines of KLH-stimulated splenocytes were analyzed by ELISA. Next, D2F2 tumor cells were grafted to the flanks of mice treated with PBS or acrolein and tumor growth were monitored. The impact of acrolein on NFkB activation was investigated using THPXBlue cells and on aryl-hydrocarbon receptor activation using the stably transfected human reporter cell line AZ-AHR.

Results: Nasal allergic sensitization of mice to KLH was prevented by addition of acrolein, and led to a significant impairment in antibody-formation (IgG1, IgG2a, IgA and IgE) as well as cytokine-response (IL5, IL13, IL10 and IFNγ). Moreover, mice exposed to acrolein had a relative accumulation of regulatory T cells in the lung and were protected from anaphylactic reactions upon challenge with KLH. D2F2 grafted tumors grew significantly faster in the acrolein-exposed mice and had a significant greater accumulation of CD3+ and Foxp3+ cells in the tumors than PBS sham-treated mice. In LPS-activated THP1X-Blue cells NFkB activation was specifically inhibited by acrolein. Importantly, acrolein activated the arylhydrogen carbon receptor in a concentration-dependent manner in AZ-AHR cells.

Conclusion: We report that exposure with acrolein protected mice from allergic sensitization and promoted tumor growth by favoring regulatory immune cells in the lung and in tumors. For the first time we could demonstrate that acrolein can act via the aryl-hydrocarbon receptor and thereby may favor the generation of regulatory T cells. This study therefore adds a novel mechanism of action of acrolein being of great relevance in allergy and cancer.

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Basophil activation with Pru p 3 may rescue negative Pru p 3 sIgE in peach allergic patients

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Background: Peach allergy in Mediterranean areas is often due to Pru p 3. In a few peach-allergic patients, serum specific immunoglobulin E (sIgE) to Pru p 3 and other commercial allergens (Pru p 1, Pru p

Gender	Age	sIgE peach (kUA/L)	sIgE Pru p 3	sIgE Pru p 1	BAT peach (%CD63)	BAT Pru p 3 (%CD63)	BAT Mal d 1 (%CD63)
F	46	1.50	0*	0*	94	95	3
F	16	1.97	0	0	60	21	6.1
M	50	0.14	0	0	54	42	2
F	42	0.19	0	0	5.7	3.6	2.5
M (control)	25	0	0	0	4.6	5.6	2.5

[Results of sIgE and BAT for the 5 patients]
*Confirmed by multiplex chip analysis(December 2015)

4) are not detectable, despite a convincing clinical history and positive skin prick tests to peach.

Objective: We made the hypothesis that basophil activation test (BAT) to molecular allergens might improve the sensitivity of Pru p 3 reactivity detection.

Method: BAT was performed on freshly drawn blood with reagents, allergen extracts (peach, cow's milk (CM)) and molecular allergens (Pru p 3, Mal d 1) from the BAT supplier. For each allergen, 4 concentrations were assayed: 62, 31, 12 and 1 ng/ml, except for Mal d 1, which is supplied at higher concentrations. BAT was considered positive when the percentage of CD63-expressing basophils was at least twice higher than the negative control. We assayed 4 severe peach-allergic patients and one peach-tolerant donor, all tolerant to CM.

Results: BAT was positive to peach and Pru p 3 in 3 peach-allergic patients, and negative in one patient and in the peach-tolerant control. BAT to Mal d 1 and to CM was negative in all 5 donors (Table).

Discussion and conclusion: The demonstration of Pru p 3 reactivity through BAT allowed 3 out of 4 patients to be assigned to the nsLTP profile. The absence of PR-10 reactivity in Mediterranean patients with severe peach allergy is in line with previous reports. Because Pru p 3 supplied by the BAT supplier is a native allergen, the presence of contaminating Pru p 7 cannot be excluded formally and is currently under investigation. Clinical relevance:

BAT to Pru p 3 may be useful for rescuing the molecular-based allergy diagnostics of peach-allergic patients with undetectable sIgE to peach Pru p 3.

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Skin prick tests: agreement among different positivity criteria

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Background: Skin Prick Tests (SPT) positivity criteria has changed over time. In 2011, EAACI recommended that SPT should be considered positive if the longest wheal diameter (Lwd) was ≥3 mm, replacing the previous recommendation to use the mean wheal diameter (Mwd ≥3 mm). Other proposed criteria for SPT positivity include the use of the area of the wheal (Aw ≥7 mm^2) and the adjustment to histamine response (AaH).

Aims: 1) to evaluate the agreement among four different SPT positivity criteria – Lwd ≥3 mm, Mwd ≥3 mm, Aw ≥7 mm^2 and AaH ≥50% (area of the allergen wheal ≥50% of the area of histamine); and

2) to describe the impact of using different criteria in the prevalence of atopy.

Methods: We selected all individuals that performed SPT in the ICAR (Impact and control of Asthma and Rhinitis; PTDC/SAU-SAP/119192/2010) study (n = 829). Each individual was tested with up to 30 allergens (GA^2LEN battery plus additional allergens), histamine (10 mg/ml) and negative control. Wheals' areas and perimeters were measured with planimetry using the software PrickFilm2006v3® (Inmunotek, Madrid, Spain). Individuals with less than 10 valid allergen measurements (n = 42) or presenting limitations to SPT interpretation [e.g. dermographism (n = 2), unreactive histamine (n = 25)] were excluded from the analysis. Atopy was defined as the presence of at least one positive SPT. Agreement was tested with Cohen's kappa; comparisons between the prevalence of atopy using different definitions were performed with McNemar and Cochran's Q tests for paired samples.

Results: Overall, 760 individuals were included, totalling 21 131 individual SPT. The proportion of positive SPT ranged between 9.7% (AaH50%) and 11.3% (Lwd). Two percent of the SPT (n = 463), had at least two discrepant classifications. The highest agreement was found between Ldw and Aw (k = 0.975; $P < 0.001$), and the lowest between Mwd and AaH50% (k = 0.889; $P < 0.001$).

The prevalence of atopy ranged between 54.1% (AaH50%) and 57.1% (Lwd; p to global difference <0.001). The use of the Lwd criteria was associated with significantly higher atopy prevalence than Mwd (55.9%) and AaH50% (P = 0.004 and <0.001, respectively), but not significantly different from Aw (56.6%; P = 0.125).

Conclusion: The agreement between different positivity criteria was high. Nevertheless, the prevalence of atopy changed significantly according to the criteria used to define skin prick tests positivity and they should not be used interchangeably.

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The diagnostic value of skin prick testing with purified LTP

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Background: More than 50 different LTPs have been described until now in foods, including legumes, cereals, nuts, and fruits, mainly in the Rosaceae family, in pollens, including trees or weeds, and in other allergenic sources such as latex. Cross-reactivity among them seems quite variable.

Food LTPs are responsible for IgE sensitization and induce allergic symptoms ranging from oral allergy syndrome (OAS) to severe systemic reactions.

The objective of this study was to evaluate if purified Pru p 3 in skin prick test (SPT) could be used as a marker of sensitization to any food LTP by analysing the agreement between SPT and LTP-specific immunoglobulin (sIgE) levels.

Method: Patients over 18 years of age who attended the Allergy Services of six Spanish hospitals, reporting symptoms after the ingestion or contact with plant foods were

selected. They were included if they had a positive SPT to Pru p 3 or to at least one of some extracts were LTP has been described: peach peel, hazelnut, apple peel, corn flour, tomato, kiwi, grapes, lettuce and peanut.

sIgE to the available purified LTPs from plant foods (Pru p 3, Cor a 8, Mal d 3, Ara h 9, Tri a 14 and Jug r 3) was measured.

Results: Of the 121 individuals included in the study (38.9±10.3 years old; 42.1% men), 113 individuals (93.4%) were positive to LTP by SPT (wheal: 52.8±28.8 mm^2). Sensitization to fruits ranged between 83.5% to peach peel to 21.5% to grapes. Symptoms were reported by 69% of the patients with peach, mainly urticaria (36%), 70% with nuts, mainly OAS (34%) and 49% with other fruits, mainly OAS (25%).

All the LTPs tested by Immunocap were negative in 15 individuals, including 5 of the 8 negative by SPT. Among the positive ones, most (104; 98.1%) were positive to Pru p 3 (11.1±20.5 kUA/l), and only 52 (49.1%) to Tri a 14 (7.7±18.8 kUA/l). Two patients were negative to Pru p 3 and positive to other LTPs (Mal d 3 and Ara h 9). It was observed a tendency to increase the sIgE to Pru p 3 when the number of LTPs involved in the sensitization increased: from 1.2 kUA/l in patients sensitized only to Pru p 3 to an average of 20.2 kUA/l in patients positive to the 6 LTPs.

Conclusion: Diagnosis by SPT with Pru p 3 is useful to identify patients sensitized to different plant food LTPs in a peach allergy predominant area.

Patients sensitized to a higher number of different LTPs had higher sIgE values to Pru p 3.

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Development and application of quantitative immunoassays for major milk allergens Bos d 5 (β-lactoglobulin) and Bos d 11 (β-casein)

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Background: Allergy to milk is one the most prevalent allergies affecting around 3% of children. At present there is no effective treatment for milk allergy and therefore strict avoidance is recommended. This however can be difficult as milk is an ingredient in many foods. The development of diagnostics and therapeutics for milk allergy depends on accurate and reliable methods for standardisation. Our aim was to develop quantitative immunoassays that could be used to accurately measure specific milk allergens for allergen standardization.

Method: Monoclonal antibody pairs recognising the major milk allergens Bos d 5 (β-lactoglobulin) and Bos d 11 (β-casein) were obtained. Subsequently two-site ELISAs and Luminex xMAP assays were developed using highly purified, IgE validated allergens as standards. The assays were used to measure milk allergens in different types of foods (powdered milk, chocolate mousse, chocolate bar, cookie) and in diagnostic/therapeutic preparations. They were also used to monitor potential milk contamination in products claimed to be 'milk free'. The Luminex assays for Bos d 5 and Bos d 11 were 'multiplexed' to allow for simultaneous quantification of both allergens in a single sample.

Results: All assays showed wide standard dynamic range. The Lower Limit of Detection (LLOD) for ELISA was 8 ng/ml for both Bos d 5 and Bos d 11. The Luminex assays proved to be even more sensitive with LLOD up to 40-fold lower compared to ELISA (0.2 ng/ml and 4.0 ng/ml for Bos d 5 and Bos d 11 respectively). Concentration of Bos d 5 in tested samples ranged from 13 mg/g of milk powder to 0.462 μg/g of cookie. Interestingly, relatively large amount of milk protein was detected in 'milk free' samples (0.306 μg/g and 0.258 μg/g of Bos d 5 in milk free chocolate mousse and chocolate bar respectively). Cocoa was one of the ingredients in those foods which can contain milk traces and explain presence of Bos d 5 in samples. No Bos d 5 was detected in samples without cocoa.

Conclusion: Immunoassays for quantification of specific milk allergens have been developed. The assays can be used separately (as ELISA) or be 'multiplexed' (Luminex assays) allowing simultaneous quantification of multiple allergens in a single sample. The immunoassays will allow standardization of milk protein levels in diagnostic and therapeutic extracts and detection of milk allergens in foods.

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A two-site immunoassay for quantification of peanut allergen Ara h 8

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Background: Ara h 8 is a minor peanut allergen and sensitization generally does not present risk for a life-threatening reaction. However, Ara h 8 is a potential trigger for oral allergy syndrome (OAS) in birch pollen allergic individuals due to structural homology with Bet v 1. Our aim was to develop a sensitive immunoassay for the detection of Ara h 8.

Method: Recombinant Ara h 8 (rAra h 8) was used to immunize mice and rabbits for development of monoclonal and polyclonal antibodies, respectively. Antibodies were screened by ELISA for reactivity to peanut allergens (Ara h 1, 2, 3, 6, and 8), as well as to Bet v 1. A two-site ELISA was developed using a monoclonal antibody for capture and polyclonal antiserum for detection, with rAra h 8 as the assay standard (concentration determined by Advanced Protein Assay).

Results: Monoclonal and polyclonal antibodies reacted strongly with Ara h 8 and showed trace reactivity to the other peanut allergens and to natural and recombinant Bet v 1. This cross-reactivity did not affect the Ara h 8 assay at antibody concentrations that were used in the ELISA. The standard curve ranged from 500 to 0.98 ng/ml with a limit of quantitation of 1.95 ng/ml.

Conclusion: A sensitive ELISA for the quantification of peanut allergen Ara h 8 has been developed. The assay has several applications, including measurement of Ara h 8 in food products and peanut extracts, and standardization of allergy diagnostic and therapeutic products. Additional applications may include measuring exposure to peanut allergen and helping to distinguish between sensitivity to Ara h 8 and Bet v 1, leading to a better understanding of oral allergy syndrome.

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Investigations with the INA-mite-detector

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Background: House dust mites (HDM) are the most important sources for allergic reactions in households. The objective was to assess the possibility of detecting HDM allergens (HDMA) on different places in the flat but also on the human body with the new INA (individual native in-vitro allergy diagnosis)-mite-detector. Furthermore, some of the performance criteria established for the INA-mite-detector should be checked.

Method: The INA-mite-detector consists of a plastic stick with a reaction field at the bottom (INA-stick), conjugate, substrate, HDM-positive and negative sera. Samples were collected by pulling the reaction field of the INA-stick over selected places: mattress, carpet, wallpaper, towel, arm, face, hair and leg. Specific IgE measurements have been performed using the tube ELISA technology with a high-titered HDM-

positive and a negative serum. The test has been done at 37°C (incubator). The overall duration including 10 min handling time with tap water washing steps (1 min each) was 2 h 25 min. The reaction obtained with the positive serum (purple colour on the reaction field) was compared with the negative reaction as yes/no evaluation. Reproducibility was tested by intra- and inter-assays ($n = 10$, each) under equal conditions. The stability of the INA-sticks (loaded with HDMA and without HDMA) was evaluated at different storage conditions.

Results: HDMA could be detected on different sample sites in the flat: on known places like mattress and carpet but also on the wallpaper, which was new for us, and on the human body, washed and not washed with soap. The tests showed a high reproducibility by intra- and inter-assays. The INA-sticks (stored in plastic tubes) either loaded or not loaded with HDMA were stable under the following conditions: 4–24°C: 4 months (will be continued and differentiated), 33–40°C: 12 weeks (terminated).

Conclusion: In a very simple and quick manner HDMA could be detected on different places using the INA technology. The detection of HDMA on the human skin could be of interest for patients with atopic dermatitis because HDM are discussed as one cause of this disease. The INA technology could be used for the detection of HDM, but also of cat and dog allergens as previously shown. Further fields of application for this new technology are conceivable and under investigation.

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Performance characteristics of specific IgE assay

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Background: A laboratory-based assay for high-throughput specific IgE measurement has been developed according to CLSI guidelines (I/LA20-A2) for specific IgE. The test utilizes a robotic *in vitro* diagnostic analyser (IDS iSYS) and measures specific IgE levels in a patient's serum in a two-step immunoassay method based on the principle of chemiluminescence. Biotinylated allergen extracts or components are incubated with streptavidin coated magnetic micro-particles (solid phase). Specific IgE in the sample then binds to the allergen and is detected with an anti-IgE antibody labelled with an acridinium ester derivative. The signal, measured in Relative Light Units (RLU), is then read from a stored IgE standard curve (standardized to the WHO 2nd IRP 75/502 with a 30 day calibration stability) to give the concentration of specific IgE present in the sample. Reagents for approximately 40 of the more common allergens have been developed to date and development of further allergen extracts and components continues.

Method: 1080 sera from patients were analysed with both the new system and an established test using 21 allergens (16 aeroallergens, 2 dust mite and 3 foods). Comparison between the assays was calculated using direct correlation (OLS regression analysis) and concordance (EAST class). Additionally, imprecision was estimated following CLSI EP05-A2 guidelines, LOD was estimated according to CLSI EP17-A2 and linearity according to CLSI I/LA20-A2. The results are expressed in kUa/l. Any values >100 kUa/l with either assay were removed from the regression analysis but retained for the concordance analysis.

Results: Regression analysis with 95% confidence intervals (952 samples) gives the new system = 0.961±0.021 * established system + 1.29±0.64 kUa/l with $r^2 = 0.88$. The overall EAST class direct concordance (1080 samples) is 73% (range 62–85% for individual allergens) and 97% for ± 1 Class. Typically the intra-assay CV is <5% and total assay CV is <10%. Assay linearity meets the criteria in CLSI I/LA20-A2. The limit of detection of the assay is <0.1 kUa/l.

Conclusion: Results for 21 allergens are comparable to those from the established system. With on-board storage for up to 126 different allergens the new system allows laboratories the flexibility to access this clinically important area without the requirement for a dedicated IgE testing system.

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Comparison of commercial skin prick test reagent using *in vivo* and *in vitro* method

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Background: Precise detection of culprit allergen is critical for selection of treatment options. Each manufacturer utilizes its own potency units and quality control program. That make clinicians confused. This study aims to compare the property of SPT reagents manufacture by European and American manufacturers.

Methods: Allergenicity of 5 allergens (*Dermatophagoides farina*, *Dermatophagoides pteronyssinus*, Oak, Ragweed, Mugwort) produced by 3 manufacturers, For comparison of *in vitro* properties, Bradford assay, SDS-PAGE, and Western-blot were used. For *in vivo* comparison, 65 allergic patients were enrolled for SPT using 13 SPT reagents (Oak and mugwort of company 2 were not available). Analysis of positivity rate and concordance rate were based on serum IgE results.

Results: Protein concentration of *Dermatophagoides farina* varies from 8.46 μg/ml company 1–846.44 μg/ml company 2; this difference was also identified on SDS-PAGE and immunoblot. Intensity at 14 kDa was highest in company 2 reagent. Concordance rate of Company 2 (kappa = 0.69) was higher than that of Company 1 (kappa = 0.67). Bradford assay of *Dermatophagoides pteronyssinus* reagents were different as follow: 87.8 μg/ml company 1, 173.1 μg/ml company 3, and 1283.5 μg/ml company 2. SDS-PAGE, Western blot and positivity rate of SPT showed the similar patterns as protein concentration. Ragweed protein quantitation showed a maximum of 6.9 folds difference: 1158.8 μg/ml company 1582.1 μg/ml company 3, and 168.0 μg/ml company 2. However, positivity rate was highest in Alleropharma, and concordance rate was highest in Hollister-Stier. The protein concentration of Oak is 1.3 folds difference between company 1 (175.6 μg/ml) and Company 3 (226.6 μg/ml). SDS-PAGE and immunoblot showed the similar tendency. However, positivity rate of SPT was not different. The Protein concentration of mugwort didn't show the difference between company 1 (400.4 μg/ml) and company 3 (408.8 μg/ml). However, on SDS-PAGE and Western blot, the 28 KDa band of company 3 presented more thicker than that of company 1.

Conclusions: Allergen potencies of house dust mites, and oak were lowest in company 1 SPT reagents and highest in company 2 reagents. These differences are critical for precise diagnosis of allergic diseases.

Poster Discussion Session PDS 9

Food allergy: From mice to men

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Comparing the sensitizing capacity of raw and processed cow's milk in a murine sensitization model for food allergy

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Background: Epidemiological studies show that the consumption of raw milk early in life is protective against the development of allergies later in life. The effect was found to be limited to raw milk consumption and was not observed when this milk was boiled or when pasteurized and homogenized shop milk was consumed. So milk processing seems to abolish the allergy protective effects of raw milk. The components and mechanisms involved are however still unknown. In this study the sensitizing capacity of raw and processed cow's milk was compared in a murine sensitization model for food allergy.
Method: C3H/HeOuJ mice were sensitized orally once a week for 5 consecutive weeks with raw milk, heated raw milk (10 min 80°C), shop milk (pasteurized and homogenized), an 80:20 mixture of casein/whey protein (equivalent to the amount in milk; sensitized control) or PBS (non-sensitized control) using cholera toxin as adjuvant. One week after the last sensitization mice were challenged both intradermally and orally with casein/whey. Clinical parameters, such as the acute allergic skin response, anaphylactic shock symptoms and changes in body temperature were assessed upon intradermal challenge and serum specific antibodies and mast cell degranulation were measured upon oral challenge. Activated Th2-, Th1- and regulatory T-cell populations were quantified in spleen using flow cytometry and *ex vivo* cytokine production was measured after re-stimulation with casein/whey.
Results: Mice sensitized with raw milk did not show any clinical symptoms upon challenge and did hardly produce specific IgE antibodies. Sensitization with the processed milk types on the contrary increased the acute allergic skin response and anaphylactic shock symptoms and caused a drop in body temperature. IgE levels were also significantly increased in these mice. No differences were observed in mucosal mast cell degranulation between groups and also T-cell populations did not differ. The production of Th2 cytokines IL-5 and IL-13 was however significantly reduced in the raw milk group compared to the processed milk groups after *ex vivo* re-stimulation of splenocytes with casein/whey.
Conclusion: In contrast to processed milk, raw milk is hardly able to induce sensitization. Allergic symptoms and IgE levels were reduced and this coincided with reduced Th2 cytokine responses. What it is in raw milk that prevents sensitization needs to be elucidated in future studies.

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PI3K inhibitor suppressed food allergic symptoms in the mouse model

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Background: Phosphoinositide 3-kinase (PI3K) pathway is involved in positive and negative regulation of several immune responses including cytokine signaling and T cell differentiation. PI3K inhibitors have been shown to alleviate airway inflammation in mice asthma models. Therefore, we hypothesized PI3K inhibition may suppress symptoms of food allergy.
Methods: Balb/c mice were sensitized with ovalbumin (OVA)/alum and then were repetitively challenged with OVA by oral administration. The development of hypothermia and diarrhea was observed after the challenges. The effects of pretreatment with PI3K inhibitor, 3-methyladenine (3-MA), on allergic symptoms, serum levels of OVA-specific antibodies and mucosal mast cell protease-1 (mmcp-1), cytokine mRNA expressions in the intestines, and OVA-specific cytokine producing ability of mesenteric lymph node mononuclear cells were analyzed.
Results: 3-MA pretreatment suppressed allergic symptoms induced by OVA challenges. *In vitro* OVA-specific IL-4 and IL-10 but not IFN-gamma production by mesenteric lymph node mononuclear cells purified from 3-MA-treated mice were decreased compared with those of non-treated mice. 3-MA pretreatment decreased the elevation of serum mmcp-1 levels after the OVA challenge and the upregulation of mRNA expression of mmcp-1, IL-4 and IL-10 in the intestines.
Conclusion: 3-MA pretreatment inhibited oral allergen-induced mucosal mast cell activation, development of Th2 responses, resulting in the suppression of food allergic symptoms.

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Influence of dietary combined vitamin deficiency on cellular immunity in rats

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Background: Dietary combined vitamin deficiency may impact cellular immunity.
Methods: Wistar male rats ($n = 18$, average weight – 58.1 ± 0.5 g) were divided into 3 groups and fed with a complete semi-synthetic diet. Vitamin deficiency was induced by 5-fold reduction of the vitamin mixture amount in the feed and complete-vitamin E, B_1 and B_2 exclusion from the mixture for 30 days. The deficiency was then corrected within 5 days. The animals of the 1st (control) group received 100% of vitamin mixture (100% Vit); 2nd and 3rd group – 20% of vitamin mixture (20% Vit). The next 5 days rats from the 3rd vitamin-deficient group were fed with diets supplemented with 80% of Vit (20% Vit+ 80% Vit). The levels of vitamins A (retinol and retinol palmitate) and E (tocopherols) in the diets and rat livers were determined by reverse-phased HPLC method using UV detection which allows separation of various tocopherols (α, γ, δ) and tocopheryl acetate after sample preparation and extraction without saponification. The levels of vitamins B_1 and B_2 in the diets and rat livers were determined by fluoromety. The numbers of lymphocytes, relative quantity of B-lymphocytes (CD45RA+) and T-lymphocyte (CD3+), subpopulations of T-lymphocytes: T-helper

(CD3+CD4+) and cytotoxic T-lymphocytes (CD3+CD8+), and NK-cells (CD161a+) in the peripheral blood of rats were determined by flow cytometry using a Beckman Coulter FC 500 (USA) cytometer.

Results: The vitamin deficient diet for 35 days resulted in reduced levels of vitamin A in the liver

($P < 0.05$) by 25- fold, vitamin E and B_1 – 2.0–2.3- fold, vitamin B_2 reduced 40%, 25-hydroxyvitamin D (25(OH)D) blood plasma concentrations reduced 21% compared with the control rats. In rats fed the vitamin deficient diet (20% Vit), lymphocytopenia (2.29 vs 3.96×10^9/l, $P < 0.01$) and reduction of relative contents of T-lymphocytes (48.2 vs 57.3%, $P < 0.05$) and T-helpers (CD3+CD4+) (60.9 vs 71.5% of CD3+, $P < 0.05$) in peripheral blood were found. Normalization of vitamin contents in the diets of the rat vitamin deficient group led to an almost complete recovery of cell immunity parameters to the levels of the rats in the control group.

Conclusion: Vitamin deficiency due to low dietary intake induces profound cellular immune changes which are reversible with reconstitution of the diet with vitamins.

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EPIT is safe and efficacious in filaggrin deficient mice sensitized to peanut

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Background: Epicutaneous immunotherapy (EPIT) has proven safe and efficacious in the treatment of food allergy utilizing allergen loaded patches applied on intact skin. The integrity of the skin may be altered by key proteins of epithelial structure, such as filaggrin (FLG). The present study investigates the association between a complete FLG deficiency and peanut EPIT efficacy in a FLG knock-out mouse model.

Method: Mice fully deficient in filaggrin (FLG-/-) and wild-type (WT) mice were sensitized by 6 weekly gavages with peanut protein extract and cholera toxin. Sensitized mice received EPIT for 8 weeks using Viaskin (100 µg peanut proteins / patch; 1 patch per week) and were then submitted to a peanut enriched regimen. Data recorded included eosinophil infiltration in esophageal mucosa, humoral and cellular responses (specific IgE production and ex-vivo stimulation of splenocytes by peanut proteins) and the different steps of allergen capture and transportation by dendritic cells following peanut EPIT patch application using flow cytometry.

Results: Sensitization of mice was confirmed by a significant increase of specific Th2 biased immunological responses. In sensitized mice, whether FLG deficient or not, EPIT significantly reduced IgE levels, Th2 cytokines secretion by splenocytes and eosinophil recruitment into the esophagus, compared to Sham. The allergen applied onto the skin of FLG-/- mice did not passively permeate the epithelium. Instead, the allergen was captured by skin $CD205^{high}$ DCs, which migrated to afferent lymph nodes, as previously described in WT mice.

Conclusion: The immunomodulatory effects of EPIT were unaltered in this mouse model of filaggrin deficiency, suggesting that in humans, EPIT would be efficacious and safe in the presence of FLG polymorphism or mutations.

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Impact of early life exposure to the mycotoxin DON on the development of food allergy

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Background: Early life exposure to detrimental compounds can have significant effects on development. Previously we showed that the trichothecene deoxynivalenol (DON), a fungal metabolite found in grain-based human diets, acts as a specific disruptor of the intestinal tight junction network and hence might contribute to gastrointestinal disorders. We hypothesized that the developing fetus and/or newborn would be exposed to DON through the placenta and breastmilk, resulting in a negative impact on gastro-intestinal development, thereby increasing susceptibility to develop gastrointestinal disorders such as food allergies.

Method: Upon arrival, C3H/HeOuJ mice were fed the control AIN93G diet and breeding pairs were formed. Upon assessment of a sperm plug, females were either kept on the control diet or a control diet containing 10 mg DON/kg feed, until 15 days after delivery of the pups. The offspring of the control and the DON-treated dams were divided into

1 sham-group,
2 oral tolerance-group and
3 food allergy group.

Mice in group 1 were given oral gavages with PBS, mice in group 2 received PBS with 40 mg/ml OVA and group 3 was given 40 mg/ml OVA and 20 µg/ml Cholera toxin (CT). After weaning, offspring were were sensitized orally with OVA + CT. Acute allergic skin responses, shock symptoms, and specific plasma immunoglobulins were measured upon intradermal ovalbumin challenge. T cell populations were analyzed with use of flow cytometric analysis in spleen and mesenteric lymph nodes (MLN).

Results: Increased intestinal permeability in the dams as a result of DON exposure was observed by increased translocation of FITC dextran across the intestinal interfaces. However, maternal exposure to DON did not affect acute allergic skin responses or shock symptoms in the offspring. Flow cytometric analysis of the MLN and the spleen revealed no clear effect of maternal DON exposure on the effector responses in the offspring. OVA-specific and total immunoglobulin levels were similar between offspring of control dams and DON-treated dams.

Conclusion: Our study suggests that maternal DON exposure in the current experimental setup does not affect the outcome of the OVA-specific food allergy in the offspring. It is possible that the DON-content of the diet was not sufficient to affect immune development in the offspring. Current research focuses on early life exposure of the developing fetus and/or newborn to different detrimental compounds in the maternal diet.

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Japanese loquat allergens share antigenic cross-reactivity (14- and 17-KDa bands) with Betulaceae pollen

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Background: We previously reported that patients who have Japanese Loquat (Eriobotrya japonica) allergies often present with oral symptoms; however, there have also been patients who experienced anaphylaxis. To the best of our knowledge, no antigenic analysis of loquat allergies has been performed to date.

Objectives: In this study, to elucidate the relationship with anaphylaxis, we aimed to identify the causative antigen of loquat

allergies through the antigenic analysis of serum from patients with loquat allergies.

Methods: We performed an immunoblot assay by using serum collected from 21 loquat allergy patients—19 patients with oral or localized symptoms and 2 with anaphylaxis. In addition, we attempted to identify the causative antigen through N-terminal amino acid sequencing and magic angle spinning (MAS). Finally, we performed inhibition assays to confirm the cross-reactivity of loquat allergens with other pollen.

Results: In 19 of the 21 patients, 2 bands (17 and 14 kDa in size) that bound to immunoglobulin E antibodies were detected on the immunoblot. N-terminal amino acid sequencing revealed that both the 17- and 14-kDa bands were almost completely homologous to Mal d 1, which is a Bet v1-like protein found in apples. MAS revealed that the 17- and 14-kDa bands were the same protein, and that the C-terminal ends were digested.

The immunoblot inhibition assay revealed inhibition by Alnus japonica (Japanese alder) and Betula papyrifera (white birch) pollen, but not by Cryptomeria japonica (cedar) pollen.

Conclusions and clinical relevance: Our studies revealed that loquat allergens, including those in cases of anaphylaxis, share cross-reactivity with Betulaceae (birch family) pollen, an allergen belonging to the Bet V1 family. Moreover, the immunoblot assay revealed 2 bands (17- and 14-kDa) that bound to the antigens.

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Allergenicity assessment of hen egg iron-free ovotransferrin and iron-loaded ovotransferrin

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Background: Food allergy is a global public health problem, and egg was considered as the second allergic food. Ovalbumin (OVA), ovomucoid (OVM), ovotransferrin (OVT) and Lysozyme (Lys) were accepted as the main egg allergens in egg white. Of them, OVT had two iron-binding sites, and iron-loaded ovotransferrin (Holo-OVT) had different tertiary structure compared with iron-free ovotransferrin (Apo-OVT), leading attention to the allergenicity change of OVT.

Method: Apo-OVT was purified by CM Sepharose Fast Flow Cation-exchange chromatography, followed by dialyzing against Fe^{3+} solution to prepare Holo-OVT. The purity of Apo-OVT and Holo-OVT were identified by Urea-PAGE. The allergenicity of Apo-OVT and Holo-OVT were evaluated in a balb/c mouse model as well as dendritic cells (DCs) based on antigen process.

Results: The purity of Apo-OVT and Holo-OVT were 98% and 68%, respectively. Histologic examination of the mouse small intestines revealed that Apo-OVT and Holo-OVT induced intestinal allergy, but no systematic allergic symbols were observed. Moreover, compared to Apo-OVT group, the specific IgG and IgG1 in serum, splenic cell proliferation and IFN-γ, which was related to food allergy, were decreased significantly. However, DCs took in less Apo-OVT than Holo-OVT. In addition, Cathepsin D was used to simulate antigen process by DCs, and both Apo-OVT and Holo-OVT showed no significant difference before and after digestion.

Conclusion: It was indicated that the allergenicity of Apo-OVT was not so strong according the mouse model and the allergenicity of Holo-OVT was weaker than Apo-OVT. Moreover, antigen uptake by DCs may be not positively related to the allergenicity of allergens. For the further study, to clearly understand well the relationship between antigen process by DCs and food allergy, the type of T cell response after antigen primed DCs should be investigated.

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Effects of enzymatic deglycosylation following ultrasound pretreatment on the structure and immunoreactivity of soybean 7S protein

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Background: The aim of this study was to evaluate the effects of enzymatic deglycosylation following ultrasound pretreatment on structure and immunoreactivity of soybean 7S protein.

Method: The 7S globulin was pretreated by ultrasound and then enzymatically deglycosylated by Peptide N-Glycosidase F (PNGase F). The processed 7S globulin was characterized by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), reversed phase high-performance liquid chromatography (RP-HPLC), UV absorption spectrum, circular dichroism (CD) spectrum, and surface hydrophobicity (H_0) analyses, which provided information related to changes in protein structure. Enzyme-linked immunoabsorbent assay (ELISA) employing human sera (IgE) of soy allergics was used to evaluate IgE-binding ability.

Results: 7S globulin after deglycosylation had slightly lower molecular weight and higher eletrophoretic mobility. The hydrophobicity of deglycosylated 7S globulin increased. The transformation of α-helix and coils to β-sheet was observed in Far-UV CD spectroscopy after single deglycosylation of 7S fractions. The UV spectrum of processed 7S globulin showed that there was a blue shift of 3 nm and a decrease in absorption intensity. The results on ELISA assay showed that individual deglycosylation of 7S globulin reduced the IgE-binding capacity, while deglycosylation following ultrasound pretreatment enhanced the IgE-binding capacity.

Conclusion: It was indicated that deglycosylation could be taken into account as an effective strategy to reduce potential allergenicity of soybean 7S globulin.

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Biomarkers for peanut allergy in peripheral blood derived from a whole mRNA screen in Ara h 2 specific T cells

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Background: Peanut allergy is a life-threatening IgE-mediated disease. Development of IgE-mediated responses requires the interaction of antigen presenting cells, mast cells, T cells and B cells. However, allergen specific CD4+ T cells are playing a pivotal role in the development of peanut allergy. Gene expression analysis of these allergen specific T-cell subsets may lead to a better understanding of the regulation/dysregulation of allergen specific T cells.

Method: Whole mRNA array (Agilent whole human genome oligo micro array) from allergen- specific T cells (sorted $CFSE^{low}$ T-cells upon exposure to Ara h 2 stimulation) from 5 peanut allergic and 6 non- peanut allergic individuals resulted in selection of 11 candidate genes (CD36, CAMK4, BMPR1a, COMMD1, TAB3,

PTPN11, GIMAP8, HEMK1, ARG2, PYCRL, CSNK1E, GFPT1 and IL-13). To test for the usability of these genes as markers of peanut allergy in PBMCs their relative gene expression in peanut allergic ($n = 12$), atopic poly-sensitized ($n = 10$) and non-atopic controls ($n = 14$) was investigated.

Results: While RNA expression of TAB3 and GFPT1 expression was significantly lower, PYCRL and HEMK1 expression was significantly higher in PBMCs of peanut allergic individuals as compared to non-allergic individuals.

Conclusion: We describe four genes derived from a T-cell based, whole mRNA search that are bearing a potential to be used as markers for peanut allergy in peripheral blood derived mononuclear cells.

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Could IL-33/ST2 pathway play a role in Pru p3-sensitized peach allergic patients?

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Background: It has been demonstrated that Interleukin-33 (IL-33)/ST2 axis is involved in the development of allergic diseases but, their contribution in food allergy is still unknown. In this study, we've measured and compared the serum levels of IL-33 and its soluble receptor (s-ST2) in Pru p 3-sensitized peach allergic patients (Pru p 3-SPAP), non-Pru p 3-sensitized peach allergic patients (non-Pru p 3-SPAP) and normal controls.

Methods: sIgE anti-rPru p 1, anti-rPru p 3, anti-rPru p 4, IL-33 and s-ST2 receptor were assessed in the sera of 53 control patients, 68 non-Pru p 3-SPAP and 47 SPAP. Within this group, the presence of rhinitis and/or asthma were evaluated. Using the basophil activation test assay (BAT), we assessed basophil activation before and after the addition of s-ST2 to the sera of Pru p 3-SPAP.

Results: All Pru p 3-SPAP were only positive to sIgE to Pru p 3. All non-Pru p 3-SPAP and control patients were negative to sIgE to all peach allergens. The highest levels of IL-33 were found in Pru p 3-SPAP compared with non-Pru p 3-SPAP and normal controls. A significant difference between non-Pru p 3-SPAP with rhinitis/asthma and Pru p 3-SPAP without rhinitis/asthma was found. Significantly lower s-ST2 levels were found in Pru p 3-SPAP compared with non-Pru p 3-SPAP. BAT analysis showed a significant decrease in basophil activation after the addition of the s-ST2 to the sera of Pru p 3-SPAP.

Conclusions: An imbalance in the IL-33/ST2 pathway has been found in Pru p 3-SPAP. This data open up new therapeutic approaches for food allergy.

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Severe profilin mediated food reactions correlate with e oral mucosa integrity

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Background: Increased exposition to grass pollen may lead to a high degree of allergic inflammation, sensitization to minor allergens, such as profilin and development of pollen associated food allergy. Our aim here was to analyze whether this high degree of allergic inflammation affect the epithelial barrier integrity of the oral mucosa.

Method: 3 groups of patients were included in the study: a healthy control (group 1) and other two groups with grass pollen allergy associated with moderate (group2) or severe (group3) food allergy. Formalin-fixed, paraffin embedded sections of oral mucosa biopsies were obtained from 20 allergic patients and 6 healthy subjects. Immunohistochemistry for tight junctions markers (claudin-1, ZO-1, ocludin 1) was performed. Additionally, DAPI and Masson staining was carried out in all the samples.

Results: Claudin-1 expression, as a marker for tight junction functionality and epithelial barrier integrity, was inversely proportional to pollen-associated food allergy severity. Moreover, oral mucosa from patients with severe pollen-associated food allergy displays significantly lower number of cells in the epithelium than healthy patients (47.43 ± 10.09 cell/pixel vs 14 ± 0.76 cell/pixel). Masson staining suggest a differential orientation of collagen fibers in allergic patients with severe pollen-associated food allergy.

Conclusion: Pollen-associated food allergy severity correlates with a damage in the epithelial barrier integrity of the oral mucosa. This suggests that the allergen might be able to penetrate through the damaged epithelia inside the mucosa and induce an inflammatory response.

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Cross-reactivity to fish and chicken meat – a new clinical syndrome?

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Background: Fish is a common elicitor of food-allergic reactions. While the patients' clinical cross-reactivity between different fish species has been addressed in many studies, allergic cross-reactions between fish and other foods are not well understood. This study aimed at analyzing the relevance of clinical cross-reactivity between fish and chicken meat in patients with allergy to chicken meat without sensitization to hen's eggs.

Method: Patients with food allergy to fish and chicken meat ($n = 29$) or chicken meat only ($n = 7$) were recruited. IgE-reactive chicken proteins were identified by Edman protein sequencing and mass spectrometry (MS) analysis as well as quantified by ELISA. Purified allergens were applied in specific IgE measurements by ELISA and skin testing.

Results: Chicken parvalbumin (12 kDa) as well as two others proteins, aldolase (40 kDa) and enolase (50 kDa) were identified as chicken meat allergens. They were recognized by specific IgE of 61%, 75% and 83% of all chicken meat-allergic patients' sera. Fish and chicken meat allergens were highly cross-reactive while high inhibition rates with fish or chicken allergens correlated with the patients' primary sensitization to fish or chicken. In cooked or roasted food samples, enolase and aldolase were detectable in chicken breast while parvalbumin was detectable in chicken legs and wings.

Conclusion: Fish and chicken meat are cross-reactive food sources. Both, fish-allergic and chicken meat-allergic patients might be at risk to develop a food allergy based on cross-sensitization to either chicken meat or fish. Thus, we propose the term 'fish-chicken syndrome' for this clinically important phenomenon, which involves the cross-reactive allergens parvalbumins, enolases and aldolases.

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Is jellyfish ingestion safe in allergic patients? Preliminary results

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Background: Jellyfish consumption is an ancient habit in China where it is used for prevention of arthritic diseases as well as appreciated for its taste. Jellyfish intake is increasing in Europe due to globalization and to the recommendation of the Food and Agriculture Organization of the United Nations among other factors. *Catostylus* tagi is an edible jellyfish native to the Portuguese coast.
Objective: To evaluate the safety of *C. tagi* in crustacean and/or cephalopod allergic patients and their willingness to introduce it in their diet.
Method: Exemplars of C. tagi were caught in the Tejo River, and prepared at the Egas Moniz laboratory as previously described. Samples for skin prick-prick tests (SPPT) were prepared 8–12 h before utilization. Two pastes were made with the small round portions of cooked *C. tagi* mixed with normal mayonnaise at 5% and 10% w/w, samples 1 and 2 respectively. The snack pastes were prepared a few minutes before consumption by spreading 100–200 mg of the paste on wheat toasts of about 3 g.

*T*en adults, 8 female with a median (interquartile range) age of 35 (22) years, allergic to fish, crustacean and/or cephalopods were included. Five had asthma and 9 rhinitis; 9 were sensitized to dust mites. All had previous history of severe systemic reactions and 5 of them anaphylaxis. First, SPPT with the crude umbrella of *C. tagi* were performed and if negative, they were invited taste the snack sample 1 and 15 min later, if negative, sample 2. A 9 point hedonic scale was applied after sensory analysis. They were also asked to answer a short questionnaire. This study was approved by the hospital ethics committee and written informed consent was obtained.
Results: All 10 patients had negative SPPT with crude *C. tagi*. All 10 volunteered to taste both snacks and no adverse reactions were observed. The tasters scored samples 1 and 2 with an average of 6 and 7 respectively. Nine out of the ten patients were willing to introduce *C. tagi* into their diet.
Conclusion: All 10 fish and/or seafood allergic patients had negative skin prick-prick tests and negative oral challenges. If this sample of patients tolerated jellyfish, it could be extrapolated to be non-allergenic to a larger population that is not allergic to seafood. These preliminary results encourage further studies with a larger sample of allergic patients.

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Plane tree pollinosis can influence the clinical and molecular expression of LTP food allergy

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Background: Lipid Transfer protein (LTP) allergy is the main cause of food allergy in adults in the Mediterranean area. It is known to be less severe in context of pollinosis. Most patients suffer from Pru p 3 (peach LTP) allergy in context of Plane tree pollinosis in Barcelona. The aim of the study was to establish the influence of Plane tree pollinosis on the clinical and molecular expression of LTP food allergy.
Method: Patients allergic to plant food and monosensitized to LTP were consecutively included and classified in two groups: A: Plane tree pollen (PTP) allergic (Pollinosis and sensitization to Pla a 1 and/or Pla a 2). B: No pollinosis neither sensitization to Pla a 1/Pla a 2.

Symptoms due to respiratory and food allergy, culprit food and molecular sensitization pattern were recorded. Skin prick test (SPT) to a standard panel of inhalants and plant foods and prick by prick to culprit food were performed. Allergen components to PTP (Pla a 1, Pla a 2) and Pru p 3 were evaluated by SPT. Pla a 1, Pla a 2, Pla a 3, Pru p 3 and other food LTP (hazelnut: Cor a 8, peanut: Ara h 9, walnut: Jug r 3, wheat: Tri a 14) were evaluated by ISAC®. Nasal challenge (NC) to PTP was performed.
Results: $N = 57$. Age 37 ± 10.8 y.o. A: 32/B: 25. Group A: 100% rhinitis, 40% had also asthma.
Number of culprit foods: A: 3 [2–7] vs B: 3 [2–6]. Clinical manifestations: oral allergy syndrome (OAS) and anaphylaxis were the most frequent manifestations in both groups. Anaphylaxis was more frequent in B.
Molecular Pattern: Pla a 3 was recognized in 66% of A o and 29% of B.
Distribution of LTP reactivity: Most patients recognized more than one LTP. Patients allergic to PTP (A) showed a broader recognition pattern of LTPs, compared to B (A/B): Jug r 3 (50%/50%), Cor a 8 (50%/33%) were the most frequently recognized, followed by Ara h 9 (31%/33%) and Tri a 14 (22%/5%).
4 patients with an exclusive sensitization to Pla a 3 without Pla a 1 or Pla a 2, showed a positive nasal challenged with PTP extract.
Conclusion: There are no differences in clinical manifestations between A and B, although anaphylaxis and OAS tend to be more frequent in B. Most of our patients are sensitized to more than one LTP. LTP allergic patients with plane tree pollinosis show a broader LTP recognition pattern than those without pollinosis. An isolated sensitization to Pla a 3, in the absence of Pla a 1/ Pla a 2, could also induce a pollinosis in patients allergic to plant food LTP.

Poster Discussion Session PDS 10

Mechanisms of immunotherapy

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Geographical variability in IgE and IgG4 in patients with allergy to *Dermatophagoides pteronyssinus* before and after treatment initiation with different concentrations of a depigmented and polymerized extract

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Background: Allergy to house dust mites has a high prevalence in Spain. 41.4% of allergic asthma (AA) and 39% of allergic rhinoconjunctivitis (AR) patients are sensitized to *Dermatophagoides pteronyssinus* (DPT). Allergen immunotherapy is an efficient and safe treatment, eliciting immunologic changes since its initial doses.
Method: We analyzed data from a phase I, multicentre, open-label, single-agent and dose-escalation study, in which a depigmented modified extract of DPT was administered subcutaneously to adult patients with allergic rhinitis and/or AR, with or without mild persistent controlled AA. In three medical centers located in distant regions of the Spanish geography (North, South and Northeast), 36 patients were treated: six with each of the dose concentrations tested: 0.11, 0.28, 0.44, 0.55, 0.66 and 0.77 mg/ml, during four visits:
*Visit (V) 1: screening.
*V2: administration of 0.1 ml, 0.2 ml and 0.2 ml
*V3: one month later administration of 0.5 ml
*V4: final assessment

IgE and IgG4 levels to DPT, Der p 1 and Der p 2 were measured at V1 and V4.
Results: An increase in IgE to DPT was observed with all dose concentrations, mean levels at V1 being 39.7 (22.3, 57.1) KU/L, and at V4 73.6 (50.1, 97.1) KU/L (95%CI). Mean IgG4 levels increased from 0.4 (0.3, 0.5) mg/L at V1 to 0.9 (0.6, 1.1) mg/L at V4 (95%CI).

We observed important geographical differences between mean IgE DPT levels in the medical centres involved, the values at V1 being respectively 74 (17.7, 130); 38.4 (9.4, 67.4) and 20.3 (4.5, 36) KU/L (95% CI). The distribution of IgE to Der p 1 and Der p 2 by centres mimicked that of total DPT. At V4 there was an increase in mean levels in all centres. Regarding IgG4 to DPT, the differences at V1 were even more pronounced between the centres in the North, Northeast and South, the values being 1.86 (0, 5.02); 0.33 (0.17, 0.5) and 0.25 (0.13, 0.37) respectively (95%CI). Interestingly, in spite of the general increase in IgG4 for all patients of the study at V 4, there was actually a decrease in the centre with the highest values (North), making the initial regional differences smaller, the final values being 1.05 (0.38, 1.71), 0.68 (0.36, 1.01) and 0.73 (0.19, 1.27) (95%CI) for North, South and Northeast centres respectively.
Conclusion: There was an important geographical variability in basal IgE and IgG4 to DPT. They exhibited a tendency to increase after treatment, presenting regional differences that resemble those observed initially.

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Dynamics of soluble forms of VCAM-1 and CD23 during specific immunotherapy in children with seasonal allergic rhinitis

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Background: The level of soluble forms of membrane-associated molecules in the serum reflects the degree of allergic inflammation underlying seasonal allergic rhinitis (SAR) but the dynamics of these molecules in the pathogenesis of SAR and its evolution during treatment, the most important of which is specific immunotherapy (SIT), remains to be established. The aim of the study was to determine the dynamics of serum levels of soluble forms of vascular cell adhesion molecule-1 (sVCAM-1) and soluble fragment of low-affinity IgE-receptor, FcεRII (sCD23) during pollen immunotherapy in patients with SAR.
Method: Two groups of children (aged 6–14 years) with SAR were examined: I – group without SIT, II – children who were treated for one – two years with specific immunotherapy using standardized allergenic extracts. Control group: children of the same age without the signs of atopy. sVCAM-1 and sCD23 concentrations were measured by a sandwich enzyme-linked immunosorbent assay.
Results: There were increased concentrations of sVCAM-1 and sCD23 in group I (without SIT) comparing to controls ($P < 0.05$). In addition, the level of sCD23 in all children with SAR correlated with the level of total IgE ($P < 0.05$). The sCD23 level did not fluctuate during the natural course of disease in untreated patients, but was significantly decreased in patients who received immunotherapy for 1–2 years ($P < 0.001$). As for the levels of sVCAM-1, they were also significantly decreased after immunotherapy ($P < 0.05$). However, the percentage of the decrease in the sVCAM-1 levels was not correlated with the duration of immunotherapy.
Conclusion: It could be concluded that decreased expressions of sVCAM-1 and sCD23 after SIT are probably related to attenuation of inflammatory reactions in SAR. Reduction in sCD23 levels during SIT is also probably involved in the working mechanisms of immunotherapy and clinical improvement in SAR, but modulation of serum sVCAM-1 levels is not likely participates in mediating of its clinical effect.

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Peanut-specific immunoglobulin levels following SCIT-treatment with a chemically modified, aluminum hydroxide adsorbed peanut extract (HAL-MPE1) in peanut allergic patients

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Background: Currently, there is no effective disease modifying treatment for peanut allergy available. Therefore, a chemically modified, aluminum hydroxide adsorbed peanut extract (HAL-MPE1) for subcutaneous administration has been developed. In a phase I study, evaluating the safety

and tolerability of SCIT-treatment with HAL-MPE1 in subjects with peanut allergy, changes in peanut specific immunoglobulin levels and basophil histamine release were assessed.

Method: In a randomized, double blind, placebo controlled, single-centre, Phase I study 17 Caucasian subjects (mean age: 20.9±2.6 years, 53% female), with a history of systemic reactions after peanut ingestion, a positive food challenge test and an Ara h 2 IgE > 0.7 kU/l, were randomized to receive 15–20 weekly incremental doses of either HAL-MPE1 (11 subjects) or placebo (6 subjects). At baseline and end of study IgE, IgG and IgG_4 to peanut extract, peanut allergens (Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, Ara h 9) and basophil histamine release were determined.

Results: Increased IgG and IgG_4 levels specific for peanut extract, Ara h 1, Ara h 2, Ara h 3, Ara h 6 were observed following active treatment as compared to placebo, whereas no clear changes in Ara h 8 and Ara h 9 specific IgG and IgG_4 levels were observed compared to placebo. No clear pattern in IgE levels specific for peanut extract and peanut allergens (Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, Ara h 9) was observed. Furthermore, a trend towards a difference in basophil histamine release between the placebo group and the active treatment group was observed.

Conclusion: The results indicate that subcutaneous administration of HAL-MPE1 is capable of inducing immunological changes following 4–5 months of weekly dose escalations.

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EPIT-induced bystander effect mainly conferred by naive Tregs via soluble factors and cell-cell contact in a murine model

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Background: Only epicutaneous immunotherapy (EPIT), as compared to oral or sublingual immunotherapy, induces naive Tregs in a model of food allergen sensitized mice (Dioszeghy et al., 2015) and prevents the induction of anaphylaxis to further allergens via regulatory T cells (Tregs) (Mondoulet et al., 2015). This study is an in-depth investigation of the role of naive Tregs in this bystander effect.

Method: Following milk sensitization, mice were treated with milk EPIT or Sham. CD4+CD25+ T cells (Tregs) were isolated with an additional CD62L+ surface marker defining naive Tregs. Naive and effector Tregs were adoptively transferred into recipient mice, which were then subjected to peanut sensitization and IV challenge with peanut. In a second experiment, TGF-b, CTLA-4 and OX40 were inhibited by the injection of blocking antibodies 24 h before their adoptive transfer into milk-sensitized mice or 24 h before an IV challenge in peanut-sensitized mice. Outcome markers included a drop in rectal temperature, hypersensitivity reactions and serum mouse mast cell protease-1 (mMCP1) measurements after IV challenge with peanut.

Results: In recipient mice sensitized to peanut and previously infused with naive Tregs induced by milk EPIT, there was no induction of peanut s-IgE but s-IgG2a was significantly increased and animals were fully protected against anaphylaxis after IV injection of peanut ($P < 0.001$). However, in mice receiving effector Tregs and then sensitized to peanut, peanut s-IgE increased and s-IgG2a was unchanged, and mice were not protected against anaphylaxis after IV injection of peanut (ns). Noticeably, this protection was lost by blocking TGF-b, CTLA-4 orOX40 before the adoptive transfer ($P < 0.05$, $P < 0.01$ and $P < 0.01$ respectively) and before the IV challenge ($P < 0.05$, $P < 0.01$ and $P < 0.01$ respectively).

Conclusion: Naive Tregs induced by EPIT might play a central role in the bystander effect, via soluble and cell-cell contact interaction.

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Inhibition of CD23-mediated Serum IgE-facilitated allergen presentation by allergen-specific immunotherapy with Japanese cedar pollinosis

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Background: Subcutaneous immunotherapy (SCIT) with Japanese cedar pollen (JCP) extract has proven its effectiveness in both adults and children who suffer from Japanese cedar pollinosis. The treatment is associated with increases in allergen-specific serum IgG4 which is thought to compete with IgE bind to allergens. The IgE receptors expressing on surface of antigen presenting cells have been shown to facilitate the presentation of allergens. It is interesting to investigate whether SCIT with JCP extract alters IgE-facilitated allergen binding, which might relate to the mechanisms of action in Allergen Immunotherapy (AIT). The cell free enzyme-linked Immunosorbent facilitated antigen binding (ELIFAB) was chosen in this study.

Methods: Various concentrations of human recombinant soluble CD23 (sCD23) (low affinity IgE receptor; FceRII) were immobilized overnight on a 96-well microtiter plate. Serum containing JCP-specific IgE preincubated with allergen JCP extract was transferred to the 96-well plate to measure binding of JCP-IgE to coated sCD23. JCP-IgE bound to sCD23 was detected by biotin-conjugated anti-human IgE antibody. The study used sera from Japanese cedar pollinosis subjects with or without SCIT (12 subjects received standardized allergen extract of JCP at a dose of 2000 JAU) for at least 48 months.

Results: The optimal binding to sCD23 occurred between 0.3 and 1 mg/ml of JCP allergen extract. The specificity of JCP-IgE binding to sCD23 was confirmed by using an anti-CD23 blocking antibody. Dependency of IgE for binding was also confirmed by means of heat denaturation of serum. In ELIFAB assay, JCP-IgE complexes bound to sCD23 was significantly reduced with sera from JCP allergic patients treated with SCIT compared to sera from non-treated patients.

Conclusion: This is the first report on the use of ELIFAB for measuring IgE-facilitated allergen binding with sera from JCP patients. ELIFAB assay provides a cell free, simple and reproducible method to measure the ability of allergen to cross-link IgE-CD23 complexes. Reduced binding of allergen-IgE to sCD23 in the patient treated with SCIT suggests that ELIFAB can be one of simple methods to assess the immunological response to AIT.

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EPIT-induced Tregs suppress T cell proliferation in specific and bystander conditions in a model of food allergen sensitized mice

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Background: Epicutaneous immunotherapy (EPIT) on intact skin induces sustained desensitization in mouse models of food allergy. Mechanistic analyses show that EPIT significantly increases the Foxp3+ Tregs population. Adoptive transfer of EPIT-induced Tregs protects sensitized mice from anaphylaxis and prevents further sensitization to other allergens (bystander

effect). This study investigates the suppressive properties of EPIT-induced Tregs with a focus on specific/bystander effects.
Method: Milk-sensitized BALB/c mice were treated with milk EPIT or not (Sham). Tregs ($CD4^+CD25^+$ T cells) from milk EPIT, Sham or non-sensitized groups were sorted, as well as effector T cells ($CD4^+CD25^-$) from milk or peanut sensitized mice, and co-cultured for 4 days at different ratios with allergen-pulsed $CD11c^+$ antigen-presenting cells using either anti-CD3 or allergen stimulation. Anti-CTLA-4 and anti-TGF-b antibodies were used to determine whether EPIT-induced Tregs act via cytokines or cell-contact dependent mediation. Suppression was analyzed by tracking divided $CD4^+CD25^-$ with CFSE by flow cytometry. Supernatants were also collected to quantify cytokine secretion.
Results: With anti-CD3 stimulation, Tregs were able to suppress effector T cell (from milk or peanut sensitized mice) proliferation whatever the experimental groups (up to 90–98% of proliferation inhibition in EPIT and Sham groups). In contrast, with allergenic stimulation, only EPIT-induced Tregs significantly inhibited effector T cells proliferation in specific or bystander conditions (i.e. 20–25% proliferation inhibition) compared to Sham or non-sensitized Tregs. Interestingly, blocking CTLA-4 and TGF-b abrogated the suppressive capacity of EPIT-induced Tregs in both conditions. IL-2 was barely detectable in supernatants of EPIT Tregs compared to Sham Tregs, suggesting that EPIT-Tregs highly consume this cytokine.
Conclusion: With allergenic stimulation, EPIT-induced Tregs inhibited effector T cell proliferation with the same potency in specific and bystander conditions. Suppression induced by EPIT-induced Tregs might use 3 complementary pathways:
(i)a high consumption of IL2 reducing its availability for effector T cells,
(ii)TGF-b secretion and
(iii)CTLA-4 cell contact mediation.

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Intranasal delivery of a nanoemulsion vaccine suppresses T_H2 immunity and inhibits allergic responses in a mouse model of allergy

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The pathology associated with allergy is most often the result of a Th2-skewed immune response and increased IgE production, triggering the release of histamine from mast cells, which can lead to anaphylaxis. Immunotherapies to shift immune responses from Th2 to Th1 have generally required prolonged immunization protocols and have not induced long-lasting Th1 responses. We have demonstrated that nanoscale emulsion (nanoemulsion, NE), a novel mucosal adjuvant, induces robust IgA and IgG antibody responses and Th1/Th17-polarized cellular immunity, resulting in protection against a variety of respiratory and mucosal infections. We hypothesized that this strong induction of Th1/Th17 by nanoemulsion has the potential to modulate the Th2 immune responses associated with allergy, by re-educating the immune system to have a more balanced response when encountering the allergen. A Th2-biased allergic phenotype was established by intraperitoneal sensitization with ovalbumin (ova) and alum. Subsequent IN immunizations with NE-ova resulted in increased Th1 associated immune responses (IFN-γ, TNF-α, IgG2a and IgG2b) and IL-17, while decreasing Th2 cytokines (IL-4, IL-5 and IL-13) and IgG1. Additionally, the NE immunization also significantly increased IL-10 production as well as regulatory T cells, suggesting the suppression of Th2 immune responses may be mediated by regulatory cells. Mice that received the therapeutic NE immunizations also had reduced IgE and increased IgA. Importantly, following inhalation challenge with ova, NE-treated mice had significant reduction in lung inflammation and mucus production, suggesting a strong reduction in allergic hypersensitivity. These data demonstrate that NE-based vaccines can modulate Th2 allergic responses to promote Th1/ Th17 immunity and suggest the therapeutic use of NE vaccines for diseases associated with Th2 immunity.

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Human monocyte-derived suppressor cells control graft-vs-host disease while preserving graft-vs-leukemia effect and acquire clinically relevant qualities

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Background: Graft-vs-host disease (GvHD) heavily limits the curative outcome of allogeneic bone marrow transplantation (BMT). Immunosuppressive agents currently used to control GvHD diminish the efficacy of the immune reconstitution. Immunosuppressive cell based therapy is a relatively recent alternative strategy for such conditions. We have previously reported on a novel subpopulation of human monocyte-derived suppressive cells (HuMoSC) as a prospective approach for controlling GvHD. The objectives of our current study are to explore the therapeutic relevance of HuMoSC in clinical conditions of allogeneic BMT and to evaluate the clinical benefits of such therapy on the curative graft vs leukemia (GvL) reaction.
Method: Therapeutic efficacy of HuMoSC was evaluated in the xeno-GvHD conditioned mice (NOD/SCID/IL2-R$\gamma_c^{-/-}$) with pre-established plasmacytoid dendritic cell neoplasm (Cal-1). HuMoSC properties were explored in different inflammatory environments and in presence of immunosuppressive drugs usually used in patients during allogeneic BMT. Clinical grade HuMoSC were assessed.
Results: HuMoSC are highly potent at controlling GvHD symptoms while preserving graft-vs-geukemia effect with prolonged survival *in vivo*. HuMoSC acquire the distinct genomic signature of immunosuppressive cells. In addition to their own anti-inflammatory effects, HuMoSC are endowed with the peculiar ability of polarizing FoxP3+CD8+ and FoxP3+CD4+ Treg populations from their effector counterparts and of augmenting the regulatory properties of naturally occurring Tregs. Of great clinical relevance, HuMoSC inhibitory function against effector T lymphocytes is preserved in different inflammatory environments and is not affected by anti-inflammatory and immunosuppressive agents currently used in clinical practice. Importantly, HuMoSC can be generated in GMP conditions and they sustain long term preservation with unaltered phenotype and function.
Conclusion: HuMoSC-based cell therapy represents an auspicious targeted approach for controlling GvHD, its efficacy is not altered by the immunosuppressive drugs and, therefore, may take a rapid pace toward the clinical implementation in leukemia patients undergoing allogeneic bone marrow or stem cell transplantation.

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Towards a non-allergenic peptide mix containing the T cell epitopes of the clinically most relevant house dust mite allergens for tolerance induction

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House dust mites are one of the most important allergen sources. Der p 1, Der p 2, Der p 5, Der p 7, Der p 21 and Der p 23 are the clinically most important house dust mite (HDM) allergens. The aim of

this study was to define a mix of non-allergenic T cell epitope-containing peptides of these allergens for tolerance induction. According to the amino acid sequences of these allergens, we synthesized and purified 33 overlapping peptides covering the complete sequences of Der p 1, 2, 5, 7, 21 and 23. The peptides were tested for IgE and IgG reactivity with sera from HDM allergic patients in ELISA. PBMCs from 27 HDM allergic and 10 non-HDM allergic individuals were incubated with the synthetic peptides and T cell proliferation was measured using a CFSE dilution-based assay. The peptides could be purified in large amounts. They lacked secondary structure but most of them remained soluble in physiological buffers. ELISA assays indicated that most peptides from Derp 1, 2, 5, 7, 21 and 23 lacked IgE reactivity and thus were non-allergenic. T cell proliferation assays identified 12 predominant epitopes in the Der p allergens. Our data indicates that a reasonable number of non-allergenic peptides including the sequences and thus T cell epitopes of the clinically most relevant house dust mite allergens can be defined for prevention of HDM allergy by tolerance induction.

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Development and validation of a sandwich enzyme-linked immunosorbent assay (ELISA) for the quantification of Ara h6 in peanut flour, peanut extract, and patches for epicutaneous immunotherapy (EPIT)

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Background: EPIT to treat peanut allergy is currently in late stage clinical investigation. Validated analytical assays are needed to accurately quantify major allergen in pharmaceutical allergenic products.
Method: Polyclonal antibodies were produced by immunization of rabbits with purified Ara h6. Antibodies were purified by affinity chromatography. A first portion was used as coating antibody and a second part was labeled with biotin for use as detection antibody. The ELISA was optimized for signal-to-noise ratio and assay sensitivity. Matrix specificity was assessed by spiking purified Ara h6 into the different allergen materials: peanut flour, peanut extract, and patch. Validation was designed according to the International Conference on Harmonization Q2 (R1) and using the tolerance intervals approach, for each allergen product. For API and Patch, accuracy part of the validation was applied on reconstituted form with 108 individual determinations (6 concentration levels × 6 series (various operators and days) × 3 determinations per series).
Results: The calibration curve ranges from 0.391 to 12.5 ng/ml Ara h6. No relevant cross-reactivity was observed; neither with the Ara h6-homologous allergen Ara h2 (not detectable; <0.1%) nor with Ara h1 allergen (0.62%). Using this ELISA, the Ara h6 content in peanut extract was determined to be around 3% (w/w), in line with literature.

For Peanut extract quantification, the method is linear ($r = 0.955$), precise (coefficient of variation for repeatability (CVr) < coefficient of variation for intermediate precision (CVR) ≤15%), true (relative bias ≤8%) and accurate over the range 40% to 200% (1.0–5.1% (w Ara h6 / w extract)).

For Peanut patch quantification, the method is linear ($r = 0.998$), precise (reconstituted samples: CVr < CVR ≤17%, authentic patch samples: CVr < CVR ≤6%), true (relative bias ≤8%) and accurate over the range 40% to 200% (2.7–13.5 µg Ara h6 per 250 µg peanut protein patch).

Absence of matrix effect was demonstrated for all three tested matrices.
Conclusion: An Ara h6 ELISA was developed for major allergen quantification in peanut products from the source material to the treatment patches. The validation demonstrated that this ELISA is suitable as quantitative assay for release and stability testing.

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A hypoallergenic vaccine of Der p 23, a new major house dust mite allergen, for immunotherapy

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Der p 23, a new, major house dust mite (HDM) allergen which is recognized by more than 70% of HDM-allergic patients has high allergenic activity and therefore must be considered as an important component for HDM-specific immunotherapy. We constructed and characterized a hypoallergenic Der p 23 vaccine for HDM immunotherapy. Three non-allergenic peptides from the C-terminal IgE epitope-containing part of Der p 23 (P4, P5) and P6, a mutant peptide containing serines instead of cysteins were identified. Peptides were fused to the hepatitis B virus-derived PreS domain as recombinant fusion proteins (i.e., PreS-2XP4P5 and PreS-4XP6) which were expressed in *Escherichia coli* and purified to homogeneity. PreS-2XP4P5 as well as PreS-4XP6 showed no relevant IgE reactivity and exhibited considerably reduced allergenic activity in basophil activation tests using blood from HDM allergic patients compared to Der p 23. Upon immunization of rabbits, only PreS-2XP4P5 induced high levels of Der p 23-specific IgG antibodies that inhibited binding of patients′ IgE to Der p 23 comparable to IgG antibodies induced with Der p 23, whereas antibodies induced with PreS-4XP6 had only low blocking capacity. Additionally, IgG antibodies induced with PreS-2XP4P5 inhibited Der p 23-induced basophil activation comparable to IgG antibodies induced with Der p 23. Compared to Der p 23, PreS-2XP4P5 induced lower T-cell proliferation but higher levels of the tolerogenic cytokine IL-10 and the Th1 cytokine IFN-gamma in PBMCs from HDM allergic patients, indicating an immunomodulatory capacity of the fusion protein. Therefore, PreS-2XP4P5 represents a promising candidate for immunotherapy of HDM allergic patients.

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Characterisation of a new method for quantitative determination of house dust mite allergen specific IgE-blocking factor for monitoring allergy immunotherapy with SQ HDM SLIT-tablet

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Background: Allergy immunotherapy (AIT) targets the underlying mechanism of allergic disease and induces allergen-specific non-IgE antibodies that interfere with the binding of allergen to IgE (IgE-blocking factor).

A new method for determination of house dust mite (HDM) IgE-blocking factor in human serum was developed for use in clinical AIT trials with the newly developed SQ HDM SLIT-tablet

(ALK (ACARIZAX®), Torii (Miticure®), Merck (MK-8237)). The method provides a measure of the IgE-inhibitory activity exerted by non-IgE antibodies including IgG_4.

Previously, levels of AIT-induced HDM IgE-blocking factor were determined in independent tests for *D. pteronyssinus* (*Der p*) and *D. farinae* (*Der f*). In contrast, in this new method HDM IgE-blocking factor is determined in a single test, utilizing a 1:1 mixture of *Der p* and *Der f* extracts, the two HDM species contained in the SQ HDM SLIT-tablet.
Method: The clinical trials MT-02 and MT-04 investigated the efficacy and safety of daily treatment with the SQ HDM SLIT-tablet in subjects with HDM respiratory allergic disease.To assess the accuracy of the

new IgE-blocking factor assay, serum samples from MT-02 analysed in 2008 were re-analysed in 2015 with the new method (n = 145).The-blocking factor assay is based on measurements of HDM-specific IgE made in the absence or presence of AIT-induced non-IgE antibodies. To assess the accuracy of the IgE measurements in the new IgE blocking factor assay, serum samples from MT-04 were analysed and compared to the reference method for specific IgE (ImmunoCAP) (n = 699).

Results: A significant correlation ($P < 0.001$) between the new and the previous IgE-blocking factor assays was observed when analysing samples obtained before and after SQ HDM SLIT-tablet treatment.A significant correlation ($P < 0.01$) between IgE measured by the new IgE-blocking factor method and the reference ImmunoCAP IgE method was observed.

Conclusion: The new method for quantitative determination of HDM IgE-blocking factor using a mixture of *Der p* and *Der f* extracts is comparable to the previous methods and is suitable for measurement of IgE-blocking factor induced during SQ HDM SLIT-tablet AIT.

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Pollen-food syndrome – a novel way to tackle the problem

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Background: In the Northern hemisphere one of the main causes of winter/spring pollinosis is birch pollen allergy induced by Bet v 1. Most patients reacting to this protein also suffer from adverse reactions after the ingestion of various fruits, nuts, and vegetables. The oral allergy syndrome (OAS), a class 2 food allergy, is caused by food allergens structurally related to Bet v 1, which are able to cross-link IgE originally generated against Bet v 1. Birch pollen extracts administered during allergen immunotherapy are not always suitable to ameliorate OAS. Therefore, the aim of this study was to design a novel hybrid protein with reduced IgE-binding capacity for the combined treatment of birch pollen and associated food allergies towards apple and hazelnut.

Method: After the design of the 'birch-apple-hazelnut' allergen hybrid molecule MBC4, all proteins (Bet v 1 from birch, Cor a 1.04 from hazelnut, Mal d 1 from apple, and MBC4) were produced recombinantly and purified to homogeneity. IgE ELISA and mediator release assays were used to determine the IgE binding capacity. To further investigate the immunological behavior of MBC4 T cell proliferation assays were performed and an *in vivo* mouse model was established. Moreover, the susceptibility towards proteolytic processing of the proteins was examined.

Results: After purification all four proteins were characterized physico-chemically. Measurements displayed an increased hydrodynamic radius and a reduction of secondary structural elements of MBC4 compared to the parental allergens. Further, IgE-binding of MBC4 was significantly reduced. *In vitro* as well as *in vivo* data revealed abrogated cross-reactivity on IgE level, but retained immunogenicity on T cell level, as well as the induction of cross-reactive IgG antibodies.

Conclusion: MBC4 is a promising vaccine candidate for the combined treatment of birch pollen and associated food allergies towards apple and hazelnut. It displays reduced IgE-binding capacity, conferring a good the safety profile, whereas it is still able to stimulate an allergen-specific T cell response.

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Oral immunotherapy in combination with a non-digestible oligosaccharide supplemented diet in a peanut allergy mouse model

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Background: Although the prevalence of food allergy is increasing, no curative treatment is available yet. Improving oral immunotherapy (OIT) for food allergy is necessary to reduce side effects and achieve tolerance. Non-digestible oligosaccharides, like scFOS/lcFOS (FF), have been shown to reduce allergic symptoms in murine models of allergy. This study aims to evaluate the capacity of FF to support OIT in an established peanut allergy mouse model.

Method: After sensitization (d0–d35) using peanut extract (PE), mice received a 1% FF (9:1) or control diet for the rest of the study and were treated with PE or PBS intragastric (5 times/week) for three weeks (d41–d59). Hereafter, mice were exposed to PE via an intradermal (d64), intragastric (d70) and intraperitoneal (d77, i.p.) challenge to determine clinical efficacy (acute allergic skin responses, anaphylactic shock symptoms and body temperature). Furthermore, antibody levels, cytokine production and number of various immune cells were measured at different time points during the study (d0, d35, d50, d63, d71 and d78).

Results: OIT on its own, was able to reduce allergic symptoms upon PE challenges, in addition, serum levels of IgE, IgG1 and IgG2a, were raised after OIT. OIT+FF was able to lower the acute allergic skin response and mast cell degranulation after peanut exposure. FF did not show an additive effect on antibody levels. On d63 and d78, the production of cytokines IL-5 and IL-10 by splenocytes, and on d63 by MLN cells was elevated in the OIT+FF group compared to the OIT group. After therapy (d63), percentage of B cells in the spleen was lower in the OIT+FF group compared to the OIT group. On d78, Th1 cells were higher in the MLN and Th2 cells were lower in the spleen in the OIT+FF group vs the OIT group. Also, activated CD8+ T cells were lower in the MLN and CD103+CD11b+ DCs were higher in the spleen. During therapy (d50), the short-chain fatty acid (SCFA) content in the caecum showed a shift by the FF diet, to a higher butyrate, lower acetate ratio.

Conclusion: These data show that in a mouse model for peanut allergy, OIT+FF protect against allergic responses upon peanut exposure. However, the OIT dose seemed too low to protect against the i.p. challenge. Furthermore, cellular parameters suggest Th2 suppression after OIT+FF, compared to only OIT. FF also altered the cytokine production and influenced SCFA content in the caecum. In future experiments, we will focus more on the working mechanism.

Poster Discussion Session PDS 11

Infections and microbiota in allergy

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IgA responses to the gut microbiota in infants in relation to allergy development

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Background: The increasing allergy prevalence in affluent countries may be caused by reduced exposure and diversity of microbial stimulation, resulting in abnormal postnatal mucosal immune maturation. While a reduced gut microbiota diversity and low mucosal total IgA levels in infancy have been associated with allergy development, IgA responses to the gut microbiota have not been studied.
Method: The proportion of the gut microbiota, bound to IgA or not, was analyzed by flow cytometry-based sorting of faecal samples, collected at 1 and 12 months of age in 20 children developing allergy and 28 children staying healthy up to seven years of age. Furthermore, the microbial composition and diversity of bacteria, bound or not bound to IgA, were analyzed with barcoded 16S rDNA 454-pyrosequencing. Together with the analysis of total secretory IgA load, the bacterial load of the stool samples of healthy and allergic children was also determined.
Results: IgA-coating patterns decreased from 1 to 12 months of age in both allergic children and children staying healthy up to 7 years of age, reflecting the contribution of maternally derived IgA antibodies in breast milk during the first period of life. Children developing allergic manifestations, particularly asthma, during childhood had a lower proportion of IgA bound to faecal bacteria at 12 months of age compared to healthy children. This cannot be attributed to differences in IgA levels nor to differences in bacterial load, which was shown to be higher in healthy children. However, the bacterial targets of early IgA responses (including the coating of genus *Bacteroides*) as well as the IgA recognition patterns, detected by *Principal Component Analysis*, seem to differ between healthy children and children developing allergic manifestations.
Conclusion: Allergy and asthma development associates with an altered IgA responsiveness to the gut microbiota during infancy, possibly indicating an impaired mucosal barrier function.

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Lower gut microbiota diversity is associated with higher susceptibility of BALB/c mice to allergic sensitization

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Background: The hygiene hypothesis suggests that neonatal alterations of microbial exposure promote the development of allergic diseases by impairing the maturation of the host immune system. In previous works, we observed that conventional (Cv) BALB/c mice experimentally sensitized to cow's milk (CM) could exhibit a rather high inter-individual variability for the production of CM-specific IgE antibodies. Here, we aimed to determine whether this level of sensitization could be correlated to the early gut microbiota composition.
Method: Five-week-old BALB/c mice Cv (n = 92) were orally sensitized to CM with cholera toxin as adjuvant. Fecal samples were collected one week before sensitization in order to analyze the gut microbiota composition by 16S rRNA gene sequencing. IgG1 and IgE responses against β-lactoglobulin (BLG), a major CM-allergen were measured in sera. Analysis of lymphocyte populations by flow cytometry were performed in spleen cells.
Results: As expected, a great variability of the BLG-specific antibody responses was observed among sensitized mice. 13 high-IgE responder (HR) mice and 13 low-IgE responders (LR) mice were then selected for further analysis of the gut microbiota composition. A lower gut microbiota diversity was observed in HR mice compared to LR mice. Even though the dominant microbiota was not significantly different, the relative abundance of the genus *Lachnospiracea incerta sedis* and of several bacterial isolates was significantly different between HR and LR mice. Surprisingly, HR mice also displayed a higher frequency of T regulatory cells (CD4+ CD25+ Foxp3+) than LR mice.
Conclusion: These results confirmed that the gut microbiota could actually influence the host susceptibility toward an allergic sensitization, even in mice that shared the same genetic background (inbred BALB/c mice), the same diet and the same housing. They are also in agreement with results from human studies reporting a lower gut microbiota diversity in allergic children.

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Decreased microbial conversion of lactic acid into butyrate in infants developing eczema

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Background: The development of the early gut microbiome is a dynamic process significantly influencing health throughout life. Aberration in its early development has been associated with the development of allergic diseases, but the exact patterns remain unclear. In this study the temporal dynamics of the infant gut microbiota were investigated and associated with the development of eczema in the first 18 months of life.
Method: This arises from a parent registered study (ISRCTN65195597) investigating the effects of a partially hydrolysed formula containing specific oligosaccharides (pHF-OS) on the prevention of eczema in infants at risk for atopy. Gut microbial composition was investigated in a set of vaginally born infants (n = 138). Faecal bacterial compositions were analysed by 16S rRNA gene sequencing of DNA extracted from stool samples in the first 6 months of life. In addition major microbial metabolites (lactate and SCFAs)

and stool pH were determined. Statistical analyses involved multivariate explorative data analysis using Canoco 5 software and differential abundance testing using the R-package MetagenomeSeq. All comparisons between infants developing and not developing eczema were corrected for the type of feeding, ethnicity and having siblings.

Results: Infants developing eczema in the first 18 months of life showed aberrant gut microbiome development in the first 6 months of life with significant temporal differences of *Parabacteroides* and genera of Enterobacteriaceae. These genera decreased in time in subsequently healthy infants, but the decrease was less pronounced for infants that developed eczema. Furthermore over time, eczematous infants showed decreased acquisition of lactate-utilising bacteria known to produce butyrate, namely *Eubacterium* and *Anaerostipes* spp., which was supported with significantly increased faecal concentrations of lactic acid and decreased concentrations of butyrate at 6 months of age.

Conclusion: The differential temporal dynamics and development of key bacterial species and metabolites in the gut of infants developing eczema may reflect a suboptimal implementation of the intestinal microbiome already early in life. The aberrances identified may prove to be useful as biomarkers for later atopic diseases and could aid the development of optimal nutritional strategies to support timely gut colonisation of key species, such as lactate-utilising and butyrate producing bacteria, in the gradually diversifying infant gut.

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PGE2 released by alveolar epithelial cells protects the pulmonary endothelial barrier integrity

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Background: One of the hallmarks of acute respiratory distress syndrome, a condition caused by a wide variety of factors (e.g. infection or trauma) is the disruption of the blood-air-barrier formed by pulmonary microvascular endothelial (PMVEC) and alveolar epithelial cells (AEC) which are closely neighbouring. A recent paper demonstrated that the lipid fraction of supernatants from AEC strengthened the PMVEC barrier function [*1*]. We have shown that PGE2, leads to increased endothelial barrier function via EP4-receptor activation [*2*]. Therefore, we hypothesized that the reported effect was mediated via PGE2 and investigated this by (I) measuring PGE2-levels in supernatants of AEC (II) assessing cyclooxygenases (COX) expression (III) analysing PMVEC-barrier function and (IV) using a selective EP4-antagonist in order to investigate the involvement of the EP4 receptor in barrier-enhancement.

Method: Primary AECs were isolated from Balb/c-mice and seeded at a density of 1 million cells/cm² in plates coated with laminin. After 6 days in culture, cells were treated with vehicle or lipopolysaccharide (LPS 10 μg/ml) with or without COX-inhibitors, incubated for 8 h and supernatants were collected. PGE2-levels were measured by radioimmunoassay. COX 1 and COX-2 was determined by immunofluorescence and Western blots. Barrier function was investigated using electric cell substrate impedance sensing. One way- and two way-ANOVA for repeated measurements followed by Bonferroni post-tests were used for statistical analysis.

Results: We found that AEC release high levels of PGE2, which was significantly increased by stimulation with LPS (average of 7.5 ng/ml ± 1.2 vs 15.6 ng/ml ±1.4, $n = 4$, $P < 0.0001$). COX-1 and COX-2 were constitutively expressed in AQP5 (a marker for type 1 AEC)-positive cells ($n = 3$). Treatment of PMVEC with supernatants of AEC strengthened the barrier function and this effect was significantly inhibited by a selective EP4-antagonist ($n = 4$) and absent when AEC were treated with COX inhibitors.

Conclusion: In conclusion we found that AEC constitutively express COX-2 and release PGE2 into the supernatant which increases the PMVEC barrier function via PGE2 mediated EP4 receptor activation. Therefore, EP4 receptor activation by strengthening the barrier function is a promising approach for future therapeutic options in ARDS.

1 Wang L et al. PLoS ONE 2013, 8(2) e55311

2 Konya *et al. J Allergy Clin Immunol*, 2013, 131(2)532–540

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Spleen tyrosine kinase induces MUC5AC expression in human airway epithelial cell

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Background: MUC5AC is a major secreted mucin and is increased in chronic inflammatory airway diseases. Spleen tyrosine kinase (SYK) is a mediator, which acts as an important regulator of intracellular signal transduction in the inflammatory response. Originally, SYK was identified in hematopoietic cells, but it was recently demonstrated that SYK is also expressed in some non-hematopoietic cells, including respiratory epithelial cells. However, the effects of SYK on mucin-secretion in human airway epithelial cells has not been studied.

Method: In mucin-producing human NCI-H292 cells and primary cultures of human nasal epithelial cells, the effects and signaling pathways of SYK on MUC5AC expression were investigated by reverse transcriptase-polymerase chain reaction (RT-PCR), real-time PCR, enzyme immunoassay, and immunoblot analysis with several specific inhibitors and small interfering RNA (siRNA).

Results: SYK induced MUC5AC expression. SYK significantly activated the phosphorylation of ERK1/2 and p38 MAPK signaling pathways. SYK-induced MUC5AC expression was significantly attenuated by pretreatment with U0126 (ERK1/2 MAPK inhibitor) and SB203580 (p38 MAPK inhibitor). In addition, the knockdown of the ERK2 and p38 MAPK by ERK2 and p38 MAPK siRNA significantly blocked SYK-induced MUC5AC expression.

Conclusion: These results show that SYK increases MUC5AC expression via ERK2 and p38 MAPK signaling pathways in human airway epithelial cell.

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Development of a mouse model of RSV-induced asthma exacerbations

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Background: Bronchial asthma (BA) is one of the most common chronic inflammatory disorders of respiratory tract. Respiratory syncytial virus (RSV) is one of the main causes of BA exacerbations. The aim of this study was to develop the mouse model of RSV-induced asthma exacerbations.

Method: Female BALB/c mice were divided into 4 groups. The groups 1 and 2 were i.p. sensitized with 20 μg/mouse ovalbumin (OVA) mixed with 2 mg/mouse aluminum hydroxide on days 1, 14 and 27 followed by i.n. challenged with 500 μg/mouse OVA on days 40, 41 and 42. The group 2 was additionally infected with 5×10^6 $TCID_{50}$/mouse RSV strain A2 on day 38. The group 3 was infected with RSV in the same regimen but without OVA sensitization and challenge. The

group 4 was naïve mice. 24 h after last OVA challenge airway hyperresponsiveness (AHR) to methacholine was measured by whole-body plethysmography. The left lung was taken for histological analysis. The right lung was used for viral RNA (vRNA) evaluated by qPCR. Bronchoalveolar lavage (BAL) was collected for the total cell count by flow cytometry.

Results: The infected animals (groups 2 and 3) lost 9% and 13% of body weight, respectively,compared to naïve mice (groups 4), indicating successful RSV infection. The infection of respiratory tract was confirmed by RT-PCR analysis, which detected from 2×10^6 to 7×10^6 vRNA copies/1 μg total RNA, respectively. Animals received OVA (group 1) and OVA followed by RSV infection (group 2) demonstrated significant increase in AHR by 118% and 79% compared to naive mice. Furthermore, the RSV infection of OVA treated mice resulted in a 2.2-fold increase in the number of total leukocytes in BAL compared to naïve mice and 1.6-fold increase compared to OVA sensitized and challenged mice. Histological examination of the lung tissues revealed predominantly eosinophil infiltration after OVA sensitization and challenge and lymphocyte infiltration in animals received RSV only. The mice received OVA followed by RSV infection demonstrated both eosinophil and lymphocyte infiltration to the lungs, that indicate combined allergic and viral inflammation.

Conclusion: Developed model of RSV-induced BA exacerbations reflected the main features of the disease: RSV replication in the respiratory tract, increased AHR and combined eosinophil/lymphocyte mediated inflammation. Described model can be used for the revealing of molecular mechanisms of the disease and for pre-clinical studies. Supported by RSF No14-15-00894.

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Respiratory syncytial virus up-regulates lung IL-33 expression during allergic pulmonary inflammation

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Background: IL-33, an IL-1 family member, is crucially expressed and involved in pulmonary disorders, but its regulation in virus-induced exacerbation of allergic airway inflammation remains unclear. We hypothesized that respiratory syncytial virus (RSV) infection of the allergic lung would increase IL-33 expression.

Method: In order to develop allergic airway inflammation female BALB/c mice were sensitized (on days 0, 14 and 27) and challenged (on days 40, 41 and 42) with ovalbumin (OVA) (OVA group). For development of RSV-induced exacerbation of allergic inflammation, mice were additionally infected with RSV strain A2 on day 38 (OVA/RSV group). Third group of mice was treated with RSV alone (without sensibilisation – RSV group). Untreated mice were used as control (Control group). Airway hyperresponsivness (AHR) was assessed in 24 h after the last OVA challenge. Next day mice were sacrificed for endpoint analysis. Histological analysis of the lung, evaluation of IL-33 mRNA expression and assessment of amounts of viral RNA (vRNA) in lung tissue homogenates by qPCR were performed. Intracellular IL-33 protein expression in different cell types in lung was assessed by flow cytometry using fluorophore labeled antibodies.

Results: RSV-induced exacerbation was characterized by increased mixed eosinophilic, lymphocyte and macrophage infiltration, compare to uninfected OVA allergic mice with predominantly eosinophilic infiltration and RSV group of animals characterized by lymphocyte/macrophage airway inflammation. AHR was significantly increased in all 3 groups of animals (OVA, RSV and OVA/RSV) compare to control group. vRNA was detected in RSV and OVA/RSV groups only. In RSV-infected mice, IL-33 mRNA expression in the total lung was up-regulated compare to OVA, OVA/RSV and control groups. According to flow cytometry data the percentage of IL-33$^+$ T-cells and neutrophils was increased in groups OVA/RSV and RSV compared to control mice by 26% and 40% for T-cells and by 110% and 28% for neutrophils, while the percentage of IL-33$^+$ T-cells and neutrophils in OVA group did not changed. Controversially the amounts of IL-33$^+$ epithelium cells of OVA/RSV and RSV groups was decreased 2-fold compared to OVA and control groups.

Conclusion: RSV-infection of allergic mice lead to induction of IL-33 production by T-cells and neutrophils and decrease of IL-33 expression by epithelium cells. Supported by RSF No 14-15-00894.

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Urinary leukotriene E4 in preschool children with acute rhninovirus wheeze

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Background: Cysteinyl Leukotrienes (cystLTs) are important mediators of wheeze in asthma, but the role of cystLTs in the pathogenesis of preschool viral wheeze is unclear. The purpose of the study is to assess the relationship of urinary Leukotriene E4 (U-LTE4) to particular asthma in preschool children with episodic viral wheezing following upper respiratory tract infections with or without atopic predisposition.

Method: U-LTE4 and serum total IgE were measured in same children (1–6 years) during an acute attack ($n = 50$) and in the convalescent phase ($n = 50$). Exacerbation was defined on clinical basis (wheeze in the presence of coryzal symptoms). Atopy was determined by specific serum IgE measurement and skin-prick testing. U-LTE4 was determined by enzyme immunoassay. Respiratory viruses were identified by multiplex PCR.

Results: During exacerbation, U-LTE4 was significantly higher comparison to convalescent phase ($P < 0.001$). Viruses were detected in 100% specimens overall: 68% were rhinoviruses. Rhinovirus infection patients demonstrated significantly higher levels of U-LTE4 compared to other virus infection during exacerbation ($P < 0.001$). During convalescent phase, a highly significant difference of U-LTE4 was found when low IgE was compared to high igE patients, $P < 0.001$.

Conclusion: U-LTE4 is strongly associated with the wheeze exacerbation phase in preschool children, more so in rhinovirus infection and high IgE level. This suggests a potential role of U-LTE4 as a marker of exacerbation phase, virus-induced wheezing, and atopic in preschool children.

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Innate immunity changes in relation to age between atopic and non-atopic subjects

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Background: Immune responses develop in early life; profound changes in T-cell

responses during the first year of life have being linked to the development of atopy. However, whether innate immunity undergoes progressive maturation and whether this can influence the development of allergic responses is not well known. In this study we aimed to investigate whether atopic and non-atopic individuals show age-related changes in Toll-like receptor (TLR)-mediated responses.

Method: Peripheral blood mononuclear cells (PBMCs) were isolated from 76 individuals: 38 atopic and 38 non-atopic stratified by age, ranging from neonates to 50 years old. Atopy was assessed by questionnaire and skin prick testing. PBMCs were stimulated for 24 h with or without TLR3 ligand Poly(I:C). Cell-free supernatants were then harvested and analyzed for cytokine and chemokine production using Luminex multiplexing technology.

Results: Both atopic and non-atopic subjects develop similar age-associated increases in Th2-related cytokine IL-5 ($P < 0.05$) and Th17-related cytokine IL17A ($P < 0.001$) upon stimulation with TLR3. Of note, non-atopic subjects show significant age-associated increases in Th1-related IFNγ ($P < 0.05$), innate-related IL1β ($P < 0.05$), TNFa ($P < 0.05$), MIP1B ($P < 0.05$) and Th17-related IL23 ($P < 0.05$) cytokine responses. In contrast, atopic subjects exhibit age-related increase in Th2 cytokine IL9 ($P < 0.05$) upon TLR3 stimulation.

Conclusion: Our findings suggest that atopic subjects show altered innate (TLR-mediated) immune responses compared to non-atopic. This altered cytokine profile reflects differences in the innate immune maturation process and may contribute to the increased susceptibility of the atopic patients to viral infections.

Poster Discussion Session PDS 12

Innovations in allergen-specific immunotherapy

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Grass pollen subcutaneous immunotherapy results in faster and greater suppression of allergen-induced skin responses compared to sublingual immunotherapy: a randomised controlled trial

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Background: We undertook a randomised, double-blind, double-dummy, controlled trial of subcutaneous (SCIT) and sublingual (SLIT) grass pollen immunotherapy in participants with moderate-severe seasonal allergic rhinitis.
Methods: 106 participants were randomised to one of three arms: active-SCIT (Alutard SQ, ALK), active-SLIT (Grazax, ALK) or double-placebo. Early (EPR, 15 min) and late (LPR, 8 h) skin responses to intradermal injection with grass pollen extract (Aquagen SQ, *Phleum pratense [10 SQ units containing 7ng Phl p 5]*, ALK, Denmark) were recorded at baseline, at year 1 and year 2 during treatment and at year 3 (one year after stopping treatment). Serum was assessed for ability to block IgE-facilitated grass pollen allergen binding to EBV-transformed B-cells (IgE-FAB) *in vitro*.
Results: At year 1, SCIT suppressed skin EPR compared to both placebo (26.1%) and SLIT (22.6%), $P < 0.05$. SLIT had a suppressive effect at year 2 (18.2% vs placebo), but the effect of SCIT was greater (17.4% vs SLIT), both $P < 0.05$. Both active treatments had significant persistent effects at year 3 (SCIT 18.6%, SLIT 8.4% vs placebo, both $P < 0.05$). Both treatments reduced skin LPR at all 3 years, peaking at year 2 (SCIT 63.5%, SLIT 39.7% vs placebo), persisting at year 3 (SCIT 49.7%, SLIT 32.5% vs placebo); SCIT had a greater effect than SLIT throughout (all $P < 0.01$). SCIT sera showed strong inhibition of IgE-FAB at year 1 ($P < 0.05$ vs placebo and SLIT), whereas SLIT sera showed modest, but significant inhibition ($P < 0.05$ vs placebo). By year 2, the effect of SLIT had increased, showing no difference from SCIT. A significant effect was maintained at year 3 by SCIT and SLIT sera.
Conclusion: SCIT compared to SLIT results in faster and greater suppression of skin EPR and LPR, and faster suppression of IgE-FAB. Two years with SCIT or SLIT result in persistent suppression (albeit of lower magnitude) of skin EPR, LPR and IgE-FAB one year after discontinuation.

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Safety of ultra rush subcutaneous immunotherapy using an infusion pump

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Background: Ultra-rush schedules of up-dosing phase with subcutaneous immunotherapy (SCIT) have been described mainly with hymenoptera venoms. However, to our knowledge there is no experience using a subcutaneous infusion pump with aeroallergens.
Method: We selected 60 patients with clinical indication of SCIT with allergic rhinitis and/or asthma who were sensitized to cat or dog. The extracts used were Alutard® extract (ALK, Denmark). A portable infusion pump (IP) (Medis Infusa T®, Italy) was used for subcutaneous administration of the extracts. The infusion lasted 4 h in the same day using the maintenance dose (1.2 ml total dose). Adverse reactions were monitored during 24 h and grading according EAACI guidelines.
Results: Sixty doses were administered. Immunotherapy-related adverse reactions (ARs) were reported in 15 patients. By doses, 5% were local reactions (LRs) and 21.6% systemic reactions (SRs). All LRs were delayed. Of the SRs, 46.2% were immediate while 53.8% delayed SRs. Symptoms associated with SRs were rhinitis (61.5%), urticaria (38.5%), conjunctivitis (30.8%). Most of SRs were grade I (92.3%) and responded to the treatment.
Conclusion: Administration of a maintenance dose of SCIT in 4 h using a subcutaneous infusion pump is a feasible and safe way of SCIT's administration of aeroallergens extracts. Though, adverse reactions are higher than conventional schedules, all were mild.

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Characterization of rBet v1 produced in CHO cells for the development of a new birch pollen vaccine

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Background: In Europe and North America up to 20% of the population is suffering from IgE-mediated allergies (type I) leading to allergic rhinitis (AR) and asthma. AR can be treated using allergen-specific immunotherapy (AIT), in which natural allergen extracts are administered. Disadvantages of current AIT products include large variation in source material and long treatment periods, leading to inconsistent product quality and poor patient compliance respectively. A possible solution for these disadvantages is the use of recombinant allergens. One of the major allergens responsible for IgE binding in more than 95% of birch pollen allergic patients is Bet v1, a 17 kD protein from *Betula verrucosa*.
Method: Recombinant Bet v1 has been expressed in a Chinese Hamster Ovarian (CHO) cell line and has been purified. In order to investigate the structural and immunological properties of the recombinant Bet v1 the molecule was fully characterized, using SDS-PAGE, MS, IgE/IgG Immunoblot, inhibition ELISA and CD.
Results: CHO cells expressed two rBet v1 variants with a molecular weight of 17 kD and 22 kD respectively. MS data confirmed identity of both rBet v1 variants, with 97% and 78% sequence coverage respectively. CHO cells are capable of post-translational modification and analysis of the 22 kD variant of rBet v1 after treatment with PNGASE F, showed the presence of glycan groups. CHO cells expressed equal ratios of glycosylated and non-glycosylated variants, with equal IgG but reduced IgE binding in comparison to rBet v1 expressed in *E. coli* and native Bet v1. Structural analysis with CD showed no structural difference between both rBet v1 variants expressed in CHO cells. This suggests that modification occurs after protein

folding and the addition of the glycan group might block the epitope or cause steric hindering leading to reduced IgE binding.

Conclusion: The reduced IgE binding observed in rBet v1 expressed in CHO cells compared to expression in *E. coli*, suggests that CHO cells might produce a hypoallergenic variant of Bet v1. This hypoallergenicity might be caused by the production of the glycosylated variant of rBet v1 and could provide a novel modification platform for the development of birch pollen vaccines and AIT overall.

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Allergic sensitization to Cannabis ruderalis: prevalence, clinical and immunologic characteristics, subcutaneous immunotherapy

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Background: Cannabis sativa var. ruderalis Janisch with low content of Delta-9-tetrahydrocannabinol (not a source of drugs) is widely distributed in the Eurasia and Russia. Allergen immunotherapy (AIT) is widely used, however, the quality of evidence for individual AIT products (mixed and single-allergen preparation) is unclear. Multiallergen immunotherapy in polysensitized patients requires more supporting data to validate its efficacy in clinical practice. Aim. To determine the prevalence of allergic reaction induced by weed pollen and C. ruderalis (C) and to evaluate the efficacy and safety of subcutaneous AIT for patients with persistent allergic rhinitis (AR).

Method: Aerobiologic, epidemiologic monitoring .102 polysensitized to weed pollen and C. patients (18–64 years; never smoked marijuana) with AR were recruited. Clinical examinations, skin tests (ST) were used for diagnosis. Patients were randomly divided into 2 groups which received SCIT of mixed aqueous PNU standardization extracts of weeds pollen with (group A) or without (group B) C – major allergen associated with C. was not identified. The efficacy of treatment was evaluated with a combined symptom plus medication score (CSMS).

Results: The prevalence of pollen allergy in population increased: pollen AR – 12.3%, pollen asthma 2.4%. Weeds pollen dominates in the atmosphere from July to October. More than 80% of patients with pollen allergy have hypersensitivity to weeds and positive ST: with Ambrosia – 49%, C – 54%, Helianthus- 54%, Chenopodium – 56%, Artemisia – 66%, Cyclachaena – 68%. The concordance between positive ST to C. and other pollen ranged from 12 to 96% (to Artemisia – 96%, Chenopodium – 96%, Ambrosia – 84%, Helianthus – 76%, Cyclachaena – 84%, Betula – 16%, Corylus-12%). Monosensitization to C. was only 1%. CSMS in group A was lower than in B (1.79 points vs 2.57, $P < 0.05$). The frequency of local reactions was higher in group A than in B (57.5% vs 13%). Adverse systemic reactions in the setting of AIT were not registered.

Conclusion: Weed pollen sensitization is commonly observed in polysensitized patients, while monosensitization to C. is rare. Treatment weeds mixtures without C. allow some patients to achieve better results faster and with less risks of adverse reactions. Observed sensitization profiles suggest that C. ruderalis sensitization may be mediated by cross-reactivity. Molecule-based allergy diagnostics is required for a more effective AIT.

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Mobile exposure chamber: safety of controlled allergen challenge

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Validation studies have shown the efficacy of a mobile exposure chamber in clinical allergy trials as an alternative to natural pollen exposure in an outdoor setting. However, data regarding safety parameters and results are limited.

Objective: To determine the safety of provoking patients with allergic rhinoconjunctivitis to pollen allergens in a mobile exposure chamber.

Method: In a randomized, single-blind, placebo-controlled study, a total of 132 non-smoking adult volunteers with seasonal allergic rhinoconjunctivitis have been tested in a mobile exposure chamber. The participants have been exposed to different concentrations of grass or birch pollen up to 240 min in and outside the pollen season. As objective safety parameters, a spirometry (FEV1) was performed before and after the exposure, the peak nasal inspiratory flow (PNIF) and peak expiratory flow were measured before, at 30 min intervals and after the challenge. Symptoms of the eyes, nose and bronchia were rated (Scale of 0–3) and recorded by the participants every 10 min and a visual analogue scale (VAS) reflecting the general well-being was completed before, every 30 min during and after exposure. During all challenges, the investigator and a Study Nurse were present and emergency medication was on-site. All participants were contacted 24 h after the challenge to report any potential adverse events or late reactions.

Results: A significant difference between active and placebo exposures could be documented for the Symptom score and VAS, in and outside the appropriate pollen season [$P < 0.0015$]. No adverse events or late reactions within 24 h after provocation were reported. Measurements of FEV1 and peak expiratory flow showed no clinically significant decrease neither for active nor placebo challenge. One participant was treated with Salbutamol aerosol after complaining of an obstructive bronchial sensation even though the FEV1 decreased only slightly by 1%. Apart from this, no emergency medication was required and all exposures were well tolerated.

Conclusion: Using a mobile exposure chamber for allergy trials is at present a safe and well-tolerated method as long as safety parameters and monitoring are implemented. However, the long-term security aspects need to be investigated further.

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Gut homing receptors designate epicutaneous immunotherapy as the most appropriate route for the treatment of food allergy in a model of peanut sensitized mice

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Background: Allergen specific immunotherapy is showing promise in the treatment of food allergy. In the 3 different routes being investigated, epicutaneous (EPIT), oral (OIT) and sublingual (SLIT), regulatory T cells (Tregs) are believed to play a pivotal role. Differential expression of homing receptors determines specific migration patterns of Tregs and could modulate efficacy *in vivo*. This study evaluated the effect of EPIT, OIT and SLIT on Tregs expression of gut homing receptors and their long term maintenance.

Method: BALB/c mice were orally sensitized to peanut and then treated with EPIT, OIT, SLIT or not treated (Sham). The proportion of Tregs in spleen and their expression of gut homing receptors (CCR9, CCR6, and CCR3) were analyzed by flow cytometry in spleens after 8 weeks of treatment or 8 weeks after treatment termination. The *in vivo* suppressive activities of Tregs were evaluated by examining the decrease of peanut-specific cytokine

responses and by the protection against esophageal eosinophil infiltration after oral peanut administration (Mondoulet, PLoS One 2012) in peanut-sensitized mice receiving adoptively transferred Tregs.
Results: In all treatment regimens, Foxp3+ Tregs increased at the end of immunotherapy ($P < 0.001$ compared to Sham), significantly more with EPIT ($P < 0.01$ compared to OIT and SLIT). EPIT-induced Tregs were both CD62L+ and CD62L- whereas OIT and SLIT mainly induced CD62L- Tregs. EPIT induced higher expression of the 3 gut homing receptors CCR9, CCR6 and CCR3 whereas OIT induced CCR9 and CCR6 and SLIT did not. Following transfer of Tregs isolated at the end of treatment, EPIT, OIT and SLIT Tregs decreased Th2 cytokine production in recipient mice, but only EPIT-Tregs protected them from eosinophil infiltration in the esophagus following intensive peanut oral administration. Eight weeks after the discontinuation of EPIT, the level and phenotype of Tregs were sustained, but not after OIT and SLIT. Moreover, transfer of Tregs isolated 8 weeks after the end of EPIT decreased Th2 cytokine production and protected mice from eosinophil infiltration in esophagus, but not after OIT or SLIT.
Conclusion: The greater increase of Tregs and their greater expression of gut homing receptors and longer sustainability suggest the potential superiority of EPIT over SLIT and OIT for the treatment of food allergy.

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Allergoids of *Phleum pratense* conjugated with mannan are immunogenic by the sublingual route. A comparative study with native (non-modified) allergens

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Background: Sublingual immunotherapy (SLIT) is a well-established treatment of allergic diseases. In most cases, SLIT is performed with non-modified (native) allergen extracts. In previous studies we have shown that the conjugation of high molecular weight allergoids with mannan derived from *S. cerevisae*, significantly increases allergen uptake by dendritic cells. The aim of this proof of concept study was to assess the immunogenicity of mannan-conjugated polymerized allergens of *Phleum pratense* administered by the sublingual (s.l.) route, in an experimental mouse model system, in comparison with the same amount of its native counterpart.
Methods: *P. pratense* pollen allergens were prepared in two forms:
inative,
iipolymerized and conjugated with mannan (PM).

BALB/c mice (groups of 6 animals) were treated sublingually with 5 µl (200 µg) of native, or PM on days 0, 7, 14 and 21. A control group treated with the same volume of PBS was also included. To prevent swallowing during s.l. administration, mice were anaesthetized with ketamine/medetomidine. The immunogenicity of each allergen preparation was evaluated 5 days after the last dose by measuring specific IgG1 and IgG2a serum levels by ELISA and specific spleen cell proliferation (CFSE assay) to the native allergen extract.
Results: The humoral antibody response of mice immunized with PM was positive compared with controls for both IgG1 ($P < 0.01$) and IgG2a ($P < 0.05$) levels. By contrast, no IgG1 or IgG2a response could be observed in mice immunized with the native allergen extract. The specific proliferative cell response was positive in both groups of mice immunized with PM ($P < 0.001$), or native ($P < 0.05$) over controls. This cell response was significantly higher in mice immunized with PM than in those immunized with native allergens ($P < 0.01$).
Conclusion: Polymerized allergens conjugated with mannan induce a systemic immune response when administered through the sublingual route. This response, at both humoral and cellular levels, appears stronger than that obtained with native (non-modified) allergens.

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Epicutaneous immunotherapy but not oral immunotherapy prevents eosinophilic infiltration in the esophagus in a model of milk sensitized mice

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Background: We have developed a model of sensitized mice with eosinophilic inflammation in the esophagus following extensive exposure to allergens (Mondoulet et al., 2012). Peanut epicutaneous immunotherapy (EPIT) prevents the induction of esophageal eosinophilic inflammation induced by oral peanut exposure. This study evaluates the prevention of oral milk-induced esophageal eosinophilic inflammation by milk EPIT, compared to milk oral immunotherapy (OIT).
Method: BALB/c mice were orally sensitized to milk, then treated with milk EPIT ($n = 10$), OIT ($n = 10$) or Sham ($n = 10$) for 8 weeks before being re-exposed to milk 2 weeks later. Esophageal samples were then taken for histological analysis. Specific IgE, IgG1, IgG2a were monitored following immunotherapy. Splenocytes were harvested for *ex vivo* Treg staining and *in vitro* stimulation for cytokine measurement (IL4, IL5, IL13).
Results: Mice were effectively sensitized to milk with a high induction of specific IgE ($P < 0.001$). EPIT and OIT did not modify specific IgE but significantly increased specific IgG2a ($P < 0.001$). At a cellular level, EPIT, but not OIT, significantly induced Foxp3+ Tregs ($P < 0.05$), whereas both techniques significantly decreased Th2 cytokines ($P < 0.05$). Esophageal eosinophilic infiltration (measured in 6 high power fields) was greater with Sham (66±3) than with EPIT (14±2, $P < 0.05$) and in naive mice (9±4, $P < 0.05$). With OIT, the infiltration was greater (63±4), but not significantly different from Sham.
Conclusion: EPIT, but not OIT, is effective in preventing esophageal milk-induced eosinophilic infiltration in a model of milk-sensitized mice.

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Evaluating the suitability of house dust mite raw material for exposure chamber tests

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Background: Allergen exposure tests in clinical trials shall be set up to challenge subjects in the most natural way possible. For house dust mites, that would be exposure with full mite bodies, body parts and feces. The primary objective in this venture was to evaluate the technical possibility of using house dust mite raw material (body particles and feces) for allergen challenge tests in the mobile exposure chamber, considering possible concentrations and clinical relevance thereof as well as particle distribution, including the chance for individual exposure, resulting contamination areas and general safety issues.
Method: A 1:1 mixture of Dermatophagoides farinae (D.f.) and Dermatophagoides pteronyssinus (D.pt.), feces and body particles, was used as a base material. The base material was pre-processed to ensure that no particles below 10 µm and above 120 µm were dispersed. The base material was pre-packed between two foils, with 6 mg per meter.

Initial disperse rate was set to generate a resulting concentration of 250 µg per m^3, based on primary measurements with other aeroallergen particles. Two 60 min runs, with 9 known atopic (incl. 3 asthmatics) subjects (>3 mm wheal in prick test with D.f. and D.pt. extract) have been completed to indicate the adequacy of the selected concentration. Also 3 non-atopic subjects were included to identify general safety issues for healthy patients. FEV1 and peak expiratory flow (PEF) were measured before and after the exposure test.

Rapid Der f2 and Der p2 tests were used to determine allergen contamination of certain areas, including walls, ceiling, active and non-active seats after 2 h of continuous dispersal. The rapid test used could discriminate three different Der f2 and Der p2 concentration categories (>1 µg, 1 µg to 0.2 µg and <0.2 µg).

Results: Rapid tests of the walls did show an accumulated allergen concentration below 0.2 µg Der f2 and Der p2 per two square meters or not recognizable at all. Rapid tests of the non-active seats showed no recognizable amount of allergen in that area.

Atopic subjects reached a symptom plateau after 40 min, with an average nasal symptom score of 5.8.

Asthmatics had no significantly reduced FEV1 or PEF after the test. Non-atopics showed no symptoms.

Conclusion: It has been shown, that using raw material of Dermatophagoides farinae and Dermatophagoides pteronyssinus for exposure tests in the mobile exposure chamber is effective and safe.

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The 'Hub-and-Spoke' approach to large multicenter allergy trials utilizing the mobile environmental exposure chamber system (mEEC): setup and screening for an immunotherapy study conducted in the US Northeast and US Midwest

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Background: Performance of successful allergen-specific trials for immunotherapies/novel immune modulators are increasingly difficult with a traditional field approach due to:

1) Climate change which results in erratic pollen seasons and may result in poor pollen exposure;

2) Reliance on standard screening criteria such as skin testing which may result in inclusion of patients who are minimally symptomatic or who have confounding allergies or non-allergic symptoms.

A controlled allergen challenge in a mEEC where the allergen exposure is consistently studied across geographically diverse regions will de-risk and improve data quality in allergy trials.

Method: Set-up of multiple mEECs were performed towards the conduct of a Phase II dose-ranging study for a grass immunotherapy to randomize 250 patients. The phenology of grass species in the US was examined and two locations in the US were identified in the humid subtropical climate (CFA Köppen): US northeast (mid-atlantic): New Jersey, Pennsylvania, and New York regions and US Midwest (eastern north central): Ohio, Indiana and Upper Kentucky state (east south central). 20 Investigators located around the mEECs medically screened their patients for eligibility to attend the mEEC for symptomatic screening. Patients were screened for 4 consecutive days in the mEEC (3500 ± 500 grass pollen/m^3). A score of 6/12 on TNSS was required for eligibility to proceed. A Regional Co-ordinating Manager (RCM) liaised with Investigators to coordinate patient logistics to the mEEC hub. An electronic Patient Data Acquisition Tablet (ePDAT) was used while patients were in the mEEC to record nasal and non-nasal symptoms.

Results: 2 mEECs were validated and requalified *in situ* rapidly in each jurisdiction. 396 patients will be screened by study completion. Patients who are more distant are accommodated in hotels during screening. Due to the demographics of the Northeast and Midwest, 14 investigators were refering patients to the Neptune, NJ site and 6 to the Cincinnati site. To-date, 105 patients have been screened with a 13% screen failure rate. Screening will be closed in mid-February for those patients with grass allergy and no interfering tree allergies.

Conclusion: A hub-and-spoke approach is effective for rapid patient symptom screening and inclusion in clinical trials without confounding by patients' concomitant allergies. The multicenter mEEC approach is a feasible approach to conducting pivotal allergy trials.

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Development of an aptamer-based tool for quality control of a birch pollen immunotherapy vaccine

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Background: Birch pollen allergy represents the main cause for winter and spring pollinosis in the temperate climate zone of the northern hemisphere. Over 95% of birch pollen allergic patients are sensitized to the major allergen Bet v 1. Allergen-specific immunotherapy (AIT) is the only causal and effective treatment targeting the underlying immune mechanism. Within the EU-funded project 'BM4SIT – Innovations for Allergy' a hypo-allergenic but hyper-immunogenic mutant of Bet v 1 (termed BM4) was created for therapeutic use to replace current natural allergen extracts in AIT in order to make treatment safer and more effective.

Objective: A major aspect of vaccine development is the quality control of the BM4 drug substance. Therefore, we aimed at producing BM4-specific aptamers to use within an ELASA experimental set-up, thus providing higher reproducibility with a decreased batch-to-batch variation during a tightly monitored production process. Aptamers are short specific DNA oligonucleotides, that are able to bind a target molecule because of their three dimensional shape and thus have a comparable specificity like antibodies.

Method: The BM4-specific aptamers were selected by a biotin-monitored in-vitro Mag-SELEX (Systematic Evolution of Ligands by Exponential Enrichment) selection procedure towards the purified BM4 protein. After the last cycle, PCR products of aptamer sequences were cloned into a bacterial host followed by sequencing of the aptamer sequence-containing plasmids. The identified aptamer sequences were biotinylated and used in a dot blot to determine their specificity against the BM4 protein.

Results: Within the sequencing results of 45 'aptamer-containing' plasmids, five duplicate sequences, which were 100% identical, and two duplicate sequences with sequence identity higher than 50% were obtained. Of the five duplicate sequences, two showed a high reactivity to the BM4 protein in a dot blot assay, whereas the other three were less reactive. No reactivity to Bet v 1 was observed.

Conclusion: Five BM4-specific aptamer sequences were successfully identified and proven to bind to the BM4 molecule. This will allow the set-up of an ELASA for quality control of the BM4 drug product.

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Alternaria alternata depigmented-polymerized extract: biochemical and immunological characterization

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Background: Sensitization to *Alternaria alternata*, one of the most common fungi worldwide, is associated with the development of asthma. Prevalence to *A. alternata* varies among countries, but sensitisation rate might be as high as 20%. Chemically modified allergens (allergoids) for their use in allergen immunotherapy have a reduced allergenicity respect to native extracts, improving the safety while maintaining their immunogenicity assuring their efficacy. Whereas allergoids of pollen and mites have been extensively evaluated, there is little evidence of those from fungi.

The objective was to develop and characterize an *Alternaria alternata* allergoid previously purified (depigmentation process) and to evaluate its *in vitro* safety.

Method: Depigmented-polymerized extracts (Dpg-Pol) were produced from native extracts (NE) from *A. alternata*. The presence of the relevant allergens in the modified molecule was determined by mass spectrometry and the profile of polymerization was determined by HPLC. Immunogenicity of native and modified extracts was determined by immunization of New Zealand White rabbits. ELISA-competition was performed to asses IgE-binding activity using a pool of human sera, whereas ELISA-inhibition was carried out for IgG-binding activity using rabbit's polyclonal antibodies. Abnormal toxicity and genotoxicity test (Ames Test) have also been performed.

Results: Peptide sequencing confirmed the presence in the Dpg-Pol of Alt a 1, Alt a 3, Alt a 6 and Alt a 8. The HPLC profiles showed a range of high molecular weight components, most of them higher than 1.500 kDa, and an elevated consistency between batches. As result of polymerization IgE-binding respect to NE was reduced in more than 99%, whereas IgG-binding capacity was not significantly affected (variation in values of 50% inhibition lower than 20%). Similar IgG antibodies titers were induced by both native and depigmented-polymerized extracts, suggesting a similar immunological stimulation. Preliminary toxicity studies showed that Dpg-Pol are not toxic nor genotoxic.

Conclusion: A depigmented-polymerized allergen extract of *A. alternata* has been developed, maintaining its immunogenicity while reducing its allergenicity, suggesting similar immunological characteristics and higher safety than native extracts.

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Safety and efficacy of immunotherapy with mannan-polymerised mite allergen extract in sensitized allergic dogs

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Background: Mannan-conjugated polymerized allergen extracts have reduced allergenicity and improved uptake by dendritic cells. Acute and repeat-dose toxicity studies in rodents and the administration in rabbits have demonstrated its safety. The objective was to evaluate safety and effectiveness of one year treatment with the polymerised mannosylated extract of *Dermatophagoides farinae* (DF) in dogs suffering of Canine Atopic Dermatitis (CAD) due to the sensitization to this mite.

Methods: Sixteen dogs with CAD, clinically diagnosed and with specific IgE to DF (PET ELISA™; Alergovet, Madrid, Spain) were selected. Four were monosensitized to DF and the others were co-sensitized to *Lepidoglyphus putrescentiae* (LD) and/or *Acarus siro* (AS). The median age was 3 years (range 1–7). The polymerised mannosylated DF extract (Inmunotek,SL) was supplied at a concentration of 10 000 TU/ml (2 μg of Der p1/ml) and was administered subcutaneously. The first dose was 0.2 ml, the second dose was 0.5 ml one week later, followed by a maintenance dose of 0.5 ml monthly. The primary effectiveness outcome was the combination of the symptom pruritus scored by owners and the support medication dose (SM), being the maximum value 1 and the minimum 0. Friedman's test was used for comparative statistics and Wilcoxon for the comparison of the SM score before treatment and the SM score of rest of the visits. Safety was evaluated by grading and recording and any adverse reactions after each administration.

Results: The median value of SM score in the first day, before the administration of the first dose, was 0.55; 0.46 after 1 week, before the second dose and 0.30 after 1 month, before the third. The values decrease below 0.2 in the remainder visits ($P < 0.0001$, Friedman′s test). The improvement of SM after the first week of treatment was 16% ($P = 0.0152$, Wilcoxon's test), 45% ($P = 0.0008$) after the second administration, achieving the value of 91% ($P < 0.0001$) from the visit 7 until the end of the study. Regarding safety, one dog had a mild increase in pruritus after injections 2 and 3; these reactions were mild and resolved spontaneously without the need of medication.

Conclusions: Immunotherapy with mannan-polymerized DF extract is safe and provides a fast and high effectiveness response in dogs affected of CAD due to hypersensitivity to *D. farinae*. In these dogs it seems that DF was the responsible of the sensitization because all of them improved, even those polysensitized to other mites.

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Comparison of two mite extracts for specific immunotherapy using bronchial provocation testing

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Background: The aim of this prospective study was to evaluate the response of two different mite immunotherapy extracts in patients with allergic asthma and house dust mite allergy (HDM).

Methods: A total of 95 patients (age 8.45 ± 2.8 years) with bronchial asthma and HDM underwent bronchial allergen provocation (BAP) which is considered the gold standard for the diagnosis of clinically relevant allergen-specific asthma.

Subcutaneous immunotherapy (SCIT) was performed either with a non-modified mite allergen (ALK-Arabello) or with a modified mite extract (Allergopharma). After one year, 62 patients (65.3%) underwent a second BAP. Of these, 20 patients (32.3%) received a non-modified allergen, and 18 patients (29.0%) a modified extract. 21 patients (33.9%) without SCIT were controls. Three patients (4.8%) received a SCIT with other preparations, and were excluded from further analyzes. Also excluded were four patients (6.5%) of the control group who had a negative BAP both times. The following parameters were compared before and after SCIT: Early and late asthmatic response (EAR and LAR), exhaled NO, total IgE and specific IgE and IgG against mites.

Results: Before SCIT, the EAR was comparable in the two treatment groups (FEV1 decrease Mean 28.4% ± 8.4% vs 27.1% ± 6.0%). After SCIT there was a significant improvement of EAR ($P < 0.001$) with both mite extracts (FEV1 decrease: Mean 15.5% ± 10.8% vs 16.6% ± 12.6%; ns.), whereas the decrease in FEV1 in the control group remained unchanged (23.4% vs 25.8% after one year).

Conclusion: After one year of SCIT, 70.0% (non-modified) and 72.2% (modified mite extract) of the patients exhibited significant improvements, as defined by BAP. Thus, BAP is a useful and objective method of estimating the effectiveness of different mite preparations.

Poster Discussion Session PDS 13

New trends in hymenoptera venom allergy and anaphylaxis

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Mismatch of history and diagnostic tests in Hymenoptera venom allergic patients

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Background: The diagnosis of Hymenoptera venom allergy is based upon patients history and the results of diagnostic tests. In diagnostic procedure we are trying to find out the culprit insect from the patients history and then confirm sensitization with the diagnostic tests. Sometimes the data from patients history do not match the results of diagnostic tests.
Method: The study is retrospective. Inclusion criteria were: treatment due to Hymenoptera venom allergy in 2014, proven sensitization to Hymenoptera venom and mismatch of history and the results of diagnostic tests.
Results: In 2014 we treated 453 patients due to Hymenoptera venom allergy. Out of 453 patients 7 patients (1.5%) fulfilled inclusion criteria. All 7 were men, aged 35 – 72 years, average age was 49 years. They all experienced severe anaphylaxis (Mueller grade III 3 patients and Mueller grade IV 4 patients) after Hymenoptera sting.

6 patients had a history of anaphylaxis after honeybee sting, but had negative honeybee sIgE and elevated wasp sIgE. Skin tests with wasp venom were positive in 1 patient and negative with honeybee venom in all patients. According to history all of them found sting left in the skin. In diagnostic workup BAT was single positive to wasp venom only in 5 patients, while in 1 patient BAT was double negative.

1 patient had a history of anaphylaxis after wasp sting, but had negative wasp sIgE and elevated honeybee sIgE. Skin tests were negative with both venoms. BAT was single positive to honeybee venom.
Conclusion: Mismatch of history and diagnostic test is not a frequent problem. It is useful to bear in mind that all patients do not differentiate between honeybee and wasp. The history data about sting in the skin does not necessarily indicate on honeybee sting, since after aggressive removal of the biting insect some remnants of the stinger could be find in skin also after wasp sting.

In the case of history – diagnosis mismatch extensive diagnostic workup is needed with cellular tests and recombinants in order to find out the proper venom for specific immunotherapy. The results of the diagnostic tests should be re – discussed with the patient to clear up mismatch.

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Molecular recombinant allergens in the diagnosis of hymenoptera venom allergy

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Background: The diagnosis of Hymenoptera venom allergy (HVA) is routinely based on the clinical history and detection of IgE-mediated sensitization by skin testing and/or by *in vitro* detection of venom-specific IgE. Double positivity of diagnostic tests to both bee and vespid venoms is frequently observed. Aim of this study is to differentiate the venom extracts for immunotherapy (VIT) through component-resolved diagnosis analysis with recombinant species-specific major allergens (rSSMA).
Methods: 42 patients were involved, 32 males and 10 females, with HVA documented by a history of systemic adverse reaction (SAR) to Hymenoptera, positive skin tests and sIgE. The specific immunotherapy treated patients were 8 patients with Apis mellifera venom (Group 1), 25 patients with Vespula spp. venom (Group 2) and 9 patients with Polistes spp. venom (Group 3). Sera for diagnostic tests were obtained in all patients and the rSSMA phospholipase A2 (Api m 1) of Apis mellifera, phospholipase A1 (Ves v 1) and antigen 5 (Ves v5) of Vespula spp., antigen 5 (Pol d5) of Polistes spp. and crossreacting carbohydrate determinants (CCDs) (MUXF3) were estimated by ImmunoCAP (Phadia).
Results: In Group 1 we observed positive result of IgE to Api m1 in 6 patients, to Ves v1 in 2 patients, to Ves v5 in 2 patients and to Pol d5 in 3 patients. In Group 2 positive value of IgE was found in 17 patients to Ves v1, in 16 patients to Ves v5, in 8 patients to Pol d5. There was no patient with positive IgE to Api m 1. In Group 3 all patients had positive result of IgE to Pol d5, 2 patients to Ves v1 and 8 patients to Ves v5. Dosage of IgE to Api m1 was always negative. IgE to MUXF3 had positive result in 2 patients of Group 1 and in 1 patient of Group 2.
Conclusion: Our study confirms the high specificity of sIgE to rSSMA of Hymenoptera venoms to distinguish between true double sensitization, indicating VIT with both venoms, and cross-reactions in patients with double positivity. In future, the sensitivity of component-resolved diagnosis could be improved by the addition of other important rSSMA.

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Novel recombinant IgE and BAT AUC based diagnostic algorithm to dissect bee and wasp allergy

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Background: Diagnosis of insect sting allergy is often difficult. We aimed to establish better in-vitro diagnostic algorithms for identification of the disease-causing insect.
Method: Hundred and seventy-seven consecutive sting allergic patients were included. Testing for sIgE to venoms and SSMA (rApi m1 and rVes v5) and the BAT were performed for each participant; the majority was also tested with skin tests.
Results: One hundred and thirty-three patients with unequivocal culprit history were analysed.

In the case of venom sIgE single positivity (sIgE+/−; $n = 73$), the agreement between different test results and culprit histories was excellent (90–96%).

In the venom double positive group (sIgE+/+; $n = 56$), we constructed diagnostic algorithms that relied on the ratio of bee/wasp SSMA and/or BAT area under the curve (AUC) test pair results. All of the algorithms outperformed the standard cut-off tests when used separately or stepwise. The best algorithm was the SSMA and BAT AUC algorithm, which correctly diagnosed more than 80% of patients in comparison to the diagnostic approaches

that used standard cut-offs (50% BAT-only/SSMA-only; 60% stepwise). The significant improvement was confirmed with ROC curve analyses.

In the venom double negative (sIgE−/−; $n = 4$) group, sensitization was confirmed in all patients with the BAT but in only 1 patient with SSMA-antibodies.

Conclusion: We established a novel ratio based diagnostic approach that significantly enhanced diagnostic accuracy, raising it to more than 80% of correctly diagnosed Hymenoptera venom allergic patients (compared to only 50% with the standard [SSMA or BAT] approaches) in a diagnostically challenging venom double positive group.

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Severity of systemic sting reactions differs between children and adults

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Background: Allergic reactions to Hymenoptera stings in children range from local reactions to severe systemic reactions, similar to those in adults. However, severity and the prevalence of systemic reactions are reported to be less common than in adults. Since there are only a few studies comparing symptom severity in children and adults, we aimed to identify the main differences between these two groups.

Method: 1044 patients (624 adults, 420 children) from two European countries with confirmed Hymenoptera venom allergy were included. Patients were asked about symptoms using standard questionnaire. While data about adults were mainly derived from an Austrian database, most data about children were from Poland.

Results: Based on previous studies, we expected more frequent severe reactions in adults. In general, sting reactions were predominantly (71.9%) mild or moderate. However, 31.6% of adults but only 21.9% of children had a severe systemic reaction ($P = 0.002$). While only 3.1% of children lost consciousness, this happened to 22.3% of adults ($P < 0.001$). Mild symptoms were more frequent in children: 73.8% of children suffered from urticaria but only 42.8% of adults reported this symptom ($P < 0.001$) and angioedema was present in 48.4% of children and only 31.7% of adults ($P < 0.001$). We also detected a difference according to the type of venom: while 74.6% of persons allergic to wasp venom were adults, bee venom allergy was more frequent in children (65.0%, $P < 0.001$).

Conclusion: We were able to confirm that severe systemic reactions to Hymenoptera stings are less common in children than in adults. Mild symptoms are more frequently seen in children and loss of consciousness happens more often to adults. Additionally, we found that bee venom allergy was more frequent in children while wasp allergy dominated in adults.

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C1q-like protein and PVF1 from honeybee venom show IgE-reactivity but do not activate basophils

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Background: Component-resolved diagnosis has evolved in the past years to the standard procedure for an advanced diagnosis of hymenoptera venom allergy. The combination of recombinant Ves v 1 and Ves v 5 enables the diagnosis of more than 95% of yellow jacket venom-allergic patients. In contrast, the molecular components so far available for the diagnosis of honeybee venom allergy are not able to reach this sensitivity. Therefore, the availability of additional non-cross-reactive honeybee venom allergens would be desirable to increase diagnostic sensitivity. In this study we addressed the diagnostic potential of PVF1 and a C1q-like protein (C1q), two yet immunological uncharacterized venom components.

Methods: PVF1 and C1q were identified as honeybee venom components by MS/MS-based approaches. To analyze the immunoreactivity of C1q and PVF1, both mature proteins were produced as his-tagged fusion proteins by baculovirus infection of Sf9 (*Spodoptera frugiperda*) insect cells. The proteins in the supernatant were purified by affinity chromatography. The cross-reactive carbohydrate determinant-free (CCD-free) proteins were tested for sIgE reactivity by ELISA using sera of honeybee venom-allergic patients. Additionally, the capacity of recombinant C1q and PVF1 to activate basophils was tested in bee venom allergic patients showing positive sIgE to one or both proteins.

Results: C1q and PVF1 could be obtained as secreted and soluble proteins. Coomassie staining and immunoreactivity of SDS-PAGE separated proteins showed their purity after isolation. Out of the honeybee venom-allergic patients approximately 30% showed specific IgE-reactivity to C1q and PVF1. Interestingly, both proteins were unable to activate basophils, even though the tested patients displayed positive sIgE-reactivity.

Conclusion: This study could demonstrate that the successfully produced proteins C1q and PVF1 show positive sIgE-reactivity in a minor group of honeybee venom allergic patients. Thus these proteins might be able to improve the sensitivity of the current diagnostics for honeybee venom allergy. However, both components are unable to activate basophils, questioning their relevance in the context of clinically relevant sensitization. This observation requires further investigation and makes further analyses on the epitope spectrum of PVF1 and C1q very interesting.

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Expansion of FOXP3-expressing regulatory T cells in beekeepers during the beekeeping season

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Background: Hymenoptera venom specific immunotherapy (VIT) is known for its efficiency, which has been related to an expansion of regulatory T cells (Tregs). Our previous in-vitro studies showed that bee venom (Bv) per se is able to induce the expansion of Tregs and the Treg differentiation from non-regulatory CD4 T cells and thymocytes. Beekeepers are known to be exposed to Bv, particularly during the beekeeping season due to the significant number of bee stings. Therefore, we asked whether the circulating Treg compartment expands in non-allergic beekeepers during the beekeeping season.

Method: A cohort of 20 beekeepers was studied inter-season and in the peak of the subsequent beekeeping season, and compared with 20 age-matched non-exposed healthy individuals from the same region. We documented a significant increase in the levels of Bv specific IgG4 during the beekeeping season, in agreement with the

beekeepers' exposure to bee stings. Intracellular staining was performed in whole blood immediately after collection, and FOXP3+ CD4 T cells quantified by flow cytometry.

Results: We found a statistical significant increase in both the proportion of Foxp3+ cells within the CD4 T cell subset and the absolute number of circulating Tregs during the beekeeping season, as compared to the levels documented inter-season. In agreement, a significant expansion was observed in the $CD25^{bright}$ CD4 T cell subset. The Treg levels achieved were also significantly higher than those found in controls. Importantly, the beekeeping season was not associated with changes in the expression of activation markers (e.g. HLA-DR) or memory markers (e.g. CD45RO) within T cells.

Conclusion: Bv exposure was associated with an expansion of circulating Tregs in beekeepers. Our data further support a direct effect of Bv in the homeostasis of the Treg compartment with implications for the mechanisms of tolerance induction in patients under VIT.

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Insect's identification by children treated with venom immunotherapy and their parents

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Background: Culprit insect identification by the patient might be helpful in diagnostic process of insect venom allergy. Insect's charts should be the part of patient's education process. The aim of the study was to evaluate correctness of insect's identification in groups of VIT-treated children and their parents.

Method: Thirty seven pairs child (73% males, mean age 11.8, SD 3.2)/parent (38% males, mean age 41.1, SD 6.6) were for the first time asked for insect identification using standardized, designed for this survey, questionnaire and chart of insects' (wasp, honey bee (hb), hornet, bumblebee, syrphid fly) of their natural size. More than half (58%) of children attended primary school, 24% of parents had higher education. Rural origin predominated (73%). Children were treated with VIT for mean time 1.5 years (SD = 1.8, range 0–6 years), with slight predominance of bee venom allergy (53%).

Results: Children were most often stung by wasp or hb (Me = 2, IQR = (1–3) and Me = 2, IQR = (1–5), respectively), whereas parents were more often stung by hb (Me = 5, IQR = (2–20)) than by wasp (Me = 3, IQR = (1–5)).

In children's group vs parent's group proper identification of insects ranged from 87% vs 87% for syrphid fly, to 89% vs 92% for bumblebee, to 95% vs 95% for wasp, to 97% vs 97 for hornet, to 95% vs 100% for hb.

In wasp venom VIT-treated children and their parents wasp was identified by 94% of children and 88% of parents. In bee venom VIT-treated children and their parents hb was identified by 90% of children and 100% of parents.

In children who reported SR before treatment due to wasp sting proper identification of wasp equaled 92% in both children and parents. In children who reported SR before treatment due to hb sting 100% of parents, while 94% children identified properly hb.

In 97% of cases both child and parent properly identified hornet, 95% hb, 89% wasp and 87% bumble bee, 78% syrphid fly.

Gender, level of education both of parents and children as well as place of residence did not affect the identification of insects. Only children who properly identified syrphid fly were older than those who did not identified this insect (Me = 12 years, IQR = (10–15) vs Me = 10 years, IQR = (8–10), $P = 0.036$).

Conclusion: General level of insects' identification was high in children and their parents. Rate of hb identification was higher than wasp identification in both children treated with respective venom and their parents, respectively.

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Intradermal skin test reactivity to wasp and bee venom correlates to venom-specific IgE and decreases during venom-specific immunotherapy

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Background: Intradermal skin test with hymenoptera venom is a sensitive method to assess wasp or bee venom sensitization and qualification for venom-specific immunotherapy (SIT) in supposedly allergic patients. We aimed to evaluate

1 skin test reactivity before and during SIT and
2 correlations between intradermal skin test reactivity with venom-specific antibodies.

Method: We retrospectively analysed files of patients undergoing Ultrarush Induction (UR) of SIT and consecutive SIT with wasp and/or bee venom between 2010 and 2012 at the Allergy Unit, Department of Dermatology, University Hospital of Zurich. Intradermal skin test reactivity and hymenoptera venom specific serum IgE levels were determined before UR and in median 3 years after UR (interquartile range (IQR) 3–5 years) during or at the end of SIT.

Results: Of 86 patients (41 females; median age 44 years, IQR, 27–52 years), 54 patients received UR and consecutive SIT with wasp venom, 24 with bee venom and 8 with both wasp and bee venom. Before UR, patients with clinical suspect of wasp venom sensitization reacted to lower concentrations of wasp venom (0.01 mkg/ml) than bee venom (1 mkg/ml)($P < 0.001$) in intradermal skin tests. Likewise, bee venom sensitized patients reacted to lower concentrations of bee venom (0.01 mkg/ml) than wasp venom (1 mkg/ml) ($P = 0.01$). Reactivity to wasp or bee venom skin tests accordingly decreased after UR. Before UR, skin tests in sensitized patients were positive in median to 0.01 mkg/ml of wasp or bee venom, while after UR patients were positive in median to 0.1 g/ml of wasp or bee venom (wasp venom sensitized patients, $P < 0.001$; bee venom sensitized patients, $P = 0.1$). Consequently, intradermal skin tests were positive to similar concentrations of wasp or bee venom in either wasp ($P = 0.1$) or bee ($P = 0.3$) venom sensitized patients after UR. Patients with positive skin tests to lower venom concentrations had higher serum levels of venom-specific IgE before UR (wasp venom SIT patients: rho −0.26, $P = 0.06$; bee venom SIT patients: rho −0.61, $P = 0.002$) and after UR (wasp venom SIT patients: rho −0.53, $P = 0.008$; bee venom SIT patients: rho −0.78, $P = 0.005$).

Conclusion: Intradermal skin test reactivity is a good marker for wasp or bee venom sensitization and correlates well to serum titers of venom-specific IgE. The skin test reactivity decreases after UR with the respective venom. It might be speculated that this decrease is a marker for an allergy-protective effect of SIT.

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Use of tryptase levels to detect adverse reactions during honeybee venom immunotherapy

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Background: Adverse reactions to venom immunotherapy (VIT) are frequent,

especially during the build-up phase and with the use of bee venom. Tryptase measure is a useful tool in anaphylaxis diagnosis. Severe reactions to VIT have been associated with increased baseline serum tryptase levels, among other factors. We investigated the changes in serial serum tryptase during the build-up phase of VIT to monitor the adverse systemic reactions (ASR) during VIT.

Method: Serum tryptase was serially measured the first day of immunotherapy in patients diagnosed with bee venom allergy undergoing bee VIT in a cluster schedule: before the first dose of VIT and 90 min after the last dose. Adverse reactions to VIT were recorded during the first year of treatment.

Results: Sixty nine patients received bee venom immunotherapy. Eighteen patients (26%) developed ASR with VIT: 4 the fist day of VIT, 8 the second day and 10 with maintenance doses. Five patients experienced severe anaphylaxis, none of them in day 1.

Tryptase level decreased during the first day of VIT in 53 patients and increased in 16 patients, twelve of them experienced ASR. Only 2 out of 12 patients suffered the ASR the day 1. Tryptase level didn't increase in 4 patients, all of them with minor reactions ($P < 0.001$). The results show a 62% sensitivity and 92% specificity.

Conclusion: An increase in serum tryptase level at the beginning of VIT, even in absence of adverse reactions, could predict future systemic reactions to VIT.

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cardiovascular medication during venom immunotherapy: still an open question?

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Background: The effect of beta-blockers (BB) and angiotensin-converting enzyme inhibitors (ACEi) during venom immunotherapy (VIT) is still matter of debate and in literature there are conflicting data regarding their use in patients undergo VIT. According to some authors BB should be discontinued, when possible, as they can exacerbate systemic allergic reactions making them more difficult to treat. Also ACEi use has been associated with a risk of systemic allergic reactions during VIT and a reduction in the efficacy. However, recent studies did not confirm these results.

Objective: Our study was designed to evaluate the effect of BB and ACEi on the safety and the outcome of VIT in a cohort of patients affected by venom hypersensitivity.

Methods: We retrospectively collected data from 511 patients underwent VIT between 2003 and 2014.

Results: of 511 underwent rush VIT protocol (two days for yellow jacket and four days for honey bee) 88 patients (17%) were taking cardiovascular medication: 31 (6%) only BB, 57 (11%) only ACEi and 12 (2%) both of them. In patients treated with cardiovascular medication during VIT we did not detect any systemic allergic reactions. We subsequently considered the risk of field sting-related relapse in re-exposed subjects taking ACEi or BB during VIT. Of re-stung patients treated with ACEi or BB (respectively 34 and 18) none showed objective systemic reactions and they have been completely protected.

Conclusions: According to our data neither the use of BB nor ACEi impair the safety and the efficacy of VIT. Taking BB and ACEi does not seem to represent a contraindication for VIT. Considering the importance of these drugs their possible suspension in subjects candidates VIT should be carefully evaluated.

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Reasons for declining venom immunotherapy: the how's and the whys

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Background: Hymenoptera stings are responsible for significant morbidity and deterioration in health-related quality of life and patients with hymenoptera venom allergy are at risk for life threatening reactions. Venom immunotherapy (VIT) is safe and the only effective treatment in allergic individuals. However, some patients prefer not to undergo VIT.

Objective: We aimed to ascertain the reasons why patients decline VIT.

Method: All patients who declined VIT since 2006 and all patients currently undergoing VIT in our department were included. Clinical records were reviewed and the patients who did not undergo VIT were contacted by phone and interviewed using a short structured questionnaire.

Results: A total of 40 subjects were enrolled, with a mean (SD) age of 41.5 (15.5) years, 30 (75%) male and 23(57.5%) refused VIT. There were no significant differences regarding sex, education, age at time of VIT proposal, severity of reactions, type of symptoms, culprit insect, comorbidities or beekeeper status between patients refusing VIT and undergoing VIT. Six patients refused VIT from 2006 to 2011 and 17 from 2011 to 2015. Nineteen of the 23 patients who declined VIT were interviewed by phone; 16 were beekeepers; 5(26.3%) avoided outdoor activities because of this allergy; 18 (94.7%) considered VIT a very important treatment; 14 (73.7%) stated the cost of VIT as the major reason for declining this treatment, 4 (21.1%) indicated difficulties in getting time off work once a month for hospital visits. If VIT was fully or 50% reimbursed, 17 (89.5%) and 10 (52.6%), respectively, would have accepted VIT. Since refusing VIT, 11 (57.9%) were re-stung and 9 of them had another anaphylactic episode.

Conclusion: In this group the cost of immunotherapy was the major obstacle for patients to undergo VIT, leaving them at risk. The number of patients declining VIT almost tripled since 2011. Interestingly, immunotherapy stopped being reimbursed in Portugal in 2011, when we were at the edge of the economic and financial crisis. The mean monthly wage in Portugal is €984 and 12.9% of the population receive the minimum salary of 505€. On the other hand, when all direct and indirect expenses are considered, VIT has a total mean yearly cost of 1400€. Our findings illustrate how economic decisions can impact patients' safety and health and reflect negatively on patient care. Doctors are the ultimate patient advocates and they must be aware of these consequences.

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Immunological factors associated with VIT treatment failure due to SSR and the influence of omalizumab treatment

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Background: Systemic side reactions (SSRs) during venom immunotherapy (VIT) frequently require emergency interventions and are cause of discontinuation of treatment -VIT failure.

Our objectives were to identify immunological factors associated with VIT failure due to SSRs and to evaluate the influence of omalizumab treatment for VIT continuation.

Methods: In years 2005–2015 1453 patients underwent VIT (516 honey-bee venom (HBV), 865 yellow jacket venom (YJV), 72 both venoms) in our clinic. VIT was, despite several attempts and emergency interventions, discontinued due to SSRs in 19/687 (2.7%) HBV VIT and 0/937 YJV VIT. Those patients were characterized according to various immunological factors including sIgE, basophil CD63 response, baseline tryptase and number of FcεRI

and IgE on basophil surface. 6/19 VIT failure patients were pre- and co-treated with omalizumab during another attempt of VIT. We tried different schedules of omalizumab treatment (dose, time interval, duration) to establish the most successful protocol.

Results: The only immunological factor associated with HBV VIT discontinuation due to SSRs was high basophil CD63 response to allergen. Basophil sensitivity was significantly higher in 19 VIT failure patients in comparison to 275 patients with successful HBV VIT (median response at 0.01 μg/ml: 38 vs 6.4%, 0.1 μg/ml: 79 vs 53%, respectively). No difference was evident for baseline tryptase.

Pre- and co-treatment with omalizumab markedly decreases basophil responsiveness and number of FcεRI and IgE on basophil, this cellular changes correlated with diminishing of SSR. VIT was successfully continued in 5/6 patients treated with omalizumab. Most favourable immunological and clinical outcomes were observed if omalizumab 300 mg pre-treatment was administered four-times before VIT in two weeks interval and if co-treatment was long lasting, at least 6 months during VIT.

Conclusions: Increased basophil allergen sensitivity is strongly associated with HBV VIT treatment failure due to SSRs. Pre-treatment with omalizumab decreases basophil responsiveness and enables successful initiation of VIT. Co-treatment with omalizumab should be long lasting.

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Anaphylaxis caused by mosquito allergy in systemic mastocytosis: a causal relationship

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Background: Anaphylactic reaction to mosquito bites is extremely rare. We reported 4 patients who experienced systemic anaphylaxis from mosquito bites. All of them were diagnosed with an indolent systemic mastocytosis.

Case 1: A 20-year-old man presented a flush on his face associated nausea, vomiting and dizziness after a mosquito bite. Out of any anaphylactic reaction, he had an elevated serum tryptase of 89 μg/l. His bone marrow biopsy confirmed the diagnosis of systemic mastocytosis. His skin prick test (PT) reaction was positive (5/25) and specific IgE serum to mosquito (sIgE) was also positive 0.66 KUA/l.

Case 2: A 32-year-old man presented a dozen of mosquito bites, and 10 min later, he had anaphylaxis. Because of delayed resuscitation, he had hypoxic brain damage resulting in pyramidal syndrome. PT was positive (5/15) and sIgE was also postive 0.44 kUA/l.

He had an elevated serum tryptase of 23.8 μg/l on daily basis and he was diagnosed with an indolent systemic mastocytosis with bone involvement.

Case 3: A 47-year-old man presented a pruritus on his ankles after a non-identified insect bites, following, 2 h after, by a loss of consciousness. PT was negative and sIgE was also negative. Mosquito allergy was confirmed by basophil activation testing (71.5%). Mastocytosis was evocated because of an elevated serum tryptase (51.3 μg/l) away from this episode and was confirmed on a bone marrow biopsy.

Case 4: A 56-year-old woman felt dizzy and nauseous after mosquito bites since she was a child. Recently, in 2014, she presented an anaphylactic reaction (loss of consciousness, urticaria) after a multiple mosquito bite. PT was positive (2/15). Out of any anaphylactic reaction, she had an elevated serum tryptase of 20.3 μg/l and is waiting for a confirmation by a bone narrow biopsy.

Discussion: We reported a case series of 4 cases of anaphylaxis to mosquito bite and mastocytosis. In the literature, there was only one case report on this association (lancet, 2013). In our cases, immunotherapy was a good and a safe option of treatment.

Conclusion: The association between mastocytosis and anaphylactic reaction to mosquito bites is probably not a fortuitous association. It is important to highlight the atypical clinical reaction and weakness of the PT and sIgE. These characteristics are similar in the association mastocytosis and hymenoptera venom. anaphylaxis.

Poster Discussion Session PDS 14

Diagnosis and treatment of urticaria

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The role of component resolved diagnostics for assessing hidden allergen of acute idiopathic urticaria n childhood

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Background: Urticaria is a common disorder, occurring in 15–25% of individuals at some point in life. Many etiological factors have been associated with the onset of urticaria. But the cause is unknow in approximately 50% of patients with acute urticaria with conventional allergy skin test or serum test, so called acute idiopathic urticaria (AIU). The aim of this study was to evaluate hidden allergen components of AIU in childhood by evaluating Component-resolved diagnostics (CRD), which is a tool that characterizes each patient's IgE antibody profile to individual allergen components discriminating between genuine sensitization to specific allergen sources.
Method: 165 children with urticaria were recruited from Hanyang University Medical Center for 5 years. Of them, 74 children with AIU were selected. The diagnosis of allergy was based on case history, allergy skin prick test and IgE serology performed. Cases with positive allergy skin test and/or serologic test were excluded as allergic urticaria. Precise identification of relevant sensitizers in the case of AIU be achieved by conventional diagnosis with complete extracts. Children with AIU presented no positive results from allergy skin test or serum test. Specific component detection for profilin, PR-10, and LTP were measured using ImmunoCAP for allergen component resolved diagnostics (CRD; Phadia AB, Uppsala, Sweden).
Results: Mean age of subjects was 7.6±3.4 years old and male was 41 among subjects.

1 PR-10: Two subjects were positive to rGly m 4 for soybean, 3 were positive to rMal d 1 for apple, 3 were positive to rCor a 10 401 for hazel nut, 2 were posive to rPru p 1 for peach, 2 were positive to ara h 8 for peanut, 3 were positive to rBet v 1 for birch, 3 were positive to rCor a 1 for hazelnut, one was rAln g 1 for alder.
2 LTPs: 2 were positive to rPru p 3 for peach, one was nArt v for mugwort.
3 profilins: 2 were positive to rBetv 2 for birch, 2 were postive to rPhl p 12 for timothy.

Conclusion: The results of this study may suggest that some hidden allergen components of AIU could be figured out by using CRD in childhood. However, there were still remained to further evaluate hidden causing factors to AIU.

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Urticaria and gliadin allergy

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Background: ω-5-gliadin (Tri a 19) has been described as one of the allergens involved in food allergy frequently associated to cofactors.

One of the most frequent clinical manifestations is urticaria (U). Gliadin allergy (GA) has to be considered in the diagnosis of spontaneous or inducible chronic urticaria (CU). The aim of the study was to describe the clinical pattern of U due to gliadin allergy.
Method: Patients were selected when having a compatible history of GA, positive skin prick test (SPT) to a gliadin enriched gluten extract (GEGE) and/or sIgE to Tri a 19 > 0.35 kU/l.

Clinical manifestations, initial diagnosis, time from debut to final allergological diagnosis, results of STP to GEGE and levels of total IgE, wheat sIgE and Tri a 19 sIgE were recorded.
Results: $N = 20$ patients were included (12 men, age = 44.5 [38, 0–57, 0] yo). The time until diagnosis was 7 [2, 0–8, 5] years.

Clinical manifestations: 19/20 had had at least one episode of U. U and/or angioedema (AE) where the debut manifestations in 16/20.

9/19 had had only cutaneous symptoms: 6/9 only U, 3/9 U and AE. The features of the U in this group were: 7/9 recurrent acute U (RAU) (3 with AE), 2/9 spontaneous and cholinergic CU.

10/19 had had U/A and other clinical manifestations: 10/10 anaphylaxis, 1/10 gastrointestinal disorders. The features of the U in this group: 10/10 RAU (1 with AE).

Regarding the initial diagnosis, 10/19 patients had been misdiagnosed as NSAID hypersensitivity.

Cofactors were involved in all cases: 7/19 exercise, 4/19 NSAIDs, 8/19 exercise + NSAIDs.

14/20 had positive result to SPT GEGE.

Patients with negative SPT showed lower levels of Tri a 19 sIgE.
Conclusion: Gliadin food allergy may be expressed as urticaria alone, usually RAU, with or without AE. In the differential diagnosis of not only RAU, but also in spontaneous CU or cholinergic CU, gliadin allergy has to be evaluated, specially when exercise or NSAIDs are involved.

In our series, Tri a 19 sIgE determination has shown to be more sensitive than SPT in the diagnosis of gliadin allergy.

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Immune responses to the cat flea allergen Cte f 2 in humans with papular urticaria

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Background: Papular urticaria (PU) caused by insect bites is a common skin hypersensitivity in the Tropics. Fleas at home have been associated with the disease presentation in Bogotá-Colombia, a city located in high tropical altitudes where 20% of children develop PU. Cte f 2, from cat flea or *Ctenocephalides felis*, is a salivary allergen for dogs, but there is no information about its importance as human allergen.
Method: Cte f 2 was obtained as a recombinant protein in *E. coli* (Genscript, USA). IgE and IgG levels against this recombinant and cat flea whole-body extract (Cf) were evaluated by ELISA in 61 PU patients recruited from Bogotá-Colombia. CFSE-labeled peripheral blood mononuclear cells were incubated with Cte f 2 or flea extract for 7 days to analyze T cell proliferation in 4 patients and 1 healthy control. A positive antigenic response was

considered when >5% of CD3+CD4+ T cells were $CFSE^{low}$. A bioinformatics search was performed to identify potential cross-reactive allergens between cat flea and mosquitoes (other important causal agents of PU) and a Cte f 2-like protein identified in *Aedes aegypti* was expressed in our facilities. To explore cross-reactivity with Cte f 2, IgG western blot was performed with an anti-Cte f 2 polyclonal serum (pAb) obtained from immunized mice after repeated intraperitoneal injections of the recombinant protein.

Results: Specific IgE against cat flea and Cte f 2 were significantly correlated (S. rho: 0.31, $P = 0.01$) but frequency of sensitization was higher with recombinant antigen (32.8%) than with Cf (13.1%). IgG against Cte f 2 and Cf was found in 63.3% and 51.7%, respectively. Cte f 2 induced stronger proliferation than Cf and T cell responses were observed in half of patients (50%). Ctef2-pAb recognized Cte f 2 and the Cte f 2-like protein from *A. aegypti*, but not the unrelated antigen AscarisGST.

Conclusion: Cte f 2 is recognized by T cells and is also allergenic for humans. It was more sensitive than complete extract to detect sensitization in patients with PU. The high rate of IgG response to Cte f 2 indicates frequent exposure to cat flea in this population. Cross-reactive antibody response against Cte f 2 and its homologous in *A. aegypti* was detected.

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Indirect basophil activation test in the diagnostic work-up of chronic urticaria

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Background: Chronic spontaneous urticaria (CSU), previously known as chronic idiopathic urticaria or chronic ordinary urticaria, is characterized by recurrent itchy wheals and angioedema that appear without any identifiable cause. The condition is defined as recurrent urticaria, occurring for at least 6 weeks. Approximately 40% of patients with chronic idiopathic urticaria show antibodies directed against the IgE high-affinity receptor subunit. The aim of this pilot study was to investigate the role of the indirect basophil activation test in the diagnosis of autoimmune urticaria (AIU) in children with chronic urticaria.

Methods: We evaluated patients' serum through indirect basophil activation test (BAT) using flow citometry. Anti FcERI Ab was evaluated by basophil stimulation with patient serum, and subsequent analysis of released tryptase and endocellular tryptase after basophil lysis. Results were compared to negative and positive controls, and expressed as percentages. We included sixteen patients, aged from 2 to 15 years, all suffering from CSU of unknown etiology. Donor basophils were obtained from non-atopic donors. Positive control sera were artificially prepared to simulate autoimmune urticarial patients' sera. We also evaluated tryptase level, ran auto-immunity tests, performed skin prick tests for common pneumo- and tropho-allergnes and autologous serum skin test (ASST).

Results: Three out of sixteen patients (18.8%) showed positive autoimmunity tests, all for antinuclear antibodies. Seven children (43.8%) showed a positive indirect BAT. ASST was negative in 2 patients and positive in one out of three patients tested through this method. Nevertheless, the patients with negative ASST showed a positive result with the indirect BAT, while the one who had a positive ASST was negative to the BAT. Seven children had elevated levels of tryptase. Nine patients were sensitized to pneumo- or tropho-allergens.

Conclusions: It's important to emphasize the necessity to a proper diagnosis of autoimmune urticaria, as it represents the most severe form of CSU. Indirect BAT seems characterized by a better specificity than ASST. Therefore, it is a promising tool for AIU diagnosis and may be usefully employed in the diagnostic work-up of CSU. Further study are needed to evaluate both sensibility and specificity of the serological test, compared to autologous serum skin test, considering that in our cohort the results given by the two tests were not correlated.

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A broad patient directed online survey reveals high burden of chronic urticaria and under treatment in a real life setting

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Introduction: Chronic urticaria (CU) is a common recurrent skin disorder that can strongly impact quality of life. Most previous publications report on patients treated at specialized centres, which tend to suffer from severe disease and may not reflect the average patient. Thus, it is of major interest to investigate patients with CU in a real life setting.

Objectives and methods: The ATTENTUS online survey was designed to obtain self-reported patient data on the real-life impact of CU and current use of the health care system in Germany. The 30-item survey was conducted from August to November 2014. Participants filled out an online questionnaire rating their current symptoms, medical treatment, impact on daily life and types of doctor(s) consulted.

Results: Here, the results of 17 508 received questionnaires are presented. The mean (± SD) CU duration was 11.6 (± 11.5) years. Half of the participants (56%) reported that their CU symptoms 'strongly affect' or even 'extremely affect' their daily lives. Almost 2 in 3 participants (62.3%) said that this was true during the last CU episode they experienced. 29.4% of participants reported continuous symptom development, throughout the year. Most participants had seen a general practitioner (57.2%) and or a dermatologist (81.2%) because of their CSU and 33.6% had been treated in clinics. Only 30.3% of CU affected individuals who were symptomatic at the time of the study were in physician care. 70% of participants with CSU duration of 15 years or longer had given up on consulting a physician – resigning themselves to self-treatment. As key reasons for this, 52.9% reported that they felt that their doctor was unable to help them, and 38.9% stated that they knew how to treat CU symptoms themselves. However, when asked how well their CU symptoms were actually controlled, 16.6% of participants stated that their medication had little or no effect at all on symptom control, and additional 36.5% of participants indicated that their CU symptoms were insufficiently controlled. Lastly, over half the participants stated that they had been prescribed cortisone products, which importantly, did not appear to improve overall ratings in daily activity scores.

Conclusions: Our findings indicate that in Germany, chronic urticaria is not well managed in many patients. Better awareness of the burden of disease and available treatment options is needed and may help to improve real life patient care.

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Prevalence and severity of urticaria in South Korea based on national health insurance data

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Background: Urticaria has tremendous impact on patient's quality of life.

However, its epidemiology and prevalence have not been well known. We investigated the prevalences, epidemiology and severity of various urticaria in South Korea over 5 years.

Method: We used the data of nationwide, population-based Health Insurance Review and Assessment Service claims database from 2010 to 2014. Diagnosis of urticaria was based on physician-certified diagnoses using ICD-10 codes, which included cold and heat urticaria (L502), dermographism (L503), cholinergic urticaria (L505), chronic urticaria (L508) and angioedema (T783). The severity of urticaria was assessed by hospitalization rates.

Results: The annual prevalence of all-type urticaria increased from 4.6% in 2010 to 5.3% in 2014. Among various types of the specific groups, the prevalence of angioedema and chronic urticaria increased from 22 to 35 per 100 000 people, and from 347 to 469 per 100 000 people, respectively. On the contrary, cholinergic urticaria showed relatively no changes in prevalence while prevalence of cold and heat urticaria decreased over 5 years. Chronic urticaria and dermographism were presented most commonly in female patients aged 60–79 years, otherwise cholinergic urticaria commonly presented in male patients aged 10–29 years. Angioedema and chronic urticaria showed higher hospitalization rates than other types of urticaria.

Conclusion: The prevalence of general urticaria is assumed to increase in South Korea annually. Particularly, angioedema and chronic urticaria showed a remarkable increase in prevalence and relatively higher severity.

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Prevalence and risk factor of urticaria, focusing on the chronic type in children – population based cross-sectional study

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Background: Urticaria including the chronic type is one of the most common skin diseases, but there is limited data on the prevalence and risk factors in the pediatric population. To determine the prevalence of acute and chronic urticaria and to identify the risk factors for chronic urticaria in children aged 4–13 years.

Method: This study is a population-based study consisting of 4111 children attending three preschools and six elementary schools enrolled in the prospective Seongnam Allergy Project in the year of 2015 (SAP 2015). We conducted a survey using questionnaires completed by the parents, including questions inquiring about urticaria such as chronic symptoms, triggering factors, and family and past history. Blood sampling ($n = 464$) was performed to measure vitamin D, total eosinophil count (TEC), and total IgE levels, and skin prick tests ($n = 503$) were done on the children. Logistic regression analysis was conducted to analyze risk factors of urticaria.

Results: The urticaria-related prevalence was as follows: life-time urticaria, 22.5%; current urticaria (defined as life-time urticaria together with the presence of symptoms in the past 12 months according to the questionnaire), 15.3%; and among the current urticaria, acute urticaria, 13.9%; chronic urticaria, 1.8% (persistent type 0.7% and intermittent type 1.1%) in 4–12 years children. Acute urticaria was significantly associated with allergic disease and parental allergic history ($P < 0.001$), but not chronic urticaria. After adjusting for age, sex, BMI and parental history of allergic history, chronic persistent urticaria was associated with being in a high income category (odds ratio [OR] = 5.03, 95% CI 1.49–16.91, $P = 0.009$) and living in a new house (OR = 2.37, 95% CI, 1.09–5.16, $P = 0.029$).

Conclusion: We found that 1.8% of the Korean pediatric population has chronic urticaria and increased risk of chronic persistent type was found to be living in a new house and being in a high income category.

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Hospital compared to private practice dermatologist treatment of chronic spontaneous urticaria: baseline characteristics and treatment patterns from the German AWARE (A World-wide Antihistamine-Refractory Chronic Urticaria Patient Evaluation) study

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Background: AWARE is an ongoing prospective non-interventional study to assess chronic urticaria (CU) in the real-life setting. This analysis compares chronic spontaneous urticaria (CSU) patients treated by hospital centers (HC) and private practice dermatologists (PPD) in Germany.

Method: Demographics, healthcare utilization, diagnostic tests, pharmacological treatment, disease control (urticaria control test [UCT]) and health-related quality of life (QoL; CU QoL questionnaire [CU-Q_2oL] and dermatology life quality index [DLQI]) were assessed. Data were compared between groups using parametric risk-ratio-tests (binary) or t-tests (continuous).

Results: Overall, 2252 CU patients were assessed and 2095 CSU patients (597 HC and 1498 PPD) were included in the analysis population. Demographic data were similar for HC and PPD patients: mean (± standard deviation [SD]) age (46.3 [14.8] vs 46.3 [15.9], respectively), gender (female: 72% vs 69%) and mean (±SD) body mass index (27.6 [5.4] vs 26.8 [7.4] kg/m^2). Rates of angioedema were higher in HC patients (65.3% vs 37.7%; $P < 0.0001$) and mean (±SD) time since diagnosis was longer for HC patients (5.4 [7.4] vs 4.4 [6.9] years; $P = 0.0035$). Comorbidity rates were similar in both treatment groups; however, HC patients had higher rates of Hashimoto's thyroiditis (10.4% vs 3.5%; $P < 0.0001$). Prior healthcare utilization was higher for HC patients vs PPD patients (e.g. hospitalization: 38.9% vs 27.1% [$P < 0.0001$] and use of further dermatologists or allergists: 77.9% vs 39.6% [$P < 0.0001$]). HC patients were more likely to receive the autologous serum skin test (20.4% vs 7.1%; $P < 0.0001$), whereas PPD patients were more likely to receive a prick test (44.4% vs 25.9%; $P < 0.0001$). Rates of pharmacological treatment were higher for HC vs PPD patients, e.g. any treatment: 69.3% vs 55.3% ($P < 0.0001$); non-sedative H_1-antihistamine: 57.6% vs 44.6% ($P < 0.0001$); montelukast: 8.9% vs 0.9% ($P < 0.0001$) and omalizumab: 6.9% vs 0.9% ($P < 0.0001$). Mean (±SD) UCT (7.8 [4.6] vs 7.6 [4.1]) and QoL scores (DLQI: 8.8 [7.3] vs 8.2 [6.8] and CU-Q_2oL: 38.2 [20.9] vs 36.1 [19.5]) were similar for HC and PPD patients, respectively.

Conclusion: HC patients showed higher rates of angioedema, Hashimoto's thyroiditis and a longer mean time since diagnosis vs PPD patients. Patient characteristics and QoL impairment were similar between both groups, which may be due to the higher rates of pharmacological treatment received by HC patients.

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Omalizumab is effective and safe in symptomatic dermographism: results of UFO, a multicentre randomized, placebo-controlled trial

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Background: Symptomatic dermographism (SDerm), also known as urticaria factitia, is a very frequent form of physical urticaria. SDerm is chronic, debilitating, and often resistant to antihistamines, the first line treatment. Case reports suggest that omalizumab may be effective. As of now, there are no controlled trials with omalizumab in SDerm, or any other form of physical urticaria.
Method: We conducted an investigator-initiated, prospective, randomized, double-blind, placebo-controlled phase 2 study, evaluating the efficacy and safety of omalizumab in patients with antihistamine-resistant SDerm. 61 patients aged 18–75 years were enrolled at three urticaria centres in Germany to receive three subcutaneous injections, spaced four weeks apart, of 150 mg or 300 mg omalizumab, or placebo. Patients were randomized in a ratio of 1:1:1 without stratification. The primary efficacy outcome was the change in trigger thresholds from baseline to week 10 using FricTest® (to assess critical friction thresholds, grade 0–4). Primary analyses were performed in the per protocol population, safety analyses were performed in all participants who received at least one dose of study drug. The study is registered with ClinicalTrials.gov, number NCT02169115.
Results: Patients were recruited between Dec 1, 2012 and Dec 31, 2014. At week 10, mean changes (± SEM) in trigger thresholds from baseline were −0.6±0.3 in the placebo, −1.8±0.4 in the omalizumab 150 mg ($P < 0.05$), and −2.0±0.4 in the omalizumab 300 mg group ($P < 0.005$). There was no difference in changes of trigger thresholds between the two omalizumab groups. 8 (44.4%) and 10 (52.6%) of the omalizumab 150 mg and 300 mg treated patients showed complete response, respectively, vs only 2 (11.1%) in the placebo group. The frequency of adverse events was similar across groups. The frequency of serious adverse events was low, with one event in the placebo group, one in the omalizumab 150 mg group and one in the omalizumab 300 mg group, none of which led to the discontinuation of study treatment.
Conclusion: Treatment with omalizumab is effective and safe in symptomatic dermographism patients who are unresponsive to antihistamines.

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Home self-administration of omalizumab is a safe alternative to hospital administration in chronic spontaneous urticaria

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Background: Omalizumab is a monoclonal antibody that targets IgE and has a license as an add-on therapy in patients with chronic spontaneous urticaria (CSU) who have had an inadequate response to H1-antihistamines. The product license currently states that it should be administered by a healthcare professional. To date we have treated 101 CSU patients with omalizumab since 2010, with 83 patients currently on active treatment. Due to lack of adverse reactions, good adherence to treatment and patient preference we explored home treatment with self-administration of omalizumab as an alternative to hospital administration.
Method: All patients were treated in a single center. Our current pathway is that new patients deemed competent are transferred to home treatment after 2 hospital doses and followed up in a consultant supported pharmacist-led clinic.
Results: We identified 52 patients (58%) on home treatment. The majority of patients (81%; $n = 42$) were on 300 mg 4-weekly (dose range 150 mg 6-weekly to 600 mg 4-weekly). Patients have been on home treatment for 2 weeks up to 1 year (average 3 months; median 2 months). The numbers of doses administered in hospital prior to home treatment ranges from 2 to 45 (average 13; median 11). We have had no adverse reactions or reported problems in these patients and those transferred to home treatment sooner did not differ to those who had more doses in hospital.
Conclusion: Home, self-administration of Omalizumab for CSU is a safe alternative to hospital administration.

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Identification of parameters that could predict time to return of symptoms after stopping omalizumab treatment: exploratory analysis of Phase III data from patients with chronic Spontaneous urticaria

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Background: In patients with chronic spontaneous (CSU), who remain symptomatic despite the use of anti-histamines, omalizumab, a humanized recombinant monoclonal anti-IgE antibody, significantly improved outcomes in three Phase III randomized clinical trials (RCTs). We aimed to identify characteristics that could predict the speed of recurrence of patient symptoms after treatment discontinuation. We also aimed to explore the potential relationship between the timing of response onset and the speed of symptoms recurrence in CSU patients treated with omalizumab.
Method: This exploratory analysis was done on pooled patient-level data from two Phase III RCTs: ASTERIA I ($n = 319$ 6 injections of omalizumab 75, 150, 300 mg or placebo every 4 weeks) and ASTERIA II ($n = 323$ 3 injections of omalizumab 75, 150, 300 mg or placebo every 4 weeks). The follow-up periods lasted for 16 weeks. A LASSO regularization regression model was used to select variables that were predictive of relapse over the 16 week follow-up period. Least squares linear regression with prediction intervals was used to estimate the relapse probability for each patient based on the selected variables. Heat map visualizations were used to represent the variation of probability of relapse with the selected variables.
Results: The LASSO model identified two parameters that can jointly yield a probability of a patient's recurrence of symptoms after treatment discontinuation. These parameters are: (a) the Area Above the Curve (AAC) of the Urticaria Activity Score summed across 7 days (UAS7) over the initial 4-week treatment period and (b) baseline UAS7. The results suggest that a low baseline UAS7 and a high AAC (fast initial response) indicate that a slow return of symptoms is probable upon discontinuation, whereas a high baseline UAS7 and a low AAC (slower initial response) indicate a higher probability of fast return of symptoms.
Conclusion: This analysis suggests that it may be possible to estimate the probability of return of symptoms after discontinuing

omalizumab treatment in patients with CSU, based on the baseline UAS7 and the AAC (speed of initial response) for the early response to omalizumab treatment. A better understanding of the potential clinical relevance of this exploratory analysis is needed.

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Predictors of omalizumab response in chronic spontaneous urticaria

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Background: We aim to identify possible predictors of response to omalizumab in chronic spontaneous urticaria (CSU) patients (pts).
Methods: Retrospective longitudinal chart-review study (June 2006 – July 2015), of pts with CSU treated for ≥6 months with omalizumab at the author's Immunoallergology Department. Consent from the ethics committee was obtained. Statistical analyses included descriptive statistics, Mann-Whitney test, chi-square test, odds ratio test and generalized linear models.
Results: 23 pts (20 women, 3 men) were included. The age of urticaria onset averaged 43.2 (±13) years. Pts initiated omalizumab 5.2 (±5.7) years after urticaria onset. All were medicated with montelukast, H1-antihistamine 4id and systemic steroids. IVIg, cyclosporine and azatioprine had been tried (in 4, 3 and 1 pts respectively) with no effect. After omalizumab, no pts required systemic steroids; 13 (57%) took H1-antihistamine as SOS and 10 (43%) 1–2id; 3 (15%) took montelukast (due to respiratory atopic comorbidities).

Using generalized linear models (all the following with $P < 0.001$ except men), pts showed a reduction per session of 16% of the UAS score and 20% of the UAS7. Women had a reduction per session of 15%/17% (UAS/UAS7), compared to men 2%/8% (UAS/UAS7). Pts with baseline total serum IgE > 500 kU/l had a reduction per session of 28%/41% (UAS/UAS7), compared to IgE < 100kU/l 12%/20% (UAS/UAS7).

DLQI at baseline was 19 (min 6, max 28, IQR 8); after omalizumab, was 0 (min 0, max 6, IQR 1). The difference between mediums was significant ($P < 0.001$).

16 pts are on omalizumab (average duration of omalizumab-treatment 30±23 months) and 7 have stopped (average 30±18 months). Of them, 11 were complete responders, 4 are omalizumab-dependent (need omalizumab to maintain urticaria control) and 7 non-dependent (stopped omalizumab and had no relapse of CSU).

No adverse reactions were reported.
Conclusion: Response to omalizumab seems to be faster in patients with higher baseline total serum IgE and in women. A lack of response to immune-modulating therapies prior to omalizumab is not predictive a lack of response to omalizumab. Omalizumab is a safe and efficacious therapy in CSU. Suspension of omalizumab should always be tried as some patients achieve remission.

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Treatment response to omalizumab in patients with refractory chronic spontaneous urticaria:single-institution retrospective analysis

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Background: Previous clinical trials have demonstrated the efficacy and safety of the anti-IgE monoclonal antibody omalizumab in chronic spontaneous urticaria (CSU) not responding to antihistamine treatment. The aim of our study was to describe the response patterns of patients with refractory CSU treated with omalizumab in the real-world clinical setting.
Method: A retrospective analysis of medical records of 13 patients with refractory CSU, among a total of 55 cases of CSU, diagnosed and treated in the Allergy Department of Sotiria Athens General Hospital, between January 2014 and December 2015, was performed. Treatment data were retrieved and analyzed in correlation with demographic, clinical and laboratory parameters, mainly including age, gender, clinically relevant comorbidities, drug history, IgE levels, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and thyroid stimulation hormone (TSH) levels, antinuclear (ANA) and antithyroid antibodies, and autologous skin serum test (ASST) results.
Results: The mean age of our patient population was 60.2 years, while the majority were females (10/13 cases, 76.9%). Relevant comorbidities included Hashimoto's thyroiditis (3 cases), multiple sclerosis and pemphigus vulgaris (1 case each). ASST was performed in 8/13 patients (61.5%) and was positive in 5/8 cases (62.5%). IgE, ESR, CRP and TSH levels were elevated in 2/13 (15.4%), 10/13 (76.9%), 7/13 (53.9%) and 1/13 cases (7.7%), respectively; ANA and antithyroid antibodies were positive in 4/13 (30.8%) and 3/13 cases (23.1%), respectively. All patients had a history of chronic urticaria, refractory to high antihistamine and corticosteroid treatment, and responded favorably to omalizumab. Among all responders (13/13 patients, 100%), a direct response was observed in 7/13 cases (53.8%), while in the remaining cases (6/13, 46.2%), response to omalizumab was achieved after interval administration of a single 9-day course of methylprednisolone (total dose of 188 mg).
Conclusion: In the present series, omalizumab was highly effective in resolution of refractory CSU.Furthermore, our results suggest that lack of response to this agent should prompt administration of a short-term course of corticosteroids and continuation of omalizumab treatment as per the initial protocol.These preliminary observations should nevertheless be confirmed in larger prospective studies.

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Omalizumab in chronic spontaneous urticaria. Can individual response drive therapeutic approach?

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Background: Omalizumab is a humanized IgG1 monoclonal anti-body that has been used successfully in chronic spontaneous urticaria (CSU) treatment. However, treatment response as well as the appropriate interval for symptom control can vary significantly.
Method: We share our experience with omalizumab in 64 CSU patients, identifying patterns of response and variability between groups.

We have included 64 patients with CSU, 15 to 81 years of age, of whom 54.7% are females ($n = 35$) and 45.3% males ($n = 29$). All our patients had un-controlled disease despite treatment with maximum dose of oral H1-antihistamines (H1-AH) and/or per or corticosteroids (CS), according to published guidelines.

Initial approach to treatment with omalizumab was 300 mg per 4 weeks. Following patient's response, the interval was appropriately adjusted. ASST, anti-thyroid antibodies and IgE levels were obtained prior to treatment.
Results: Omalizumab administration resulted in:

- an excellent response (no need for other medication) in 36% of our patients (Group A), while
- 16% required occasionally H1-AH (Group B),

- 7% systematically H1-AH (Group C) and
- 5% everyday use of H1-AH and CS for symptom control (Group D).

Looking at every group independently, there were no correlations with neither ASST, disease severity, prior CS treatment, anti-thyroid antibodies or time between treatment initiation and significant response. Of interest, patients in group C had positive ASST, required oral CS with maximum dose of H1-AH and an 80% had positive anti-thyroid antibodies. Patients in this group had a modest response to treatment, observed from hours to few days after first injection. Nevertheless, the small group size renders any conclusion questionable.

Moreover, a significant number of patients in Groups A (61%), B (55%) and C (43%) could maintain a stable treatment outcome, even when expanding the maintenance interval from 4 weeks to as long as 16 weeks. In these cases, the administration of omalizumab was given when the patient needed more than two antihistamine tablets daily for more than a week in order to control his/her symptoms.

Conclusion: Omalizumab is an effective add-on therapy in difficult to treat CSU patients, in whom symptom control can be a challenge. However, not all patients respond to treatment and -moreover- an individualised treatment schedule seems to be the optimal approach.

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Omalizumab in adult patients with refractory chronic idiopathic urticaria: real-life outcomes

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Background: There are few reports about the outcomes of after omalizumab therapy in patients with refractory chronic idiopathic urticaria (CIU). The aim of the study was to evaluate the recurrence rate and its consequences with omalizumab therapy in CIU.

Method: Data of 29 patients (F/M: 20/9) followed-up by allergy clinic due to refractory CIU and given omalizumab was retrospectively evaluated. All patients had given omalizumab injections (300 mg, sc) at 4-weeks intervals. Demographic and disease characteristics such as comorbidities, UAS-7, numbers of eosinophil and basophil, levels of total IgE, tryptase and vitamin D of the patients were compared between with and without recurrence.

Results: The study population was consisted of 24 patients (F/M: 16/8) with the mean age of 40.79±10.54 (17–83)y due to the remaining 4 patients were still on omalizumab therapy. The mean duration of CIU was 83.2±114.5 (6–420) months.The mean duration of after omalizumab therapy was 14.2±3.2 (3–35) months. The recurrence rate was 58.3% ($n = 14$). Gender, age, UAS-7, number of eosinophil and basophil, total IgE and tryptase levels were not different between with and without recurrence. The mean level of vitamin D was significantly lower in recurrent group (19.37±2.3 vs 27.43±1.6; $P < 0.05$). Omalizumab was restarted in all of the recurrent patients. The interval of the omalizumab therapy was arranged according to the patients needs in second line therapy and all were had good clinical response again.

Conclusion: Despite the rapid clinical response to omalizumab in adult patients with CIU, the recurrence rate can not be ignored and vitamin D level might be a predictor for this. On the other hand, re-administration of omalizumab is also effective in this particular group of patients.

Poster Discussion Session PDS 15

Molecular diagnosis for airborne allergens

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Carbohydrate composition of house dust mite allergens and its relevance for IgE binding

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Background: Glycosylation plays an important role in the recognition and uptake of allergens by antigen presenting cells (APCs) and the modulation of immune responses. In addition, cross-reactive carbohydrate determinants (CCDs) are known to constitute epitopes for human IgE. Thus, elucidation of carbohydrate structures and investigation of their biological function is essential to understand characteristics of allergens. In the present study, carbohydrate structures of proteins in house dust mite (HDM) extracts and their interaction with IgE were assessed to elucidate physicochemical and immunological properties of HDM allergens.
Method: *D. pteronyssinus* and *D. farinae* extracts and natural major HDM allergens were investigated for the presence and identity of glycans by Periodate-Schiff staining, c-type lectins, carbohydrate-specific antibodies and mass spectrometry. To elucidate the relevance of glycans for IgE binding, periodate- and mock-treated HDM extracts were incubated with pools of sera from HDM allergic subjects and individual human sera with CCD reactivity.
Results: Glycan structures were detected by Periodate-Schiff staining to be present on high molecular weight proteins in HDM extracts from both species. Binding of the lectins GNA, PNA and DSA to proteins from both HDM species indicates the presence of N-linked mannose and O-linked glycans comprising a core Gal-GalNAc_1. Investigation by a fucose-specific antibody excluded the presence of this CCD in the investigated samples. Applying mass spectrometry, the presence of various N-linked high mannose structures linked to a core $HexNAc_2$ glycan was confirmed. IgE from pools of sera reacted with HDM allergens including the major group 1 and group 2 allergens. IgE reactivity of HDM extracts was unaltered after periodate treatment, thereby excluding that mite carbohydrate structures constitute epitopes for human IgE of HDM allergic subjects. In line with this finding, HDM carbohydrate structures were not recognized by a purely CCD reactive human serum.
Conclusion: Our results reveal a complex glycosylation pattern of proteins in *D. pteronyssinus* and *D. farinae* extracts. However, the detected glycans on HDM allergens apparently do not to constitute epitopes for IgE of HDM allergic subjects and do not comprise CCDs. The immunological relevance of the individual identified carbohydrate modifications needs to be further elucidated in continuing studies.

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The challenge towards discrimination of clinical symptoms by component-resolved diagnosis using a repertoire of recombinant house dust mite allergens

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Background: Component-resolved diagnosis (CRD) is a powerful tool for precise diagnosis of allergy and may be useful to choice the component allergens for a vaccine of allergen-specific immunotherapy. In this study, our aim is to distinguish and predict clinical symptoms by the IgE-binding profile of individual patient using a CRD technique.
Method: We produced 20 allergens registered on WHO/IUIS database and three novel allergens characterized in our laboratory from house dust mite (*Dermatophagoides farinae*) as soluble recombinant proteins using an *Escherichia coli* cold shock vector expression system. Individual IgE-binding profile against these allergens was evaluated by dot-blot analysis. We compared individual IgE-binding profile among patients with
1 only asthma,
2 those with asthma and allergic rhinitis, and
3 asthmatics complicated by allergic rhinitis and atopic dermatitis.
Results: We found that the IgE-binding profile in individuals was quite different in mite-allergic patients. We analyzed IgE-binding profile in 19 mite-allergic patients. The allergen with highest IgE-binding frequency was Der f 2 with 68.4% (13/19). The numbers of allergens with which each patient reacted was not correlated with his/her RAST score. The numbers of allergen reacted with individual patient tended to be increased with progression of symptoms. Our current dot-blot analysis indicates that IgE-binding to Der f 5, Der f 9 (collagenase-like serine protease) and Der f 14 (apolipophorin) seems to be characteristic for patients with asthma and rhinitis, and that IgE-binding to Der f 6 (chymotrypsin-like serine protease), Der f 17 (EF-hand Calcium-binding protein) and DFA22 (a new Der f 2 family member) is seen in patients with asthma, rhinitis and atopic dermatitis.
Conclusion: Our data suggest the IgE-binding signature might discriminate and predict future clinical symptoms of mite-sensitized patients by our CRD system using the repertoire of recombinant mite allergens.

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Comparing methods for collection of nasal secretions

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Background: We aimed to compare different methods for collection of Nasal Secretions (NS) and to evaluate the suitability of processing with a *fixed dilution*. Furthermore, the Immuno Solid-phase Allergen Chip was used for detection of IgE to House Dust Mite (HMD) components.
Method: Firstly, NS were collected in 15 HDM allergic rhinitis (AR) subjects and 12 non-allergic controls with Filter Disks (FD) and Sinus Packs (SP). Secondly, NS were collected in 13 AR subjects with FD,

Ear Packs (EP) and SP. During processing, saline solution was added for mobilization of the NS. In the first experiment, a *fixed dilution* was obtained by calculating the amount of saline solution to add based on the weight of collected NS. In the second experiment, a fixed volume of saline was added to each sample. IgE, IgA, IgG, IgG4 (total and HDM specific), ECP, albumin (ELISA), total protein (QuickStart Bradford Protein Assay) and IgE tot 15 HDM components (chip) were measured.

Results: Levels of total IgE and HDM IgE were higher in NS of AR subjects compared to controls. HDM IgE was Below the Detection Limit (BDL) in all NS samples of controls, but was also BDL in some samples of AR subjects. No significant differences between AR subjects and controls were observed for the other markers, although ECP tended to be higher in SP samples of AR subjects ($P = 0.059$). Albumin and total protein were higher in FD compared to EP and SP samples. The hierarchical pattern of allergenicity was similar in NS and serum (ISAC).

Conclusion: Each collection method has advantages and disadvantages. The SP method is more reproducible than the FD method (previous experiment); hence SP are more suitable for long-term monitoring. Higher amounts of NS are collected with SP, which is important when multiple measurements need to be done. However, SP stimulate the nasal mucosa, resulting in more diluted samples (illustrated by the lower concentrations of proteins). Therefore, SP are less suitable for multiple measurements on the same day. EP are similar to SP, but smaller in size and generally cause less discomfort. Therefore, EP could be of interest in a paediatric setting. *Fixed dilution* processing is associated with practical issues, but has the advantage of a fixed detection limit. This is important when measuring IgE or other markers that are present at low concentrations and often BDL. Measurements with ISAC showed a similar hierarchical pattern of allergenicity in NS and serum.

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Search for Lep d 2 reactivity – a tool for optimizing specific immunotherapy decision

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Background: *Lepidoglyphus destructor* (Lep d) has been proved to be a relevant allergenic source contributing to mite allergy in both urban and farming environments. Co-sensitization to *Dermatophagoides pteronyssinus* (Der p) is quite frequent and, on the other hand, some degree of cross-reactivity between the two species has been documented raising the question concerning inclusion of Lep d in specific immunotherapy (SIT).

The aim of this study is to verify if allergy workup available methods allow an accurate identification of patients who could beneficiate from having Lep d in SIT, using the reactivity to Lep d 2 (a major allergen) as a marker of true sensitization.

Method: Clinical files of 35 patients with allergic respiratory disease, positive skin prick test (SPT) to Lep d (wheal $\geq$ 3 mm) and undergoing SIT were reviewed regarding SPT to Der p, IgE levels against Der p and Lep d total extracts, nDer p 1 / rDer p 2 components and SIT composition.

IgE immunoblotting with Lep d extract was performed in all patients (pre-SIT sera).

Results: Patients mean age was 22.6±13.7 years, being 51.4% male.

Twenty one patients had a positive blot to Lep d 2 (16 kd band), with mean Lep d SPT 10±3.6 mm and mean Lep d IgE 64.2±94.8 kU/l.

The 9 negative blotting to Lep d 2 patients had mean Lep d SPT 7.8±4.5 mm and mean Lep d IgE 17.2±33.6 kU/l.

SPT and IgE showed no statistical difference between the two groups (T test). SPT/Lep d 2 ROC curve could not define a reasonable cut-off value to discriminate positive from negative Lep d 2. Analysing IgE/Lep d 2 ROC curve a sensitivity superior to 80% implied 28.6% of false positives.

SPT to Der p were positive in 33 (94.3%) patients. Der p IgE were positive ($\geq$ 0.35 kU/l) in 23/25 (92%). Ten Lep d 2 positive patients tested for major Der p components recognized at least one of them.

Lep d was included for SIT in 12 (57%) of Lep d 2 positive and in 2 (14%) of Lep d 2 negative patients, before Lep d immunoblot results.

Conclusion: These data show a high prevalence of Lep d-Der p co-sensitization, at least 50% – Der p 1+, Der p 2+, Lep d 2+. Also suggest some degree of immunological cross-reactivity between the two species. SPT and specific IgE were not able to discriminate true sensitization to Lep d, which reflects in prescribed SIT.

Availability of component resolved diagnosis for Lep d (major allergen Lep d 2) might prove useful to more adequate prescribing SIT.

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Utilization of recombinant *Periplaneta americana* allergens for component resolved diagnosis of cockroach allergy

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Background: American cockroach (*Periplaneta americana*) is a major elicitor of perennial indoor allergy in (sub-) tropical areas. Moreover, sensitization to *Periplaneta* has been described in Europe and cross-reactivity, e.g. with German cockroach (*Blatella germanica*) and house dust mite, was reported. Precise diagnosis of *Periplaneta* allergy is hampered by the lack of component resolved diagnosis (CRD), encompassing the complete panel of *Periplaneta* allergens and uniform methods applied for allergen characterization. Here we report the recombinant expression and molecular characterization of *Periplaneta* allergens, Per a 4* (lipocalin-like protein), Per a 7 (tropomyosin), Per a 8* (myosin light chain) and Per a 9 (arginine kinase).

Method: Synthetic genes of Per a 4 (GenBank Acc. No: AY792948, without signal peptide), Per a 7.0101 (Y14854), Per a 8 (JQ279816) and Per a 9.0101 (AY563004) were cloned to the pET-vector system and expressed in *E. coli*. Recombinant proteins were purified by anion exchange and size exclusion chromatography. Protein concentration was determined by amino acid analysis and amino acid sequence was verified by mass spectrometry (MS). Secondary structure was analyzed by circular dichroism (CD) spectroscopy. IgE-binding to recombinant allergens, *Periplaneta* and *Blatella* extract was investigated by immunoblotting and/or fluorescence enzyme immunomertic assay (FEIA) testing using sera from cockroach allergic patients.

Results: All recombinant proteins showed high expression levels and a sufficient degree of purity. Up to 5 mg protein/1 l E.coli (0.4–1.4 mg/ml) were generated. Identity and intact secondary structure of proteins was confirmed by MS and CD analysis, respectively. Per a 7, Per a 8 and Per a 9 showed strong IgE-binding, whereas only a weak IgE-binding of Per a 4 was determined by experimental FEIA testing. IgE cross-reactivity was shown for Per a 7 and Per a 9 using sera from shrimp allergic patients. Moreover, patient′s sera showed different specific IgE values to *Periplaneta* and *Blatella* extract indicating a heterogeneous sensitization pattern.

Conclusion: Recombinant Per a 4, Per a 7, Per a 8 and Per a 9 were generated, physico-chemically characterized and are suitable to complete the panel of *Periplaneta* allergens for CRD. Experimental FEIA testing provides evidence for minor significance of Per a 4, whereas sensitization to Per a 7, Per a 8 and Per a 9 seem to contribute to *Periplaneta* allergy.

*Not listed in the IUIS database.

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Assessment of dog allergen molecules in the diagnosis of dog allergy in children

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Background: Allergy towards dogs is considered to be a major risk factor for development of allergic airway disease. Yet diagnostics of dog allergy is a clinical challenge; the clinical history is often inconclusive and traditional dog dander extract based tests show variations in content of allergens and may be difficult to interpret. The introduction of molecular based allergy diagnostics offers new opportunities for improved characterization. Currently, six allergens Can f 1-Can f 6 have been identified in dog. We aimed to investigate the clinical relevance of diagnosing IgE antibodies to these dog allergen molecules among children sensitized to dog dander extract.
Method: 37 children (age 10–18 years) sensitized to dog dander extract, with or without known allergic airway disease, were recruited from pediatric outpatient clinics in Stockholm. Data on clinical history of rhinitis and asthma associated with dog exposure, nasal challenge with dog dander extract and blood samples were obtained. IgE measurement towards dog dander, Can f 1, Can f 2, Can f 3 and Can f 5 were performed with ImmunoCAP and against Can f 4 and Can f 6 by Streptavidin streptaudin-linked coupling to solid-phase. IgE levels $\geq$0.1 kU_A/l were considered positive. The sensitization profile was compared to the results of the nasal challenge and the clinical history.
Results: IgE-sensitization towards Can f 1 was most common (n = 25, 68%), followed by sensitization to Can f 5 (n = 24, 65%). Sensitization to the dog allergen molecules Can f 2, Can f 4 and Can f 6 was equally common (n = 17, 46%) and 30% (n = 11) were sensitized to Can f 3. Multisensitization to more than one allergen molecule was common; 59% were sensitized to 3 or more dog allergen molecules.

IgE-sensitization to dog dander extract only was observed in 4 children, of which only one (25%) reacted upon nasal challenge compared to 75% (n = 6/8) and 83% (n = 5/6) of patients sensitized to three and all six dog allergens, respectively. Similarly, 2/4 (50%) of the patients sensitized to dog dander extract only, compared to 100% of patients IgE sensitized to all six dog allergen molecules reported bronchial asthma.
Conclusion: Multisensitization towards several dog allergen molecules seems to be associated with rhinitis and asthma. Molecular based allergy diagnostics improve the clinical characterization of children sensitized to dog.

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The measure of specific IgE to furry animals extracts is not useful for genuine sensitization diagnosis in the youngest atopic children. The fundamental role of component resolved diagnostics (CRD)

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Background: Children with atopic dermatitis and asthma are very often sensitized to inhalant allergens and food. Due to cross reactions use of specific IgE to allergen extracts in sensitization diagnosis may lead to misdiagnosis. In this cross-sectional, prospective study the degree of cross-reactivity between furry animals serum albumins and milk serum albumin depending on children age using component resolved diagnostics (CRD) was analyzed.
Method: Serum specific IgE to 112 allergen components were measured by using multiplex microarray (*Chip*) in 297 children (age: 0–18 years) with atopic dermatitis (moderate or severe, objective SCORAD index 15–40 and >40 respectively), asthma or both. Sensitization to dog (Can f 1, Can f 2, Can f 3, Can f 5), cat (Fel d 1, Fel d 2, Fel d 4) and horse (Equ c 1, Equ c 3) allergen components and co-sensitization to milk serum albumin (Bos d 6) was analyzed depending on age.
Results: IgE reactivity to dog allergen components was found in 140 children whereof 10 children (7.1%) was sensitized only to serum albumin (Can f 3) as a cross-reactivity to milk serum albumin (Bos d 6) and was not sensitized to genuine dog allergens. IgE reactivity to cat allergen components was found in 143 children whereof 13 children (9.1%) was sensitized only to serum albumin (Fel d 2) as a cross-reactivity to milk serum albumin (Bos d 6) and was not sensitized to genuine cat allergens. IgE reactivity to horse allergen components was found in 49 children whereof 17 children (34.7%) was sensitized only to serum albumin (Equ c 3) as a cross-reactivity to milk serum albumin (Bos d 6) and was not sensitized to genuine horse allergen. In the youngest children group (age: 0–2 years) sensitization to serum albumins only as a cross-reactivity to milk serum albumin (and no sensitization to genuine animals allergens) was found in 25.0%, 39.3% and 73.3% of children with reactivity to dog, cat and horse allergens respectively.
Conclusion: In atopic children, especially in the youngest group, component resolved diagnostics (CRD) is fundamental in recognition of genuine sensitization to furry animals allergens due to widespread cross-reactivity of milk serum albumin (Bos d 6) to animals serum albumins (Can f 3, Fel d 2 and Equ c 3).

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Asp f 2, Asp f 4 and Asp f 6 but not Asp f 1 may help discriminating between A. fumigatus sensitization and ABPA in CF and asthmatic patients

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Background: Asthmatic and cystic fibrosis (CF) patients display increased susceptibility to Aspergillus fumigatus sensitization, colonization, and pathogenesis. Allergic bronchopulmonary aspergillosis (ABPA) may affect up to 10% of these patients. Early reports suggested that Asp f 4 and Asp f 6 might improve the performance of ABPA diagnostics, but recent data favor Asp f 1 and Asp f 2. Moreover, CF patients may receive pulmonary transplantation (PT), but the effect of PT on molecular profile of A. fumigatus sensitization is unknown.
Method: Thirty consecutive asthmatic or CF patients with positive serum specific immunoglobulin E (sIgE) to A.fumigatus extract were assayed for sIgE to commercial Asp f 1, Asp f 2, Asp f 3, Asp f 4, and Asp f 6. Patient groups were: CF (n = 22 including 5 children under 16), severe asthma (n = 4), ABPA (n = 2), and chronic obstructive pulmonary disease (COPD) with PT (n = 2). Among adult CF patients, 9 had received pulmonary transplantation. P values of 0.05 or less were considered as statistically significant.
Results: sIgE to A. fumigatus ranged from 1.17 to 66.7 kUA/l.Prevalences of sIgE to Asp f 1 and Asp f 2 exceeded 75% in all patient groups. Asp f 4 and Asp f 6 were less prevalent in CF patients (25% for Asp

f 6 irrespective of PT) compared with all other groups.Levels of sIgE to A. fumigatus and to Asp f allergens were significantly higher in ABPA and asthmatic patients compared to age-matched CF patients. Among adult CF patients, previous PT was associated with higher levels of sIgE to A. fumigatus and to Asp f 1, Asp f 2, Asp f 3, and Asp f 4 but not Asp f 6.The ratio of sIgE Asp f 2 / A. fumigatus extract was lower (median 0.1 vs 0.7) in asthmatic and CF patients than in ABPA patients.

Conclusion: In our pilot study, Asp f 2, Asp f 4 and Asp f 6 but not Asp f 1 seem to hold promise for discriminating between A. fumigatus sensitization and ABPA in CF and asthmatic patients. We report higher levels of sIgE to A. fumigatus extract and allergens and distinct molecular profiles in PT recipients with CF.

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Purified polcalcin skin prick tests. Stability study by *in vitro* analysis

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Background: Polcalcins are calcium-binding proteins, identified in pollen from diverse plant families and with a conserved structure and high sequence homology. Due to their cross-reactivity, they are usually included in component resolved diagnosis by *in vitro* techniques. However, polcalcins are not usually as much represented as other allergens for that reason their use as purified proteins for *in vivo* diagnosis is less common. The objective of this study was to purify the native polcalcin from *Olea europaea* pollen and to determine the stability of polcalcin skin prick test (SPT) under different storage conditions.

Method: Polcalcin from *O. europaea* pollen (Ole e 3) was purified by immunoaffinity chromatography in an ÄKTAexplorer system using rabbit polyclonal antibodies anti-rChe a 3 (a recombinant polcalcin from *Chenopodium album*). Purified Ole e 3 was characterized by SDS-PAGE, immunoblot, ELISA and mass-spectrometry. The purified protein was used to prepare SPT at a concentration of 50 µg/ml. After formulation, pricks were stored at room temperature and 4°C and analysed at time 0, 1, 3 and 6 months by SDS-PAGE and immunoblot. pH, glycerol and phenol valuation was also carried out.

Results: The immunoaffinity chromatography method allowed the obtaining of Ole e 3 with a high degree of purity. The immunogenicity of the protein was confirmed by the recognition of both the polyclonal anti-rChe a 3 antibodies and a pool of sera from patients sensitized to polcalcin. SDS-PAGE and immunoblot results showed stability of the protein at 4°C during the whole period of study (6 months). However, a loss of stability at room temperature was observed. Regarding pH, glycerol and phenol values, they were maintained in the established ranges at 4°C.

Conclusions:

- Ole e 3 was obtained by immunoaffinity chromatography with a high degree of purity. The identity and immunogenicity of the protein were also confirmed.
- Purified Ole e 3 SPT was stable after 6 months of storage at 4°C in terms of protein integrity and different physical-chemical parameters.
- Purified polcalcin SPT could be used for *in vivo* diagnosis.

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Predicting 'true' olive tree pollen allergy

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Background: Most olive tree pollen allergic patients are polisensitized to other tree, weed or grass pollen. A significant percentage of this positive olive pollen skin prick test (SPT) patients don't present the Ole 1 specific IgE, which is currently considered a marker to olive pollen allergy. Our aim was to define predictive criteria of allergy to olive tree pollen in patients with a positive SPT to this pollen.

Method: We collected the medical records, SPT results and Ole1 specific IgE levels of 78 patients with a positive SPT to olive tree pollen – wheal with a diameter equal or greater then 3 mm. Using the CHAID algorithm (Qui-squared Automatic Interaction Detection) we were able to create a Classification Tree of olive tree pollen allergy considering 'true allergy' the positivity of specific IgE to Ole1.

Results: In our study group, 37 were female and 41 male, mean age was 27.2 ±15.6 (7–71 years). Asthma was present in 5.1% of patients, rhinitis in 37.2%, asthma and rhinitis in 17.9%, rhinoconjuntivitis in 37.2% and asthma and rhinoconjuntivitis in 2.6%. In 56.4% the symptoms were seasonal and 67.9% had a positive SPT to dust mites, 97.4% to grass pollen, 64.9% to parietaria pollen and 55.1% to 'other pollens' (neither grass nor parietaria pollen).

The predictive factors of the best model encountered were the SPT wheal size to olive tree pollen, the SPT wheal ratio olive/histamine and the SPT to parietaria result.

Therefore, patients with a SPT wheal to parietaria greater than 9 mm and a SPT ratio olive/histamine greater than 1.3 had a positive Ole 1 in 100% of the cases. In the other hand, patients with a SPT wheal to olive tree pollen between 5 and 9 mm followed by a SPT to parietaria smaller than 5 mm had 86.7% probability of a positive IgE to Ole 1.

The computed tree estimated risks were: re-substitution 0.14 (standard error 0.04) and cross-validation 0.20 (standard error 0.05).

Conclusion: Basic allergological patient evaluation allowed to create a predictive model of olive tree pollen allergy with a reasonable performance.

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Ultra structural orbicules released in ash (*Fraxinus excelsior*) pollen grains and their possible role in allergic respiratory

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Background: The ash tree (*Fraxinus excelsior*) belongs to the Oleaceae family, and the pollen grains are one of the most important causes of respiratory allergy. In this research, investigate the Ultra structural Orbicules in exin pollen wall development in *F. excelsior*.

Method: The external morphology, internal structure and stainability for protein of orbicules characteristics in *F. excelsior* are examined by scanning electron (SEM) and transmission electron microscopy (TEM).

Results: A total of 100 pollen grains were examined using SEM in ash pollen grains orbicules attached to the pollen exine. Based on SEM micrographs, the number of orbicules per 100 mm^2 of the locule wall surface and were not found distributed freely in the anther locules. Since the orbicules of ash pollen grains are 1–2.8 pm in diameter and they were attached to the locule wall surface through sporopollenin ous fibrils and often in continuity with the thin layer of sporopollenin each locule. The orbicule wall stains moderately for protein. The protein is localized mainly at the pollen aperture, in a thin layer between the exine and the intine and in the core

and on the surface of the orbicules. The nitrocellulose membrane test indicates possible allergenicity of the orbicules as well as of the pollen.

Conclusion: It is concluded that orbicules from the surface of the locule wall can be significant role bronchiole of the lungs and cause allergic respiratory.

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Sensitization profiles on allergic patients in the area of Aragón (Spain)

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Background: Allergic asthma and rhinitis are diseases with high prevalence and the costs induced are extremely high. To establish a correct diagnostic strategy in polysensitized patients is crucial in order to optimize the therapeutic strategy and reducing the burden by these diseases. The objective of this study is to study allergic patients from a same geographical area, with the aim of knowing the sensitization profiles to relevant allergens by means of skin-prick test (SPT) and specific-IgE (sIgE) to major allergens component-resolved diagnostics (CRD); studying the differences between SPT and CRD in pollen-allergic patients considering as exposure factor to have SPT+ and as confusion factor to be sensitized to profilin and trying to establish the risk-factors associated to be sensitized to a profilin.

Method: 231 patients were included in the study. None of the patients must have previously received allergen immunotherapy. All patients give informed consent. The sensitization profile is obtained by SPT and CRD for major allergens for both perennial and seasonal and panallergens.

Results: 228 patients out of the total had symptoms of rhinitis, being the most frequent with perennial features (68.5%). A total of 108 patients in the study population (46.75%) had asthma, and 43 patients suffered food allergy. The most frequent perennial allergen is Alternaria, both by SPT and CRD (23%).

Regarding seasonal allergens the most frequent sensitization were: Grass (SPT:80.1%, Phl p 1:75.8%, Phl p 5:54.6%), *Olea* (SPT:55.4%, Ole e 1:48.2%), *Salsola* (SPT:45.5%, Sal k 1:23.7%) and *Cupressus* (SPT:41.1%. Cup s 1:31.1%).

Applying a logistic regression model, the unique allergen with a statistically significant risk associated to sensitization to profilin is Phl p 5 ($P = 0.0023$).

The relation between correspondent allergens measured by SPT and by sIgE was studied by means of a study of cases (patients with positive sIgE to a specific allergen) and controls (patients with negative sIgE to the same allergen). It was considered as exposure factor to be sensitized or not to the allergen measured by SPT and as confusion factor was considered to be sensitized to profilin. The outcome, in terms of odds-ratios (OR), was statistically significant for *Olea* (Ole e 1), *Salsola* (Sal k 1) and *Platannus* (Pla a 1+2).

Conclusion: The diagnosis by components seems to be a crucial tool in order to determine the sensitization profile of a particular allergic population.

Poster Discussion Session PDS 16

Risk factors and prevention of allergic diseases

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Impact of vitamin D and vitamin D-binding protein on the respiratory health of farmers

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Background: Several epidemiological studies have found a relationship between serum 25-OH Vitamin D (Vitamin D) and respiratory symptoms and diseases. While these studies have sampled large populations by have targeting urban areas, urban life may not be appropriate for studying natural levels of vitamin D. This study focused on an association of respiratory health with Vitamin D and the Vitamin D-binding protein (VDBP) among farmers, who we assume, a priori, to have higher UVR exposure due to living and working in the countryside and spending more time outdoors than the average urbanite.
Method: A cross-sectional epidemiological study was performed between October 2009 and January 2010 in the French region of Auvergne (FERMA Study Phase I). A standardized questionnaire (past-year allergic nasal and ocular symptoms, past-year allergic nasal symptoms, past-year cough, past-year regular cough and expectoration, past-year wheezing, recent shortness of breath, and asthma) was administered to participants. Lung spirometry with bronchodilator tests were performed and the serum level of Vitamin D (chemiluminescence technology) and VDBP (by ELISA) were measured.
Results: Three hundred and fifty-nine (359) farmers were selected (70% male). The mean serum level of Vitamin D was 17 ng/ml and of VDBP was 123.73 ng/ml. Past-year regular cough and expectoration were significantly inversely related with Vit D (adjusted Odds Ratio = 0.311, 95% CI: 0.103–0.937). Small airways disease (FEF25-75 < 80%) was inversely related to Vitamin D among males, aOR = 0.322, [0.259–0.649]. Allergic rhinitis symptom was positively associated with VDBP in males, aOR = 1.008, [1.001–1.015].
Conclusion: Farmers of the French region of Auvergne are not exempt from low levels of Vitamin D and indeed showed average levels below the range generally considered adequate for healthy individuals (>20 ng/ml) according to international expert societies. These low Vitamin D levels are associated with past-year regular cough and expectoration and obstruction of small airways. A high VDBP level is associated with allergic rhinitis.

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The frequency of HLA genes associated with celiac disease in Han populations from Jiangxi province in southern China

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Background: Celiac disease is an autoimmune disorder elicited in genetically predisposed individuals by the ingestion of gluten. In China, celiac disease might be more common than currently reported, especially in northern area, where wheat is the staple food. Jiangxi province lies in southern China, where rice is the staple food. However, the current positive rate of IgA anti-tissue transglutaminase antibodies, a serum marker for celiac disease, is 0.36% in the populations living in Jiangxi province. The date suggested that the risk of celiac disease was higher in Jiangxi than previously expected, which may be associated with the frequency of genetic predisposing gene for celiac disease.
Method: A total of 233 healthy Han individuals from Jiangxi province were enrolled in the study. Genomic DNA from their blood clot was isolated. HLA-DQA1 and DQB1 gene typing were performed with polymerase chain reaction- sequence specific primer (PCR-SSP) method, and HLA-DQ2.5 (DQA1*0501-DQB1*0201) and DQ8 (DQA1*0301-DQB1*0302) haplotypes associated with celiac disease were estimated using Arlequin, one human genetic data analysis software.
Results: The DQB1*0201 allele frequency was 9.7% in Han populations from Jiangxi province. The frequencies of the DQA1*0501-DQB1*0201 and DQA1*0301-DQB1*0302 haplotype were 3.9% and 6.0%, respectively, and the two haplotypes were more common among female (4.9% & 8.2%) than male (3.5% & 5.2%) without significant difference.
Conclusion: This work would firstly provide a strong support to estimate the risk of celiac disease in the populations living in Jiangxi province.

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Studies on the association of gene polymorphisms and positive SPT in the Lithuanian birth cohort

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Background: The prevalence of allergic diseases is steadily increasing across Europe. Significant differences in different populations have been observed, however, data from Eastern Europe are rather limited. This is the first study to examine single nucleotide polymorphisms (SNP) of certain allergy-associated genes in Lithuania.
Method: The participants of the study were separated into either cases or controls by the results of their skin prick tests (SPT). SPTs were performed with commercial allergens (*D. pteronyssinus*, *D. farinae*, cat, dog, grass mix, tree pollen mix, timothy grass, birch, mugworth, carrot, thistle, hazel, buckwheat, kiwifruit, egg, peanut, milk, shrimp, celery, apple, horse, codfish, soy) (ALK, Denmark) according to standard protocols. Based on data collected by previously published studies in Europe, 4 allergy-associated SNPs were selected. *FCER1A* rs2251746, *IL13* rs20541 and *IL13* rs1800925 were genotyped by allele-specific PCR and *CD14* rs2569190 was genotyped by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. Statistical analysis was performed with PLINK v1.07 software.
Results: A total of 92 children from the Lithuanian birth cohort 'Alrigen' (part of

the 'EuroPrevall' cohort, recruited in 2005–2006) were included in the study. There were 39 female (42%) and 53 male participants with a mean age of 5.2 years (range 4–7 years). Positive SPT to at least one of the allergens was determined in 35% (32/92) of the children; no significant differences were observed between the genders.

Positive SPT was associated with the presence of *IL13* rs1800925 T allele ($P = 0.03$; OR 2.64, 95% CI 1.07–6.54). The second SNP in *IL13* gene, rs20541, showed a tentative association with positive SPT results ($P = 0.05$).

Conclusion: Our study shows that positive SPT is associated with *IL13* rs1800925 T allele in a group of children from the Lithuanian birth cohort. Observed other tentative associations may lead to verifiable associations between allergy and its genetic risk factors among Lithuanian children in subsequent larger studies.

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Quantitative changes in allergen-specific IgE during young adulthood

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Background: Few studies have investigated longitudinal changes in atopic sensitization in a general adult population, and usually only looked at 'conversions' from positive to negative or vice versa, ignoring the quantitative nature of skin prick or IgE test results. Our objective was to assess whether levels of specific IgE to common allergens are stable during prolonged periods in young adulthood, and whether quantitative changes may be used as outcome parameters in population studies.

Methods: The Danish SUS cohort recruited in 1992–94 as 16–20 year old farming students and rural controls was examined twice with a 15 year follow-up period. Baseline and follow-up serum samples ($n = 1118$) were stored at −80°C, and tested in parallel in the same test runs for specific IgE against house dust mite, cat, and birch and grass pollen allergens, using the ADVIA Centaur (ALK Abellò ®) system and a sensitization cut-off point 0.35 kU/l. Various methods to express quantitative changes in specific IgE were explored: differences, ratios, or a change in an ordinal categorical variable (class). Preliminary analyses compared quantitative sIgE changes with work-related farming exposures.

Results: Allergen-specific IgE levels were remarkably stable: for mite and grass pollen allergens, 8.9% and 8.0% of the whole cohort were positive at both $t = 0$ and follow-up, and only 5.3% and 4.2% changed their sensitization status. Birch pollen and cat sensitization at $t = 0$ were less prevalent (5.0% and 2.2%), and conversion frequencies in the same order of magnitude. Among those with persistent sensitization the $t = 0$ and follow-up sIgE levels were significantly ($r = 0.56$–0.67) correlated and quantitative changes usually limited; 37% (mite), 48% (grass pollen), 27% (birch pollen) and 38% (cat) however showed a ≥3-fold increase or decrease in sIgE levels. Also in analyses of class changes (cut-offs 0.35, 1.0, 3.5, etc.) most remained in the same class, but substantial subgroups showed a more than one class increase or decrease at follow-up.

Conclusion: IgE sensitization patterns and levels in young adulthood appear to be remarkably constant during prolonged periods, but subgroups may show significant changes in levels of specific IgE. Inclusion of such increases or decreases in sIgE levels among consistently seropositive subjects – in addition to qualitative seroconversion changes – may increase the power of longitudinal analyses of atopy development.

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Presence of atopic diseases in Chagas' infected children

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Background: Chagas' infected children may develop a Th1 type immune response as it was reported, whereas allergic inflammatory diseases involve the opposite Th2 inflammatory pathway.

Objective: To determine whether Th1 response to intracellular parasites such as T. cruzi, could inhibit or reduce the response to allergens and consequent clinical expression of atopic diseases with Th2 profile.

Method: Cross-sectional study in children 4–17 years old, evaluating the presence of allergic diseases reported by parents and confirmed by investigator, both clinically and with sensitivity to environmental allergens. Besides, eosinophilia and parasitic diseases as well as socio- cultural level were considered.

They were divided into three study groups: children being born from Chagas' serologically positive mothers (+-), serologically positive children (++), and control group with negative serology both in children and their mothers (–). The analysis joined the 2 groups of positive serology vs the negative control group. Contingency tables for determining risk and Chi2 (Yates correction) were used, with significance level of <0.05.

Results: One hundred and ninety six children and adolescents, mean age 10.6 years and 50% of female and male were studied.

Groups analyzed showed no statistical significant differences on allergen sensitization and atopic diseases except for rhinitis (OR 0.23; IC 0.10–0.52. $P < 0.001$), and eosinophilia (OR 0.36; IC 0.18–0.72. $P = 0.003$). No significant socio- cultural differences have been found, as well as in IgE levels and parasites from samples obtained.

Conclusion: Present study gives to Chagas' history no protective role, neither against the current presence of allergic diseases nor to the characteristic sensitivity to aero – allergens in them.

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A survey exploring the knowledge of food handlers in the hospital restaurants and small commercial restaurants regarding their ability to identify the signs and symptoms of an acute food induced anaphylaxis and their emergency response towards a suspected allergic reaction

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Background: An acute anaphylactic reaction can be sudden and unexpected. The rapid identification of signs and symptoms followed by appropriate initiation of basic life support could be lifesaving. The Food Handlers (FH) are usually the first responders if a food induced anaphylaxis occurs in a restaurant.

Method: In this study the FH from both Hospital Restaurants (HR) and Small Commercial Restaurants (SCR) from the City of Darlington in the North East of England were asked to identify the signs and symptoms of a severe food allergic reaction and to describe their emergency response. A mixture of open and semi-structured questionnaires was administered to the FH in both HR and SCR group. A total of 49 FH responded (HR: $n = 20$ & SCR: $n = 29$).

Results: Total number of identified signs and symptoms were 83 from SCR and 45 from HR group respectively. The commonest identified symptoms were rash/ itching and flushing (25%) followed by breathing difficulty (14.6%) and facial swelling/puffiness (14.6%). Only 8% of the FH could identify diarrhoea and vomiting and interestingly less than 4.5% could identify collapse or shock as a sign of anaphylaxis. Some of the other unusual responses were high blood pressure, high blood sugar, sweating, pimples, fever and heart attack as signs of food allergy. When the emergency response of FH was analysed, it was found that in the HR group all responders had basic life support training as standard hospital policy and 55.56% (n = 10/18) followed a sequence of recovery position, shout for help, call emergency number and administer adrenaline compared to only 17.24% (n = 5/29) in the SCR group. In the SCR group, 8 out of 29 FH (27.59%) were happy to use an adrenaline auto-injector (AAI), if available during an emergency situation even without formal training.

Conclusion: On the basis of the above result it appears that the food handlers have some general understanding of signs and symptoms of an acute allergic reaction; however better understanding is necessary for swift initiation of basic management. It is recommended that FH should receive appropriate training on basic life support and AAI use to facilitate the management as soon as anaphylactic reaction is identified. The availability of AAI in the first aid box of the restaurants should be seriously considered if safe storage, usage and appropriate training could be ensured.

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Smart prevention for allergic diseases: GIS-based risk index and IT-based mobile monitoring

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Background: Over 10 million people suffer the adverse effects of allergic diseases in South Korea, causing the treatment expenses of about USD 600 million annually. As the development and exacerbation of allergic diseases are complex and multifaceted, the difficulty in identifying where the highest risk of allergic diseases occurs makes most of environmental health programs remain curative instead of preventive. The existing literature on allergic diseases have identified indoor and outdoor environmental risk factors but not considered the relative weight for each factor, or considered relative weights but not linked analysis to geographic location at disaggregated levels. Moreover, the effort to applying the IT-based smart technology to establish mobile monitoring systems for allergic diseases is still in its infancy.

Method: We used geographic information system (GIS) and spatial statistical modeling to analyze the household survey data collected in Seoul during 2014–15, including a series of indoor risk factors for allergic diseases. As for outdoor risk factors, since there are only 25 monitoring stations measuring the level of ambient air pollutants on a daily basis in Seoul, a spatial interpolation technique was used to correspond with the survey data. By integrating all relevant data using a GIS software, we were able to perform statistical analysis on all data layers together.

Results: As a result of the statistical analysis, we created risk index for allergic diseases for every household in the survey, along with district-level priority maps coded by the index covering the entire area of Seoul. The resulting maps use weighted risk factors to spatially locate modeled risk zones and highlight critical areas for targeted intervention. This GIS modeling approach can be combined with the IT-based mobile technology to develop a 'smart' monitoring system where the data on allergic disease risk factors could be collected from various mobile sources (e.g. social networking services, mobile sensors, etc.) and the information for prevention can be effectively delivered to all relevant policymakers and citizens.

Conclusion: This multi-disciplinary and technology-based approach enables environmental and public health policymakers to design and implement programs that protect people before they suffer from allergic diseases, and advance the scientific community's understanding of the spatial distribution and magnitude of allergic diseases.

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Prediction of the severity of food allergic reactions

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Background: Preventing severe allergic reactions is important to reduce the morbidity and mortality of food allergy. However, there are few known clinical factors predicting the severity of reaction (SOR) following accidental ingestion or during DBPCFCs. Furthermore, it is unknown whether severe reactions tend to occur at higher doses and whether limiting exposure would thus preferentially impact severe reactions.

Method: The study population consisted of children with a DBPCFC confirmed food allergy to milk, egg, peanut, cashew nut and hazelnut. The study data was collected from the food challenge unit database at the University Medical Center Groningen and analyzed using multiple linear regression analysis with factors known or thought to influence severity. The SOR was determined by using a 12-point severity score.

Results: 232 children with DBPCFC confirmed food allergy were included in the analysis. Using the enter method, a significant model emerged for predicting the SOR during the DBPCFC (R^2 = 0.168, $F_{6,225}$ = 7.568, $P \leq 0.001$). After associations were adjusted for the other determinants, significant predictors were: age (β = 0.197, P = 0.007), eliciting dose (ED) (β = 0.192, P = 0.012), specific IgE (β = 0.195, P = 0.004), SOR following accidental ingestion (β = 0.133, P = 0.036), DBPCFC with peanut (β = 0.238, P = 0.002) and cashew nut (β = 0.258, P = 0.001). No significant relationship with the SOR during the DBPCFC was found for sex, cumulative dose, DBPCFC with hazelnut, milk or egg, a history of atopic dermatitis, asthma or allergic rhinoconjunctivitis. The total explained variance of this model was 16,8% of the SOR, and the ED only contributed 2.1% after inclusion in the model ($R^2_{(\text{before including ED})}$ = 0.147). A significant model was also found for predicting the SOR following accidental ingestion (R^2 = 0.087, $F_{8,228}$ = 13.635, $P \leq 0.001$). Significant predictors were: specific IgE (β = 0.163, P = 0.011), the length of the time interval between ingestion of the food and the subsequent reaction (β = −0.178, P = 0.006) and SOR during the DBPCFC (β = 0.142, P = 0.028).

Conclusion: Reaction severity to foods is largely unpredictable. Although SOR during the DBPCFC is associated with a higher ED, the relatively marginal size of this effect suggests that the impact of dose limitation is unlikely to reduce severe reactions more than milder reactions.

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Costs of perennial allergic rhinitis and asthma increase with level of severity and level of disease control

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Background: Allergic rhinoconjunctivitis (ARC) is a common disorder, with a prevalence of 17–29% in European adults. ARC may cause significant discomfort, with impairment of daily activities, social interactions, school or work activities, and quality of life. Total direct medical cost of ARC is estimated to be around $3.4 billions in the US. Half of this cost is due to prescribed therapy. House dust mite (HDM) allergy (perennial ARC) is the major trigger of ARC, with around 90 million people affected in Europe, North America and Japan. HDM also induces asthma. Asthma affects around 6% of the French population with considerable Medical Resource Utilization (MRU). Our study aimed to detail MRU and related direct cost in Perennial Allergic Rhinitis (PAR), with or without concomitant allergic asthma (AA) in France.
Method: Using French Electronic Health Records (EHRs), we identified in 2010 two cohorts of patients, based on General Practitioners' diagnoses, prescribing and clinical data. The first cohort included patients with PAR but no AA, while the second cohort included patients with PAR and concomitant AA. For each patient, the medical record was linked to corresponding claims data with reimbursed MRU and costs between 2011 and 2013. In each cohort, sub-groups analyses were performed according to severity of rhinitis and level of asthma control.
Results: The mean total annual cost for a patient with PAR and no AA was 247€ in 2013. This varied from 202€ to 298€ depending on rhinitis severity. Medical consultations made up almost 50% of these costs, while allergy drug made up 10%. For patients with PAR and concomitant AA, the mean annual cost varied between 403€ and 550€, depending on the level of asthma control. Asthma drugs accounted for 20% and medical consultations for 35% of the costs.
Conclusion: This innovative study linking diagnoses from EHRs to claims data (MRU) was one of the first of its kind to be conducted in France, certainly in allergy. It collected valid data on PAR management, with or without concomitant AA, and on related costs. There was a clear relationship between MRU or associated costs, and severity of PAR and control of AA. Improved disease control for patients with PAR and AA would decrease the burden of disease.

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Remote sensing of phenology: a dynamic tool to inform allergenic grass pollen aerobiology

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Background: Grass pollen is a major aeroallergen globally to which exposure is expected to intensify with climate change. Accurate assessments of the sources, timing, and duration of grassland pollinating periods are critical for improved management of allergic rhinitis and to better understand future trends in allergenic pollen exposure. Conventional methods for monitoring airborne pollen are labour intensive, site-specific, and hampered by a sparsity of sampling sites. Satellite remote sensing offers an alternative method to overcome some of these constraints by virtue of its synoptic coverage and repeatability of measurements that enable land cover mapping as well as vegetation condition and phenophase monitoring.
Method: Land cover classes as well as satellite observations of greenness as an index of grass production dynamics and phenologies (start, peak, and end of growing season) were mapped within 5 urban centres and peri-urban surroundings across two hemispheres (in Australia and France). The satellite-based enhanced vegetation index (EVI) phenology profiles were related with in situ grass pollen count datasets spanning 5–12 years to assess the predictive capabilities of the satellite remote sensing for airborne pollen forecasting.
Results: Using general additive modelling strong predictive capabilities for forecasting periods of grass pollen release were found for both Australian and French sites, including sites dominated by temperate grass species and the Australian Sydney site with multiple grass peaks in airborne grass pollen correlating with the presence of subtropical, summer-flowering grasses.
Conclusion: Remote sensing of grass phenology revealed vital information on pollen sources for forecasting grass pollen aerobiology to aid management of public health risk.

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Use and limitations of the symptom load index as indicator for the allergy burden

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Background: The inclusion of symptom data besides pollen measurements is of growing importance in the field of aerobiology and tasks like pollen forecasting. Since clinical monitoring of pollen allergic patients is time- and cost consuming, the use of crowdsourcing services, which gather huge amounts of datasets, is advantageous to refer more directly to the status of human health.
Method: The symptom scores of entries in the free, online website www.pollendiary.com and the 'Pollen' App were normalized to values between 0 (= no symptoms) to 10 (= all and most severe symptoms) of a selected user (geographic) group in a given time frame. Those datasets, the symptom load index ('SLI') were compared to pollen measurements of the respective region (retrieved from the European Aeroallergen Network; http://www.ean-net.org) and historic allergen data (Bet v 1 and Phl p 5) of the European project 'Health Impacts of Airborne Allergen Information Network' (HIALINE) from 2009 to 2011.
Results: The daily SLI follows the daily pollen concentrations during the season as a rule, although the SLI is less variable. Calculations for whole seasons show a more complicated situation and demonstrate also higher SLIs with lower total pollen loads. Comparisons of the SLI with allergen data present better correlations than with pollen data in general. A region dependent outcome can be shown for all observations.

Conclusion: The SLI as average of a large number of datasets is a useful indicator for the burden of pollen allergy sufferers. It is useful to observe the onset and development of allergenic reactions during the season within a population, to forecast symptoms and to assess the impact of a pollen season. Limitations comprise missing knowledge about the trigger of the burden and flowering phases, which are documented by pollen monitoring. Both sources are complementary and recommended to reveal the full impact of pollen allergies.

Poster Discussion Session PDS 17

Pediatric asthma

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Visual analogue scale administered to caregivers can be used to assess rhinitis severity in preschool children

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Visual Analogue Scale (VAS) is a simple tool that can be used to assess rhinitis severity in adults; however, its use in preschool children has not been studied.

Aims:

1 To assess if caregivers of preschool children with current rhinitis could accurately fill in the VAS on rhinitis severity.
2 To describe VAS results.
3 To evaluate the level of VAS depending on the ARIA classification.

Methods: A cross-sectional, nationwide, population-based study including a representative sample of 5018 Portuguese children aged 3–5 years was performed in 2007. Data was collected by face-to-face interview to caregivers using an adapted ISAAC questionnaire. In this post-hoc analysis, we included 2179 children with at least one nasal symptom in the previous 12 months, considering sneezing and/or itchy nose, rhinorrhoea or blocked nose without having a cold/flu. The questionnaire included a VAS to classify rhinitis severity (0 to 10 – lowest to highest severity, respectively). Rhinitis was also classified according to ARIA; its impact in the daily activities was assessed using a 4-point scale: 'not at all', 'a little' [mild rhinitis], 'a moderate amount' or 'a lot' [moderate-severe rhinitis].

Results: According to ARIA, 69% of children had mild intermittent rhinitis, 6% had mild persistent, 17% moderate-severe intermittent and 8% moderate-severe persistent disease. Ninety five percent of the participants ($n = 2078$) were able to fill in the VAS, with a median(interquartile range) VAS score of 3.0(4). The median VAS score increased with the number of nasal symptoms: 2[3] in children with one symptom vs 4[4] in those with two vs 6[3] in those with all rhinitis symptoms ($P < 0.001$). Children with moderate-severe persistent rhinitis had the highest VAS score (7[3] vs 6[3] in moderate-severe intermittent vs 5[3] in mild persistent vs 2[3] in mild intermittent; $P < 0.001$). VAS was more accurate in the evaluation of rhinitis severity (mild vs moderate-severe; AUC, area under the curve = 0.903) than rhinitis persistency (intermittent vs persistent; AUC = 0.766; $P < 0.001$). Using a cut-off of >4 cm, VAS (vs severity according to ARIA) had a sensitivity of 86%, specificity of 81%, positive predictive value of 82% and negative predictive value of 85%.

Conclusion: Visual analogue scale administered to caregivers can be used to assess rhinitis severity in preschool children. However, longitudinal studies (to evaluate its reproducibility and further assess validity) should be performed.

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Effect on FEV1 of salbutamol administered in obese and non- obese children without asthma to assess bronchial reversibility

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Background: Obesity and overweight are considered a risk factor for impaired lung function, the objective of this study was to determine the effect of salbutamol in VEF1 administered in obese, overweight and eutrophic children without clinical evidence of asthma.

Method: A prospective study was performed in patients 8–16 years of age with no clinical history of asthma and no history of respiratory tract infection in the last 6 weeks. Patients were integrated into three groups according to the presence or absence of obesity and / or overweight according to their anthropometric measurements by BMI taken by reference tables in the World Health Organization (WHO), After a complete medical history and vital signs stable and a spirometry was performed according to the standards of the American Thoracic Association (AAT); after the first spirometry the administration of nebulized salbutamol was made after foregoing, a second spirometry was performed. Patients who were confirmed positive reversibility (>12% variability FEV1), will continue to follow through outpatient Allergy Service. Patients who had a previous diagnosis of asthma or other lung diseases were excluded.

Results: We included 182 patients whose average age was 10.23 ± 2.4 years old, distribution of patients after BMI assessment were: 60 obese (BMI 29.03 ± 4.3 kg/m^2), 56 overweight (BMI 22.4 ± 2.6 kg/m^2) and 66 eutrophic (BMI 17.4 ± 2.1 kg/m^2), no significant differences in FEV1 baseline quantification in all three groups, the mean FEV1 of the groups was: Obese 2.33 ± 0.63 l/s, Overweight 2.43 ± 0.89 l/s and eutrophic 2.35 ± 0.89 l/s. After assessing bronchial reversibility in the second spirometry there were differences in the three groups in the variablity percentage; in obese, the average was 13.43 ± 11.2%, overweight 7.7% ± 5.6% ± 5.46 and 6.11 eutrophic. There were statistically significant differences in the percentage of variability when comparing eutrophic patients group with obese patients ($P = 0.018$).

Conclusion: Over 23% of obese patients had asymptomatic presence of bronchial obstruction at the time of the study, significant differences with eutrophic patients. Comparing our results with similar studies in asthmatic children obese and non-obese; non-asthmatic obese patients included in our study show a similar FEV1 and percentages of non-obese asthmatics variability, which could hypothesize a 'degree' of asymptomatic bronchial obstruction in obese patients.

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Clinical symptoms and immune system activation in patients with atopic and nonatopic asthma

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Background: Aim of this study was to examine pathogenesis of immune system

activation in patients with atopic and non-atopic asthma and their influence on clinical manifestations and tissue remodelling.
Method: Immune system of 140 children suffering asthma was analysed by the means of atopy presence (total IgE, specific IgE, eozinophilia, eczema, family record of atopy), possible prenatal damage of the immune system (spontaneous abortions or deadborns during mother's previous pregnancies, intrauteral growth retardation, anomalies), autoimmune and other chronic diseases, secondary anaemia, expression of CD3, CD4, CD8, CD56, CD25, DR ab and gd TCR on the peripheral blood mononuclear cells, large granular cells (LGL), the presence of monocytes, virocytes and neutrophiles in the peripheral blood, LDH, CPK, AST or ALT as parameters of ongoing cell damage and oxidative burst of peripheral PMNC determined as spontaneous or PMA stimulated NBT test.
Results: Patients with atopic asthma (114) had no signs of other but allergic diseases, family atopy in 96%, eosinophilia in 85%, increased level of specific IgE (dermatophagoides 82%, home dust 89%, pollen 10%), and slightly depleted CD3 and CD4 positive cells, spontaneous and PMA stimulated NBT test comparing to control, increased number gd TCR, while LDH, CPK, AST and ALT were normal. Patients with nonatopic asthma (26) had common extrapulmonal clinical manifestations (vasculitis, autoimmune diseases, secondary anaemia, anomalies, CNS manifestations, lymphadenopathy, splenomegaly), common prenatal risk factors for developmental dysfunctions of the immune system (spontaneous abortions or deadborns during mother's previous pregnancies, intrauteral growth retardation, anomalies), low level of IgE, higher percentage of monocytes, virocytes, LGL, CD8, DR, CD56 and CD25 positive cells, while CD3, CD4, gdTCR positive cells were depleted, increased level of LDH, CPK, AST or ALT and severe down regulation of spontaneous and PMA stimulated NBT test.
Conclusion: Inflammatory process in patients with nonatopic asthma seems to be Th1 mediated, characterised by extrapulmonal manifestations and more severe, systemic tissue remodelling, while in atopic asthma the disease is Th2 mediated, manifesting in atopic children. It looks that inflammation in atopic children is less harmful, leading to milder clinical manifestations, less pronounced tissue remodelling, developmental and metabolic dysfunctions, compared to nonatopic ones.

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Spirometry adjusted fraction of exhaled nitric oxide performs better detecting uncontrolled asthma in children

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Background: Exhaled nitric oxide (eNO) and lung function have been show to provide complementary information when assessing asthma control. However, no studies assessed their combined value. We aimed to assess the spirometry adjusted fraction of eNO ability to distinguishing between controlled and uncontrolled asthma in children.
Method: A random sample of 858 children from 20 primary schools in Oporto was screened by a health questionnaire, physical examination, spirometry with bronchodilation test (BDT) and eNO. Each subject's weight and height were measured, lung function was performed using a MIR Spirobank, with WinSpiroPRO software and eNO was analysed using a BedFont NOBreath FENO monitor. Children previously diagnosed with asthma by a physician were selected. Exclusion criteria were inability to correctly perform spirometry or eNO. Uncontrolled asthma was defined as an increase of at least 12% of the forced expiratory volume in one second (FEV1) after BDT. Receiver operation characteristic (ROC) analysis was performed, to evaluate the ability of eNO to detect uncontrolled asthma, using three diferent equations of predicted values for eNO in children (Malmberg, Kovesi and Buchvald), the ATS clinical practice guideline for interpretation of eNO levels and proposed indices with eNO adjusted for spirometry.
Results: After applying the exclusion criteria ($n = 16$), a total of 61 children were included, 28 (45.9%) were girls and 6 (9.8%) had uncontrolled asthma. The mean (SD) age was 8.8 (0.8) years and 136.0 (6.6) cm. The median (IQV) eNO was 19.0 (30.5) ppb and mean (SD) FEV1 1.77 (0.32) L. Absolute measured eNO and cut-off points suggested by ATS failed to detect uncontrolled children (AUC = 0.674 $P = 0.164$ and AUC = 0.626 $P = 0.315$ respectively), as well as the equations suggested by Malmberg, Kovesi and Buchvald (AUC = 0.676 $P = 0.115$, AUC = 0.679 $P = 0.190$ and AUC = 0.633 $P = 0.287$ respectively). The ratios eNO/FEF2575 (AUC = 0.770 $P = 0.031$; 11.8 ppb/l/s allowing 83% sensitivity 66% specificity) and eNO/FEF75 (AUC = 0.770 $P = 0.031$; 24.3 ppb/l/s allowing 83% sensitivity and 69% specificity) evidenced a good performance in detecting uncontrolled children, and performed better than the eNO/FEV1 ratio (AUC = 0.736 $P = 0.059$).
Conclusion: Spirometry adjusted fraction of eNO performs better assessing asthma control in children. Therefore, in spite of more validation studies needed, we suggest its use on the routine clinical assessment in children with asthma.

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Potential clinical insights of $FEV_{0.75}$ measurement in preschool children lung-function assessment

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Background: In preschool children (3–5 years), airways obstruction assessment by standard spirometry is limited by expiratory manoeuvre exequibility and by intrinsic age-dependent limitations. We hypothesize that $FEV_{0.75}$ (forced expiratory volume at 0.75 s) is a more reliable measure of bronchial obstruction in this age range when compared to FEV_1.
Methods: Analysis of functional data obtained from the expiratory curves of 101 children aged 3 to 5 years-old assessed with a Jaeger® MS-IOS spirometer, between Jan/11 and Jun/15. 64 (63.4%) children had clinical asthma and presented similar characteristics to the non-asthmatic children (59.4% vs 56.8% males, $P = 0.80$; mean ± SD BMI z-score 0.72 ± 1.34 vs 0.60 ± 1.50, $P = 0.68$). Flow-volume and volume-time curves were visually inspected for acceptability criteria and disregarded if not fulfilling set parameters or if presenting a restrictive pattern. Bronchodilator responsiveness was assessed after medication with 400 μg of salbutamol. Data are expressed as z-scores (GLI-2012) or Δ%.
Results: Baseline FEV_1 and $FEV_{0.75}$ correlated positively ($r = 0.94$, $P < 0.001$). On baseline, the mean ± SD FEV_1/FVC ratio was significantly lower in the asthma group (−0.56 ± 1.23 vs 0.04 ± 1.06, $P = 0.01$), but not the $FEV_{0.75}$/FVC ratio (−0.79 ± 1.14 vs −0.43 ± 0.99, $P = 0.11$). A reduced FEV_1/FVC ratio (<−1.64) was observed in 16 cases (93.8% in the asthma group, $P = 0.006$) and a reduced $FEV_{0.75}$/FVC ratio (<−1.64) in 21 children (76.2% with asthma, $P = 0.17$). There was also a positive correlation between Δ%FEV_1 and

$\Delta\%FEV_{0.75}$ (r = 0.93, $P < 0.001$), and both parameters were significantly higher in the asthma group: median [IQR] $\Delta\%FEV_1$ (10.50 [3.00–15.00] vs 3.00 [1.50–8.00], $P < 0.001$) and $\Delta\%FEV_{0.75}$ (12.61 [5.03–18.49] vs 4.96 [1.84–10.82], $P = 0.003$). $\Delta\%FEV_1$ demonstrated to be a fair test in predicting clinically suspected asthma, with an area under the ROC curve of 0.72 ($P < 0.001$), unlike $\Delta\%FEV_{0.75}$ that performed poorly (AUC 0.68, $P = 0.003$). For the highest specificity an increase of at least 15% in FEV_1 is suggested as the cut-off, while for $FEV_{0.75}$ a minimum increase of 21% would be necessary.

Conclusion: In this preschool children sample, $FEV_{0.75}$ has no advantage over FEV_1, performing slightly lower than the last, but might be acceptable when FEV_1 is not reached, with the two measurements presenting a high concordance.

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Measuring $FEF_{25–75\%}$ and $FEF_{75\%}$ has limited usefulness in lung-function assessment of preschool and young school-age children

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Background: Forced expiratory flows at lower lung volumes might be more adequate at detecting small airways involvement in early childhood asthma. Our aim was to ascertain potential insights given by $FEF_{25–75\%}$ and $FEF_{75\%}$ measurements over FEV_1 and FEV_1/FVC in young children functional assessment, as asthma predictors.

Methods: Longitudinal study with analysis of spirometry and clinical data of children aged 3–8 years old, consecutively evaluated between Jan/11 and Aug/15. All flow-volume and volume-time curves were visually inspected for acceptability criteria and those not fulfilling set quality parameters or exhibiting a restrictive pattern were excluded. Bronchodilator responsiveness (BDR) was assessed after 400 μg salbutamol. Data are expressed as z-scores (GLI-2012) or Δ% and were analysed using IBM SPSS Statistics.

Results: Spirometry was performed in 434 children (59.7% male) – 282 (65.0%) with physician-diagnosed asthma. Based on reduced FEV_1/FVC z-score (<−1.64), 69 (15.9%) spirometries were classified as obstructive (23.0% of children with vs 2.6% without asthma diagnosis, $P < 0.001$), while 21 (4.8%) had obstruction affecting only the small airways, with $FEF_{25–75\%}$ z-score <−1.64 (6.0% of children with vs 2.6% without asthma diagnosis, $P = 0.12$). A significant $FEF_{25–75\%}$ BDR ($\Delta FEF_{25–75\%}$ ≥30.0%) was observed in 70 (23.2%) of the 302 patients with normal FEV_1/FVC and negative FEV_1 BDR (ΔFEV_1 <12.0%), only 3 of them with reduced baseline $FEF_{25–75\%}$ z-score. When asthma functional diagnosis criterion of a positive FEV_1 BDR (ΔFEV_1 ≥12.0%) was absent ($n = 327$), ROC curves demonstrated that baseline z-scores of $FEF_{25–75\%}$ or $FEF_{75\%}$, or $\Delta FEF_{25–75\%}$, were unable to predict physician-diagnosed asthma (AUC: 0.59, 0.58 and 0.52, respectively).

Conclusion: $FEF_{25–75\%}$ and $FEF_{75\%}$ z-scores, as well as $\Delta FEF_{25–75\%}$ have limited usefulness as asthma predictors over established parameters, in the studied age group.

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Spirometry in preschool children: is FEF25–75 a strong outcome to assess bronchodilator responsiveness?

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Background: Preschool spirometry has been used to assess lung function in asthmatic children. In a recent study published by our group, it was documented the importance of an adapted criteria for the bronchodilator challenge test (BDR) adjusted to this age group (FEF75 > =14%), to avoid asthma over-diagnosis in children with non-atopic recurrent wheezing (RW) or with chronic cough (CC). The importance of mid-expiratory flows (FEF25-75) in asthmatic pre-schoolers is controversial. Data about the importance of the variation of mid-expiratory flow rates according to the BDR results are lacking.

Objective: To compare spirometric parameters in pre-schoolers accordingly to their clinical diagnosis and variation of FEF25-75, in relation to the BDR test.

Method: Spirometry was performed in pre-school children between January and July of 2015, with clinical diagnosis of asthma, CC and RW. The variation of FEF25-75 in relation to BDR response was recorded and classified has being positive (FEF0.75 > =14%), 'partially positive' (FEF0.75 > =12 and <14%) or negative (FEV0.75 <12%).

Results: We included 231 pre-schoolers (median age 4 (2; 6) years, 55% male gender), 65 were diagnosed with asthma, 85 with RW and 81 with CC. Success rate to achieve FEV1 according to clinical diagnosis was similar in the 3 groups ($P = 0.506$; chi-square test). No significant statistic's differences were found in the 3 groups when compared the variation of FEF25-75 and BDR (FEF0.75 > =14%, $P = 0.832$; FEF0.75 > =12 and <14%, $P = 0.927$; FEF0.75 <12%, $P = 0.172$; chi-square test).

Conclusions: FEF25-75 variation, apart from the FEVt variation criteria, doesn't allow to discriminate children with asthma, RW and CC.

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Bronchodilator test: should it be performed regardless of the baseline FEV_1 value?

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Introduction: According to GINA poorly controlled asthma is associated with greater variability in lung function than well controlled asthma. Excessive variability may be identified by a reversibility test, which is routinely performed regardless of the baseline FEV_1 value. There is a lack of data regarding which baseline spirometric parameters could better discriminate patients with a positive bronchodilator response.

Aim: We aimed to assess the accuracy of the baseline FEV_1 percent predicted value (FEV_1%) and FEV_1/FVC ratio, in discriminating patients with a positive bronchodilator response. Additionally, we aimed to determine the optimal cut-off value of baseline FEV1% and FEV_1/FVC ratio for a positive bronchodilator test.

Methods: We conducted a cross-sectional retrospective study that included pediatric patients (6–17 years) assessed in our department by spirometry and bronchodilator test, from January 2013 to June 2015. A positive bronchodilator response was considered if there was a 12% improvement of the FEV_1 after bronchodilator. Accuracy and optimal cut-off values were studied by ROC analysis, for baseline FEV_1% (Global Lung Initiative – GLI – 2012 reference equations) and FEV_1/FVC ratio.

Results: We included 362 patients, with a mean age of 12.6 years (SD: 2.9), predominantly male (68%). The mean FEV_1% and FEV_1/FVC ratio were 96.3% (SD: 14.4%) and 0.81 (SD: 0.08), respectively. FEV_1% presented an AUC of 0.76 and its best discriminative value was 88.4% (sensitivity of 56.5% and specificity of 83.3%). For

FEV_1/FVC ratio the AUC was 0.89 and its optimal cut-off value was 0.78 (sensitivity of 83.7% and specificity of 84.1%).
Conclusions: In our sample, the FEV_1/FVC ratio was more accurate than FEV_1% to discriminate patients with a positive bronchodilator test. In pediatric patients with a FEV_1/FVC ratio above 0.78 the non-execution of the bronchodilator test might be considered.

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Annual change of airway hyperresponsiveness about asthmatic children in long term remission cases

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Aim: We reported the characteristics and clinical usefulness of airway hyperresponsiveness examination about asthmatic children at EAACI 2014 and EAACI 2015 congress. This time we studied the annual change of airway hyperresponsiveness, respiratory function and serum IgE about asthmatic children who were in remission state (no symptom and no therapy) for 1–5 years.
Subjects and methods: Remission cases of asthmatic children were studied for 1–5 years annually. We examined acetylcholine inhalation test by standard method, and the data of respiratory threshold of acetylcholine (RT-Ach) was used. FEV1%, and serum IgE level were examined for 5 years annually.
Results: Mean age of 25 cases after remission at 1 year was 12.3 years old. Male to female ratio was 1.2. They have been followed up 1–5 years annually. Geometric mean of RT-Ach at 1 year after remission was 1900 μg/ml, 2 year was 3250 μg/ml, 3 year was 4500 μg/ml, 4 year was 5000 μg/ml and 5 year was 7500 μg/ml. The mean FEV1% was 85% at 1 year after remission, 2 year was 88%, 3 year was 93%, 4 year was 90% and 5 year was 85%. There data were within normal range during 5 years. Geometric mean of serum IgE level at 1 year after remission was 290 IU/l, 2 year was 320 IU/l, 3 year was 280 IU/l, 4 year was 410 IU/l and 5 year was 380 IU/l. There were not different significantly during 1–5 years after remission. Complicated cases of atopic dermatitis decreased by age, but the incidence of allergic rhinitis did not change significantly during 5 years.
Conclusion: In spite of the remission state more than a year, airway hyperresponsiveness existed in some cases of asthmatic children. After remission, RT-Ach improved year by year gradually. FEV1% was kept in normal range for 5 years after remission. But serum IgE did not decreased to normal level during 5 years of remission. These data suggest that airway hyperresponsiveness improves gradually, but allergic tendency exists for long term in spite of the remission state of asthma.

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Fractional exhaled nitric oxide and atopy in children with asthma

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Background: FeNO (fractional exhaled nitric oxide) is non-invasive marker of eosinophilic airway inflammation and is elevated in asthma. FeNO levels correlate with presence of atopy. The aim of this study was to asses the correlation between FeNO, total serum IgE and mono and poly- allergic sensitization in steroid naïve asthmatic children.
Method: Thirty children with asthma (17 male, 13 female), age 5–15 years, and thirty healthy children (18 male, 12 female), age 5–15 years, were included in the study. Recent respiratory infections were negative in all groups. Healthy group was negative for atopy. Total of sixty children underwent FeNO measurement according to European Respiratory Society/American Thoracic Society recommendations. Skin prick testing on four classes of aeroallergens (house dust mites, animal danders, pollens, molds) and evaluation of total serum IgE were performed in asthma group. The children with asthma were divided in two groups: mono and poly-sensitized.
Results: FeNO levels were significantly higher (Kruskal-Wallis test) in asthma group (C = 47.45) vs healthy control (C = 6.25). Significant difference between FeNO in these two groups (Mann-Whitney test) was found ($Z = 6.65$; $P = 0.0001$). FeNO values were significantly higher in poly-sensitized vs mono-sensitized children with asthma ($Z = -3{,}123$; $P = 0.001$). A signifiicant positive correlation was found between FeNO and increasing number of allergen sensitization ($r = 0.649$; $P < 0.01$). Significant correlation between FeNO and total serum IgE was not found. Significant correlation between total serum IgE and the increasing number of skin prick reactions was not found.
Conclusion: Children with asthma have significant higher FeNO values vs healthy control. There is significant difference in FeNO between mono and poly-sensitized asthmatic children. FeNO levels positivly correlate with increasing numbers of allergen sensitization and don't correlate with total serum IgE. The increasing numbers of allergen sensitization do not lead to increment in total serum IgE.

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Omalizumab efficiency in severe non-allergic asthma with eosinophilia and nasal polyposis

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Background: Severe non-allergic asthma with eosinophilia is a particular phenotype, scarcely described in children, and for which prolonged systemic corticosteroids is often required, with many side effects. Some recent studies suggest potential benefits of omalizumab in non-allergic severe asthma phenotypes. The purpose of this study was to assess the effectiveness of this drug in non-allergic GINA step V asthma patients.
Method: This study was single-center, observational. Non-allergic asthma patients experiencing uncontrolled symptoms despite high doses of inhaled corticosteroids were enrolled. Due to the severity of their symptoms and the lack of control, treatment with Omalizumab was started according to a weight and total IgE rate adjusted dosage. Blood eosinophilia, forced spirometry and exhaled fraction of nitric oxide were measured at entry, after 16 weeks of treatment, and at the end of the follow up. Treatment's efficiency was assessed on clinical improvement and evolution of biological markers.
Results: The subjects were 7 children (3 female), aged 7–13 years, with the following mean values: IgE: 204 ± 213 IU/ml; eosinophils: 1182 ± 624/mm^3; FEV1/FCV: 79 ± 13 percent; NO: 95 ± 49 ppb. Evolution under treatment by Omalizumab was favorable to 5 patients, illustrated by a better control of asthma and corticosteroid sparing. For these patients, eosinophilia was significantly reduced (1355 ± 626 before, vs 479 ± 161 after treatment, $P = 0.01$). We found no significant changes in spirometry or NO values. For one patient, improvement was partial, followed by secondary therapeutic escape. One patient showed no improvement under treatment. In our series, one patient had severe eczema associated, one had vernal

keratoconjunctivitis, and four children had nasal polyposis. Every child suffering from nasal polyposis had a favorable outcome under treatment. Conversely, among the three children without poylyposis, only one was treated successfully.

Conclusion: Some recent studies suggest potential benefits of Omalizumab in adult non-allergic severe asthma phenotypes. We present here the first study evaluating efficiency of this treatment in a pediatric population of non-allergic severe asthma patients. The mechanisms of action of Omalizumab in this asthma phenotype are not yet understood, but its efficacy on reducing eosinophilic inflammation has been shown. In our series, children with nasal polyposis seemed to be good responders to the treatment by Omalizumab.

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Magnesium deficiency correction for improvement of children bronchial asthma treatment results

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Background: Hypomagnesiemia was associated with more severe asthma disease and more frequent asthma exacerbations.

Method: There were included 75 uncontrolled asthma children (13.4 ± 2.8 y.o.) with low Mg erythrocyte level (<1.65 mmol/L). They were stratified (according to GINA step therapy) and randomised by 3 groups of 6-month treatment:

- Group 1 (Mg erythrocyte level < 1.65 mmol/l) – basic treatment and Magne B6® forte tablets (400 mg/day in terms of Mg^{2+}, 30 days).
- Group 2 (Mg erythrocyte level < 1.65 mmol/l) – basic treatment.
- Group 3 (Mg erythrocyte level > 1.65 mmol/l) – basic treatment.

Main estimated parameters in a 3-month treatment were assessment of asthma control level, non-symptomatic day rate, rescue medication requirement, asthma exacerbation frequency and FeNO level.

Results: Mg erythrocyte level were increased by 17.9% ($P < 0.05$) in the group 1 compared with the initial value in a 1 month of treatment. But in a 3 months it returned to the initial level. There were no significant changing of V1, V3 and V6 Mg erythrocyte levels in the group 2 and group 3. There were clinical significant FeNO level decreasing in all groups compared with the initial values at V1-V6. FeNO at group 1 was significantly lower compared with groups 2 and 3 in a 8 weeks of treatment. In a 16 weeks FeNO level at group 2 was higher then other groups. There were no significant difference of FeNO levels between groups 1 and 2 at V6.

Group 2 as-needed medication volume was higher (77 puffs) vs group 1 (50 puffs) and group 3 (57 puffs) for the whole study period. ACQ-5 test results were decreased significantly compared with V0 levels in all groups at all visits. There were clinical significant difference of ACQ-5 results between group 2 and groups 1 and 3 only at V2.

The proportion of uncontrolled patients was three times higher in group 2 compared with group 1 in a 12 weeks and two times higher in a 24 weeks of treatment.

In a 6 month of treatment the proportion of 12-week ongoing asthma control patients (12/25) of group 1 were significantly better vs group 2 (7/25). Asthma exacerbation frequency was lower (7/25) for group 1 and group 3 (3/25) patients compared with group 2 (14/25) for the whole study period.

Conclusion: Mg deficiency correction (by 1 month Magne B6® forte tablets 400 mg/day in terms of Mg^{2+}) of uncontrol asthma children with low Mg erythrocyte level (<1.65 mmol/l) can improves astma control and decreases asthma exacerbation rate.

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How much drug leaves the spacer? *In vitro* study measuring drug output from five different valved holding chambers with and without facemask

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Background: Younger children aged between 0–6 years need to use valved holding chambers (VHC) with or without facemask for drug administration via a pressurized metered dose inhaler (pMDI). Evaluating different VHC and their facemasks is important in order to maintain safe and reliable asthma treatment to children. This study is performed using a well-established *in vitro* model to obtain reliable data about drug output from different VHCs.

Method: Measurements were performed with a breathing simulator (tidal volume 75 ml, 30 breaths per/min) and two different soft face models (casts of a 1 year resp. 4 year old child). Five different VHCs (Aerochamber plus, InspiraChamber, L′Espace, Optichamber Diamond and Vortex) were placed in a custom-built horizontal test rig. 250 µg Fluticasone propionate (FP) pMDI was actuated at the start of inhalation and 5 breaths were performed. Measurements were conducted without facemasks as well as with infant and toddler size facemasks (0.5 kg pressure to the face). In order to evaluate leakage of the different facemasks, we also performed measurements with facemasks completely sealed to the face using modeling clay. Each scenario was tested in quadruplicate. Levels of FP on filter pads placed within the mouth cavity of the model were analyzed through HPLC.

Results: Drug output varied significantly between different VHCs. The mean filter deposition of FP ranged between 68 and 221 µg for the different VHCs without facemasks, between 8 and 77 µg with facemasks and between 22 and 71 µg for sealed facemasks. All the tested VHCs showed significantly better drug output without a facemask. The mean filter deposition of FP for all the tested VHCs without facemasks was 134 µg, with facemasks 25 µg, and with sealed facemasks 46 µg. Many of the facemasks had problems fitting tightly to the face and subsequently had a much better output when the mask was sealed.

Conclusion: The design of VHC and the facemasks affected drug output significantly. It is important to choose a tight fitting mask and thoroughly instruct parents on inhalation technique. This study also supports taking away the facemask as soon as the child is able to perform the correct inhalation technique ensuring a higher drug output.

Poster Discussion Session PDS 18

Diagnosis and classification of drug allergies

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A new classification option for NSAID hypersensitivity: Kalyoncu classification

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Background: The European Network for Drug Allergy (ENDA) interest group proposed a consensus document for hypersensitivity reactions to nonsteroidal anti-inflammatory drugs (NSAIDs) in 2011. A group of patients with asthma and/or rhinitis experience urticaria/angioedema type reactions in response to NSAIDs, and this group is not defined in ENDA classification. Asthmatic patients with urticaria/angioedema type reactions in response to NSAIDs was named as having Pseudo Samter's syndrome, and there is incomplete forms of the disease; in such cases rhinitis/rhinosinusitis/nasal polyposis can accompany NSAID hypersensitivity (NH) without asthma (1, 2). Kalyoncu classification was proposed to categorize patients with NH.
Objective: To compare the ENDA classification with the Kalyoncu classification.
Method: A total of 195 patients with a history of NH were included in this study. NH reaction patterns were classified as follows: asthma, rhinitis, urticaria, angioedema, and anaphylaxis. Based on reaction history and oral provocation test results, patients were categorized according to both the ENDA and Kalyoncu classifications.
Results: Of 195 patients, 130 were female (66.3%) and the mean age was 40.32 ± 13.28 years. The most common NH subgroups were NSAIDs-exacerbated respiratory disease (NERD) (32%), and isolated NH (34.2%), and the least common NH subgroups were single NSAID-induced delayed reactions (1.5%), and pseudo Samter's syndrome (11.7%) under the ENDA and Kalyoncu classifications, respectively.
Conclusion: We found that the Kalyoncu classification is more descriptive of NERD patients showing urticaria/angioedema type reactions. According to Kalyoncu classification, Samter's syndrome develops over time in patients with incomplete Samter's syndrome or pseudo Samter's syndrome. This transition of groups towards Samter's syndrome can be determined by clinical observations. The inclusion of family history (genetic background) in Kalyoncu classification may also provide future risk assessment for progression to Samter's syndrome. For controversial cases and to gain projections for the future, Kalyoncu classification can be used as a new complementary option alone or in conjunction with ENDA classification. Currently, a study of the change over time using this new means of classification, risk analysis, and risk factors for the development of Samter's syndrome is ongoing.

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Clinical characteristics and challenge-proven diagnosis of paracetamol and ibuprofen hypersensitivity in a cohort of paediatric patients from the UK, between 2009 and 2015

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Background: Paracetamol and Ibuprofen are both important drugs consumed worldwide. They are involved in hypersensitivity drug reactions, which are often poorly studied in children. Different mechanisms are implicated, which can be IgE- or T-cell mediated. Given the importance of a correct diagnosis, a drug provocation test is often required to establish diagnosis and provide alternative choice of medication.
Method: We analysed a group of 11 children, who underwent a total of 12 drug provocation tests (DPT) with a history of hypersensitivity to paracetamol or ibuprofen, referred to the paediatric allergy team at St Thomas' Hospital, in London, between 2009 and 2015.
Results: Presenting symptoms to the allergy clinic included: angioedema in 45% of cases, urticaria in 45% and anaphylaxis in 9%. The average age at diagnosis was 10 years old. Over a third of children were atopic, and two of them suffered from spontaneous chronic urticaria. Using DPT, 81% were confirmed as having hypersensitivity to either paracetamol or ibuprofen, 89% being immediate reactions. Of the 12 DPT performed, 9 were positive (56% to ibuprofen; 44% to paracetamol), after a median cumulative dose of 504.15 mg. There were 3 negative challenges (all to ibuprofen).
Conclusion: In this studied group, immediate type reactions to paracetamol and ibuprofen were the most frequent type of hypersensitivity reaction. Ibuprofen was most often involved, and urticaria/angioedema the most commonly observed clinical presenting symptoms. No cross-reactivity to paracetamol was found. Subjects reacted after a cumulative dose intake of less than 600 mg. Adrenaline was required once. DPT, was considered a safe approach that is needed not only to confirm the diagnosis of drug hypersensitivity, but to also provide an alternative drug choice for paediatric drug-allergic patients.

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Patients' evaluation of hypersensitivity reactions to NSAIDs with visiual analogue scale

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Background: Hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most common drug hypersensitivities. NSAID hypersensitivity (NH) may affect 1–2% of the general population and it is an important public health problem that affects medical prescriptions and practices. It has also an influence on the quality of patients' daily lives.
Aim: Primary aim was to understand to what extend a patient feels, or perceives the symptoms and complaints during a NH reaction and to grade it with visual analogue scale (VAS). Secondary aim was to investigate whether there was a difference between NSAID groups in terms of reaction type and severity.
Method: A total of 174 patients with a diagnosis of NH were evaluated between September 2014 and September 2015 in our outpatient allergy clinic. NH reactions were classified as asthma/rhinitis, urticaria/angioedema (u/ae), mixed reaction (asthma/rhinitis and u/ae together), anaphylaxis and delayed hypersensitivity

reaction. Patients were asked to evaluate the severity of each drug reaction by using VAS.

Results: Among 174 patients (115 female, mean age 39.12 ± 12.34 years) propionic acid group was the leading cause of hypersensitivity reactions. Only 3% of the reactions were reported to be mild and all of those mild reactions were u/ae type whereas, 92% of anaphylactic reactions were severe. Most of the reactions were in u/ae type. Acetic acid group was the leading cause of anaphylactic reactions.

Conclusion: NSAIDs cause reactions of different types with different levels of severity. These reactions are perceived by patients at different severity levels. And VAS can provide a simple and quick assessment to evaluate NH reaction severity quantitatively. We recommend VAS to be included in diagnostic work up of patients with NH.

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Nonsteroidal anti-inflamatory drug hypersensitivity in our population. Using the latest EAACI/ENDA and GA2LEN/HANNA classification as a mirror

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Background: Hypersensitivity reactions to Nonsteroidal anti-inflammatory drugs (NSAID) is the second cause of drug allergy. We compare provocations oral test and skin test results with the latest EAACI/ENDA and GA2LEN/HANNA classification, approach and diagnosis Nonsteroidal anti-inflammatory drug hypersensitivity.

Method: A retrospective analysis of all cases with a congruent NSAID hypersensitivity clinical history and evaluated with oral provocation tests (OPT) and/or skin test to pyrazolone, between January to June 2015. Medical records were investigated (underlying diseases, symptoms at the primary reaction and during provocation test and culprit drug) and classified all NSAID drug hypersensitivity cases, using the latest ENDA classification and identifying ourselves in it.

Results: In total 60 patients were included. Of those, 44 females and 16 males. The mean age of the patients was 50.37. Ibuprofen was the most implicated drug on initial reactions 25/60 (43%); Around 50% of records described an inmediate reactions with single or more than 1 culprit drug. 44/60 (73%) of patients described cutaneous symptoms. None of non-inmediate reactions involves mucosaes or systemic symptoms There were not undelying deseases in 95% patients. Of 60 patients, only 13 patients (21%) had positive OPT and 3 patients (5%) skin test positive to pyrazolone. Six patients were classified as cross-reactive responders and 10 patients as single NSAID responders after positive OPTs or skin test with the culprit drug but not with acetylsalicylic acid. About single NSAID responders, 6 /10 patients (60%) reacted to metamizol while the 40% else involves other single NSAID, but only 1 patient was Ibuprofen positive OPT.

Conclusion: In our cohort, most cases of NSAID hypersensitivity, involves cutaneous symptoms with a single NSAID, specially metamizol, althought initially ibuprofen was most implicated. Only few cases involves cutaneous and respiratory symptoms at the same reaction. We could classify all patient according to review of the EAACI/ENDA and GA2LEN/HANNA.

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Elevated sensitivity of nasal aspirin challenges in patients with Samter′s triad

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Background: The Samter′s triad consists of chronic rhinosinusitis with nasal polyps (CRSwNP), asthma and aspirin sensitivity and patients with this disease often suffer from recurrent nasal sinus operations. The EAACI guidelines recommend nasal aspirin dosages of 16 mg. The aim of this study was to determine whether increasing the nasal acetylsalicylic acid (ASA) challenge from 16 mg to 25 mg could improve the confirmation rate of disease.

Method: Patients ($n = 35$) and healthy controls ($n = 15$) were challenged nasally with 16 mg ASA. If the test fell negative, a challenge with 25 mg ASA followed.

Results: In the group of patients challenges with 16 mg ASA resulted in 88.6% detection sensitivity, which was elevated to 97% following 25 mg ASA challenge. The specificity was 100%.

Conclusion: Nasal challenges with 25 mg aspirin effectively confirm the diagnosis of Samter′s triad.

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Effects of montelukast sodium on urine density in asthmatic children

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Background: Montelukast sodium is a selective cysteinyl leukotriene receptor antagonist with proven clinical benefit in the treatment of asthma and perennial allergic rhinitis, in adults and children. Neuropsychiatric disorders and sleep disturbances, which affect the paediatric population more often, although urine analyses variations not reported. The aim of the study was to evaluate the effects of the montelukast on urine density in children with asthma.

Methods: We performed a prospective trial between 5–12 years children suffering from asthma. Montelukast was gıven 5 mg as a single dose daily for 8 months. Urine analyses were recorded during the treatment and 4 weeks after discontuniation the drug.

Results: Montelukast increased significantly mean urine density during the treatment. One month after the treatment mean urine densities decreased. No serious adverse effects were noted during the course of the study.

Conclusion: Montelukast induced adverse drug reactions are agitation, anxiety, depression, sleep disturbance, hallucinations, suicidal thinking and suicidality, tremor, dizziness, drowsiness, neuropathies and seizures. The present study shows effect of montelukast on urine density for the first tıme. Our research has shown that montelukast can have a protective effect on children with enuresis nocturna.

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The lymphocyte activation test – clinical importance in delayed drug hypersensitivity reactions: a prospective study

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Background: Depending on their onset during drug intake, drug hypersensitivity reactions (DHRs) are classified as immediate and non-immediate(delayed) reactions. Non-immediate DHRs are frequent in our daily practice and they suppose a challenge when the patient is polimediacted. The only possibility to reject DHR still remains drug provocation test (DPT) which is not excent of risk of severe reactions.

Our objective was to evaluate the clinical importance of the the lymphocyte activation test (LAT) and its utility before proceeding to DPT when delayed DHR study is carried out.

Method: We prospectively studied 58 patients (from 1 to 84- year-old) who consult us for suspect delayed DHRs. The involved drugs were beta lactam antibiotics, non- beta lactam antibiotics, NSAID, carbamazepine, allopurinol,

dextromethorphan and omeprazole. DHRs had ocuured 4 weeks-5 yeras before testing. To all patients we performed patch test with the suspected drug, LAT and previous inofmed consent- a DPT. To carry out the LAT, peripheral mononuclear patient's cells were isolated and incubated with the studied drug during 48 h. We evaluated the surface molecule CD69 by flow cytometry as an *in vitro* indicator for T-cell lymphocyte activation.

Results: 65 LATs were performed (few drugs involved in some patients). 48 of them had a negative result and 47 of the respective DPT were negative too (negative predictive value – 97.9%). 17 of the performed LAT had a positive result and showed a low positive predictive value in our cohort of patients – when DPT was carried out, just 5 were positive too(29.4%). Only in 2 patients the patch test were positive. Both had positive LATs and positive DPTs.

Conclusion: Due to its high predicitve negative value, the lymphocyte activation test could be used as a valuable tool when DPT is planned to reject involvement of the studied drug in a delayed DHRs. LAT would be useful in a cases of severe delayed DHRs when the patient was polimeidacted and all involved drugs had to be studied cautiously to find the culprit drug and to reject the involvement of the rest drugs. A lot still needs to be done to better the diagnose of delayed DHRs.

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Detection of drug - specific T cells in allopurinol induced SCARs

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Background: Allopurinol is one of the causative drugs that induce severe cutaneous adverse drug reactions (SCARs) consisting of Stevens – Johnson Syndrome (SJS)/ Toxic Epidermal Necrolysis (TEN) and Hyper Sensitivity Syndrome (HSS)/Drug Rash with Eosinophilia and Systemic Symptoms (DRESS). Measures of INF gamma production by drug specific T cells is a safe and reliable diagnostic procedure for drug allergy. In the current study, we performed IFN gamma released ELISPOT assay and successfully confirmed allopurinol as the offending drug. These results suggested that IFN gamma released

Method: Seventeen cases of SCARs caused by allopurinol and confirmed by using established criteria, were included to the study. Peripheral blood mononuclear cells (PBMC) were isolated and cultured in the absence or presence of oxypurinol. The number of IFN gamma producing PBMC was determined using ELISPOT assay KIT (Mabtech).

Results: A total of 17 patients comprised 10 SJS/TEN (58.8%), Overlap 1 (5.8%) and 6 HSS/DRESS (35.3%) were tested. Out of 17 individuals, 14 (82.3%) patients had numbers of IFN gamma spot-forming cells (SFC) above the control value of 8–30 SFC/10^6 PBMC.

Conclusion: Our result suggests that the IFN gamma ELISPOT assay might be a useful *in vitro* tool for diagnosis of allopurinol induced SCARs and may be an interesting alternative to improve the diagnosis of drug allergy.

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Evaluation of the results of lymphocyte transformation test in patients with hypersensitivity reactions following anticonvulsant usage

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Background: Administration of anticonvulsant drugs (phenobarbital, phenytoin, carbamazepine and lamotrigine) can been associated with hypersensitivity reactions. LTT (lymphocyte transformation test) is a useful method for determination of the drug causing the reaction. This study was done to evaluate the results of LTT in patients with delayed hypersensitivity reactions following anticonvulsants usage.

Method: Twenty four patients with hypersensitivity reactions [drug reactions like Drug Rash and Eosinophilia with Systemic Symptoms (DISH/DRESS) and Stevens-Johnson Syndrome/Toxic Epidermal Necrosis (SJS/TEN)] following administration of anticonvulsant drugs and 24 patients, who had used anticonvulsant drugs without hypersensitivity reactions were included. Peripheral blood mononuclear cells were isolated. The cells were stimulated with drugs, PHA and candida. Lymphocyte proliferation was measured using Brdu proliferation assay kit (Roche, Germany). The stimulation index was calculated by dividing OD of stimulated to unstimulated cells. The results in case and control groups were compared.

Results: Of 24 patients in the test group, 14 patients had positive LTT results and10 had negative test results, while in control group, 1 patient had positive LTT result and 23 had negative results. Among patients who had received Carbamazepine and Phenytoin, there was a significant difference between the results of LTT in case and control groups ($P = 0.002$ and $P = 0.028$, respectively). Although patients receiving Lamotrigine and Phenobarbital had more positive LTT results in case group, these differences were not statistically significant. The mean time interval between drug intake and development of hypersensitivity reaction was 43.64 ± 7.10 h in test group with positive result and 81.23 ± 17.30 h in test group with negative result. This difference was statistically significant ($P = 0.016$).

Conclusion: Considering significant difference between LTT results in case and control groups in patients receiving Carbamazepine and Phenytoin, and not observing such a difference in patients receiving Phenobarbital and Lamotrigine, LTT results are more valuable for diagnosis of hypersensitivity reactions following usage of Carbamazepine and Phenytoin. We showed that there is not significant relationship between the time interval of performing LTT after hypersensitivity reaction and LTT results and this test can be used even after some years for finding the culprit drug.

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*HLA-B*58:01* and allopurinol high dose were associated with allopurinol induced cutaneous adverse drug reactions in Thai population

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Background: Allopurinol is a major cause of SCAR and has been reported as the second most frequent cause of SCARs, including SJS, TEN and DRESS, in Thailand. The aim of this study was to investigate the association of genetic factors and non-genetic factors with allopurinol-induced CADR including SJS and SJS/TEN ($n = 21$), DRESS ($n = 16$) and severe MPE ($n = 7$) conferred by HLA-B*58:01 in a Thai population.

Method: This case-control association study included 44 cases with allopurinol-induced CADR in comparison with allopurinol-tolerant control patients ($n = 100$)

and population control group (n = 1095) in Thailand. The control group comprised of patients who had received allopurinol for ≥ 6 months without any adverse cutaneous event. HLA-B allele were genotyped by using a two-stage sequence-specific oligonucleotide probe system (PCR-SSOP).
Results: Regardless of the type of CADR, HLA-B*58:01 gave the P-value less than 0.001 both in the combined phenotype analysis and in the separate analysis for all CADR phenotypes. Among 37 patients with allopurinol-induced severe cutaneous adverse reactions (SCAR) found at least heterozygous for HLA-B*58:01 (100%), while 85.7% (n = 6/7) of MPE were positive for HLA-B*58:01. The risk of allopurinol-induced CADR and SCAR were significantly higher in the patients with HLA-B*58:01 allele with an odds ratio 222.00 (95% CI: 52.76–934.08, $P < 0.01$) and 272.0 (95% CI: 57.89–1277.98, $P < 0.01$). In addition, HLA-B*58:01 was also significantly associated with the allopurinol-induced severe MPE patients (OR 144.00, 95%CI: 13.85–1497.03, $P < 0.01$). Moreover, we found that high dosage of allopurinol at the first was associated to allopurinol-induced CADRs (OR 59.00, 95%CI: 19.57–98.42, $P < 0.01$).
Conclusion: Our study is the first on Thai that demonstrated HLA-B*58:01 as a broad predictor for allopurinol-induced DRESS, SJS/TEN and severe MPE. These results suggest that screening tests for HLA-B*58:01 allele in patients who will be treated with low dosage of allopurinol will be clinically helpful in preventing the risk of developing DRESS, SJS/TEN and sever MPE in Thai population.

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HLA-A and HLA-B alleles in Turkish patients with severe anti-epileptic drug allergy

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Background and aim: Studies from Far East countries showed certain relationships with some HLA alleles and severe cutaneous adverse drug reactions due to anti-epileptic drugs. Such relationships have not been evaluated in Turkish population before. The aim of the study is to determine the possible association of severe cutaneous or systemic anti-epileptic reactions and HLA-A and HLA-B alleles.
Methods: Patients who admitted to five different allergy outpatient clinics in four cities due to severe hypersensitivity reactions to anti-epileptic drugs and the ones who can tolerate these drugs were included into the study. Additionally, healthy subjects were examined. After DNA isolation in the blood samples, HLA-A and HLA-B genotyping with PCR-SSO Luminex was performed.
Results: A total of 92 patients (33 male) and 118 healthy subjects (33 male) were enrolled. 42 patients (13 male) had experienced hypersensitivity reactions due to anti-epileptic drugs. These reactions were as follows: maculopapular eruption (26 patients), Stevens Johnson Syndrome (6 patients), drug hypersensitivity syndrome (6 patients), toxic epidermal necrolysis (1 patient), DRESS syndrome (1 patient), fixed drug eruption (1 patient), hand-foot syndrome (1 patient). The culprit drugs were as follows lamotrigine (11), carbamazepine (10), phenytoin (3), valproate (1), primidone (1), pregabalin (1), topiramate (1) and combined preparations (14). Lamotrigine was involved in all SJS reactions. Drug hypersensitivity syndrome and DRESS was mostly associated with carbamazepine. HLA-B*1502 allel was not determined in any of the study groups. However, HLA-B*3502 was determined in 4 patients with reactions while it was not observed in patients who can tolerate the drugs and was detected in only 1 healthy subject (P: 0.039). One of these four patients experienced DRESS syndrome, 1 patient had drug hypersensitivity syndrome and the other two patients had maculopapular exanthema. HLA-A genotyping results were not significantly different in any groups.
Conclusion: This study suggests the possible significance of HLA-B*3502 in severe cutaneous drug reactions due to anti-epileptic drugs in Turkish population. Larger population based studies will further elucidate this significance.

Poster Discussion Session PDS 19

Pathophysiology and management of allergic rhinitis

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Microarray profiling of long non-coding RNA expression in adult patients with allergic rhinitis

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Background: Long non-coding RNA (lncRNA) plays an important role in gene transcription, protein expression and epigenetic regulation; and altered expression results in immune dysfunction. Allergic rhinitis (AR) is an immunoglobulin E (IgE)-mediated hypersensitivity disorder characterized by chronic inflammation of the nasal mucosal tissue; and thus, to understand the molecular mechanisms of lncRNA involved in the pathogenesis of AR, this study profiled lncRNA expression in AR patients.
Methods: Human lncRNA array V6.0 (4 × 180k) was used to profile differentially expressed lncRNAs in five nasal mucosal samples obtained from each adult AR patient and normal controls. Enrichment analyses (GO and KEGG pathway analyses) were performed to construct the lncRNA-mRNA co-expression network.
Results: LncRNA microarray assay indicated that a total of 1397 lncRNAs were significantly differentially expressed in the AR patients compared to normal controls. (Fold change >2.0 or <0.5, $P < 0.01$). Among these altered lncRNAs, 678 were up-regulated and 719 were down-regulated. GO terms and KEGG pathway annotation data revealed that the potential target genes of several candidate lncRNA were enriched in positive regulation of interleukin-13 secretion, cytokine-cytokine receptor interaction pathway and chemokine signaling pathway related to AR development.
Conclusion: Many lncRNA expressions were altered in AR and differentially expressed lncRNAs appear to be involved in the development of AR. The study of lncRNAs may lead to a better understanding about the roles of identified lncRNAs in the pathogenesis of AR; this would be considered in future therapeutic strategies.

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Antagonist of histamine receptor 4 could changed the imbalance of Th1/Th2 in allergic rhinitis

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Background: Allergic rhinitis is widely known caused by imbalance of Th1/Th2 reaction;. Histamine receptor 4 was involved in almost all the immune cells. Our study is aimed to investigate the inhibition of Th2 dominated reaction by JNJ7777120, the antagonist of histamine receptor 4, in allergic rhinitis murine model.
Method: OVA-stimulated allergic rhinitis murine model was used. JNJ7777120 was given by nasal drop The RNA and protein level of Th1 and Th2 biomarker (IL-6, IL-2) in nasal mucosa was evaluated by RT-PCR and ELISA. Th1/Th2 cell in PBMCs was counted by flow cytometry. Result. IL-6 was found decreased while IL-2 increased in JNJ7777120 treatment group compared with the allergic rhinitis group (both $P < 0.05$). The ratio of Th1/Th2 cell in PBMCs was increased in the treatment group ($P < 0.05$).
Conclusion: JNJ7777120 might help to treat the allergic rhinitis by changing the imbalance of Th1/Th2 cell reaction.

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Efficacy of air purifier for adjuvant therapy of allergic rhinitis

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Background: Allergic rhinitis (AR) is a refractory disease that remarkably reduces life quality of the patients, and costs a great amount of social wealth. Studies have revealed the correlation between environmental factors and the development and progression of this disease. National guidelines recommend use of environmental control practices as a component of allergic disease management. In this study, we determined PM concentration in the air, and house dust mite (HDM) allergen concentrations in the bedding and pillows, before and after applying the HEPA air purifier, to evaluate its effect to improve air quality and its efficacy for adjuvant therapy of allergic rhinitis.
Method: Air purifier was set near the bed in the bedroom for 6 months. 13 people that were 33 ± 11 years old and diagnosed with allergic rhinitis were selected. The filters of the purifiers were replaced 5 months after application. Before application of the purifier and every month after, dust sample was collected with a vacuum cleaner and dust collector. The change in PM values was shown in PM indoor/outdoor ratio. In addition, the internationally accepted Rhinitis Quality of Life Questionnaire was used to access the symptoms of the patients. SPSS19.0 was used for data entry and analysis, measurement data was descriptively analyzed, Non-parametric test,independent t test and repeated measurement ANOVA were used to comparison among groups, $P < 0.05$ was considered statistically significant.
Results:

1 Der f1 (1.06 (1.00, 1.11) μg/g) was the most abundant allergen in air followed by Der p1 (1.00 (0.70, 1.31) μg/g); Der f1 (21.72 ± 11.37 μg/g) in bedding and pillows was higher than Der p1 (4.74 (1.18, 34.88) μg/g).
2 Der f1 and Der p1 concentration slightly decreased after application of the air purifier, but the decrease for the latter was not statistically significant.
3 PM1.0, PM2.5, PM10 concentration significantly decreased in the first 3 months, but increased in the 4th and 5th months, then decreased again in the 6th month after replacing the filter.
4 Patients' subjective nasal symptoms were in line with changes in PM concentration; score for activity limitation declined all the way; while score for practical problems dropped in the first 3 months but rose again in the following 3 months.

Conclusion: Air purifier, when properly functioning, can effectively reduce PM and HDM allergen concentrations in the air, and thereby improve clinical manifestations of patients with AR.

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Evaluation of clinical characteristics in children with rhinitis

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Background: Studies performing phenotype of rhinitis in children are scarce. The aim was to study in detail the clinical characteristics and the response to nasal allergen challenge of an ample population of children with rhinitis in our area.
Method: Patients with rhinitis aged 10–20 years were included. The evaluation comprised a detailed clinical history including rhinitis severity, comorbidities, evolution and triggers. An initial allergological study (AE) including skin prick test (SPT), specific IgE (sIgE) classified patients in AE+ or AE−. Later on, nasal allergen provocation test (NAPT) with a panel of 5 allergens using acoustic rhinometry was performed in all subjects being finally classified in systemic allergic rhinitis (SAR = SPT/sIgE+, NAPT+), local allergic rhinitis (LAR = SPT/sIgE−, NAPT+), dual allergic rhinitis (DAR = SPT/sIgE+ to seasonal allergens, NAPT+ to perennial allergens), and idiopathic rhinitis (IR = SPT/sIgE−, NAPT−).
Results: 134 subjects were included, mostly women non-smokers and urban dwelling. Symptoms of rhinitis started earlier in SAR (mean 10 years, 1–18) compared with RI (mean 14 years, 7–19), $P = 0.006$. The initial AE was positive in 89 (66.5%) and negative in 45 subjects (33.5%). Nasal pruritus was the differential symptom in subjects with AE+ vs AE− (OR 6; IC 95% = 1.79–20.02; $P = 0.003$) and in subjects with LAR ($P = 0.01$, OR = 8.07). Severity of rhinitis was moderate-severe and perennial in AE+/AE−, being milder in IR (27%, $P = 0.04$). In >50% of subjects rhinitis worsened or did not improve over the years (P = n.s.). In AE+ triggers were specific (allergens, $P = 0.03$), and in AE− were non-specific (irritants/temperature, $P = 0.011$). Conjunctivitis and asthma were the most common comorbidities. Pollen was the most common sensitizer by SPT/sIgE followed by D. pteronyssinus, being the latter the most important sensitizer when NAPT was performed. NAPT classified patients as SAR 56%, LAR 22.4%, DAR 10.4% and IR 11.2% (total of allergic rhinitis 88.8%). NAPT was positive in 30/45 subjects with AE− (misdiagnosis of 66.7% if the only the initial study was performed).
Conclusion: Rhinitis in children of our area is mostly allergic, moderate-severe and perennial, with conjunctivitis and asthma as main comorbidities. Pruritus is a distinctive symptom of allergic rhinitis including LAR. A complete allergological study with NAPT is able to correctly phenotype rhinitis in children. DAR has been described in children population for the first time.

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A solubilized aqueous budesonide formulation showed higher anti-inflammatory therapeutic efficacy in a murine pulmonary inflammation model compared to marketed products

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Background: Nasally applied corticosteroids are standard therapy for patients suffering from allergic rhinitis. Second generation corticosteroids are highly lipophilic substances that are formulated and applied as suspensions in aqueous solutions. The solid particles have to be dissolved in the nasal fluid before they can permeate into the nasal mucosa. This permeation process needs to occur rapidly after application because the compound is efficiently and rapidly transported into the pharynx due to mucociliary clearance. In contrast, already dissolved drugs permeate faster into the mucosa and are less likely washed out before reaching therapeutic levels than solid suspended drug particles. Here, we report the results of a novel biocompatible aqueous formulation containing dissolved budesonide with improved efficacy compared to standard budesonide nasal sprays.
Methods: Time-dependent permeation was investigated on 3-dimensional cultivated human nasal epithelial cells and *ex-vivo* on porcine nasal mucosa. For the respiratory murine model, anesthetized mice were intranasally treated with the dissolved or suspended budesonide formulation and the LPS induced TNFα suppression in bronchoalveolar lavage was evaluated as a surrogate parameter for inflammation.
Results: We found a significantly increased permeation of dissolved budesonide through cell layers and *ex-vivo* into porcine nasal mucosa in comparison with dispersed budesonide (12-fold and 5-fold, respectively). In the mouse model LPS induced TNFα in the respiratory tract was significantly stronger suppressed with a 300 µg/ml dissolved budesonide formulation compared with a marketed nasal spray containing 1.28 mg/ml suspended budesonide.
Conclusion: In summary, dissolved budesonide permeates faster into nasal mucosa in comparison to a suspension. Moreover a markedly reduced concentration leads to a higher therapeutic efficacy compared to marketed products. A faster onset of action is observed due to earlier availability of the drug in the nasal tissue.

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The minimally important difference in the Rhinoconjunctivitis Total Symptom Score in house dust mite-induced allergic rhinoconjunctivitis

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Background: The minimally important difference (MID) has been defined as the smallest improvement considered worthwhile by a patient. The MID has been estimated for the Rhinoconjunctivitis Total Symptom Score (RTSS) for grass pollen induced but not for house dust mite (HDM).
Method: In a prospective observational multicentre study, patients consulting for HDM-induced allergic rhinitis (AR) in France and Germany recorded a 15-point global rating of change scale (GRCS) score and the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) score on a weekly basis and the individual symptom scores comprising the RTSS on a daily basis over two consecutive weeks. The MID in the RTSS was determined with anchor-based methods (using the GRCS and the RQLQ) and a distribution-based method [based on the RTSS′ standard deviation (SD)].
Results: The study population comprised 546 patients (203 children, 133 adolescents and 210 adults). During the first week of the study, the mean ± SD RTSSs for these age groups were 8.22 ± 1.84, 8.32 ± 1.91 and 8.23 ± 2.10, respectively. For an improvement of 2 points in the GRCS or 0.5 points in the RQLQ score, the regression analysis yielded MIDs in the RTSS of −0.90, 95% confidence interval (CI95%): [−1.06, −0.75], and −0.94 (CI95%): [−1.19, −0.69], in children, −0.74, (CI95%): [−1.07, −0.41] in adolescents and −1.04, (CI95%): [−1.29, −0.79], in adults. When applying distribution-based methods, the MID ranged from 0.87 to 0.91 (based on 0.33 SDs of the first-week RTSS) and from 0.88 to 0.96 (based on 0.50 SDs of the difference in RTSSs between the first and second weeks).

Conclusion: The MID in the RTSS is very consistent (close to −0.90) whatever the method used, whatever the age group and whatever the initial severity of the disease, in patients with HDM-induced AR.

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Noninterventional study on alpha-tocopherol acetate (vitamin E acetate) nasal spray as an alternative treatment option in patients with pollen-induced allergic rhinitis in comparison to standard therapies

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Background: Alpha-tocopherol acetate (ATA) nasal spray is an oily liquid which forms a protective film on the nasal mucosa. This noninterventional study investigated the efficacy of ATA nasal spray as compared with that of beclomethasone nasal spray (glucocorticoid nasal spray) and of loratadine tablets (antihistamines) in patients suffering from birch, grass, or rye pollen-induced allergic rhinitis.
Method: The study was conducted in 10 centres across Germany between April and July 2015. The observation period lasted 1 week and included Visit 1 at the beginning and Visit 2 at the end of the week. The study planned to enrol 120 patients aged 18 years or older: 60 patients deciding to use ATA nasal spray, 30 patients preferring to use beclomethasone nasal spray and 30 patients choosing to take loratadine tablets. The evaluation of efficacy was based on rhinitis symptoms recorded in a diary, comparison of the rhinitis score (intensity and frequency of symptoms) before and during the treatment, use of additional symptomatic medication, findings of rhinoscopic examinations and overall assessments of the treatment by patients and physicians.
Results: Out of 116 patients analysed (mean age of 39.1 years; 59.5% female and 36.2% male patients), 63 subjects used ATA nasal spray, 32 subjects applied beclomethasone nasal spray and 21 subjects took loratadine tablets. The improvement in the daily rhinitis symptom score was analysed for all treatment groups. Statistical significance was observed in the ATA group beginning on Day 6 compared to Day 1, in the beclomethasone group beginning on Day 4 compared to Day 1 and in the loratadine group beginning on Day 3 compared to Day 1. Comparing the situation before and during the observation period, the rhinitis score decreased statistically significantly and the percentage of patients who did not use any additional symptomatic medication increased in all treatment groups. Statistically significant improvement in the rhinoscopy score was detected in all treatment groups between Visit 1 and Visit 2. Also, the majority of patients or physicians evaluated the efficacy of all therapy options to be good or very good.
Conclusion: ATA nasal spray represents a viable treatment option for sensitive patients who suffer from side effects of glucocorticoid nasal sprays or antihistamines as well as for subjects who prefer alternative therapies to conventional medicine.

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Factors associated to the prescription of allergen immunotherapy in patients with mite induced respiratory allergy

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Background: House dust mites (HDM) are the most common cause of respiratory allergy worldwide. The study named COMETA after 'Clinical profile, treatment and cOst of the most common alleErgy in ITAly' was aimed at evaluating the characteristics of patients with mite allergy in Italy and the treatments prescribed by physicians.
Method: Patients aged between 6 and 60 years with rhinitis and/or asthma and mono-allergy to HDM were enrolled in 17 Centres. To all patients referring to these Centres a detailed clinical history was collected and skin prick tests (SPT) with a standard panel of inhalant allergens were performed. To patients monosensitized to HDM the physicians prescribed a medical treatment according to patient's characteristics, including the kind of respiratory allergy and the severity of symptoms assessed by visual analogue scale (VAS).
Results: The study population was formed by 232 patients; of them 197 (85%) were positive to SPT with both Dermatophagoides pteronyssinus and Dermatophagoides farinae, while 35 patients were positive only to one of the two mites; 228 patients had rhinitis, 92 patients had asthma; 174 (75%) patients were prescribed only symptomatic drug treatment (136 antihistamines, 122 nasal corticosteroids, 46 inhaled corticosteroids, 43 beta-2 agonists and 11 leukotrienes antagonists) while 58 (25%) were treated by allergen immunotherapy (AIT). Patients with rhinitis receiving a prescription of AIT had a significantly higher VAS score (7.91 ± 1.80) compared to those receiving drug treatment (5.95 ± 3.13) with a P value < 0.001. In patients with asthma, AIT was prescribed significantly less frequently than drug treatment: 20/92 vs 72/92 patients ($P = 0.0001$).
Conclusion: In patients with mite-induced respiratory allergy, the physician's tendency to prescribe more frequently AIT in patients with more severe rhinitis is in agreement with updated guidelines, while the tendency to prescribe it less frequently in patients with asthma suggests a need for reappraising the actual indications for asthma from guidelines.

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Effectiveness of MP-AzeFlu* for the treatment of allergic rhinitis in real-life: meta-analysis of data from Germany, Sweden, Norway, Denmark and Romania

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Background: The importance of allergic rhinitis (AR) control has been prioritized at the EU level.[1] More effective disease management and control assessment tools have been identified as key to success. The visual analogue scale (VAS) has been endorsed as the new language of AR control, and incorporated into the most recent MACVIA ARIA (Fighting chronic diseases for active and healthy aging Allergic Rhinitis and its Impact on Asthma) guideline. The aim of this analysis was to assess the effectiveness MP-AzeFlu* (a novel intranasal formulation of azelastine hydrochloride and fluticasone propionate in a single spray) in routine clinical practice in several countries across Europe, using a VAS, in line with EU and MACVIA ARIA initiatives.
Method: 2795 patients (≥ 12 years old) with moderate-to-severe seasonal AR were recruited into 5 multi-centre, prospective, non-interventional studies carried out in Germany ($n = 1781$), Sweden ($n = 431$), Romania ($n = 253$), Denmark ($n = 170$) and Norway ($n = 160$). Patients assessed symptom severity using a VAS from 0 mm (not at all bothersome) to 100 mm (very bothersome) on Days 0, 1, 3, 7, and the last visit (~Day 14) in the morning prior to MP-AzeFlu* use. Patients' perceived level

of disease control (i.e. well-, partly-, uncontrolled) was assessed on Day 3. Results are presented as a meta-analysis.

Results: MP-AzeFlu* provided effective and rapid symptom control, reducing the mean VAS score from 73.7 mm (SD 17.3) at baseline to 23.4 mm (SD 20.3) by the last visit, a reduction of 50.3 mm (SD 26.1). In terms of patients' perception of AR control, 50.3% of them considered their symptoms to be 'well-controlled' after treatment with MP-AzeFlu* for 3 days. The results were consistent across the countries.

Conclusion: MP-AzeFlu* provided effective and rapid AR symptom control in a real-life pan-European setting. One in every 2 patients felt their AR was well-controlled after just 3 days of treatment. These results align with EU and MACVIA ARIA aims for improved disease control and support MP-AzeFlu*'s position as the drug of choice for the treatment of AR.

***Dymista:** (1) Bousquet J, et al. J Am Med Dir Assoc 2015;16:1020-6.

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Comparison of the rhinitis control assessment test with more complex surveys for the evaluation of symptoms in patients suffering from allergic rhinitis

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Background: The Rhinitis Control Assessment Test (RCAT) [Meltzer EO et al. 2013] is a basic rhinitis score based on a six-item questionnaire. It serves the physician's evaluation of patients' allergic symptoms in daily clinical practice. More complex scores are the Quality of Life Questionnaire (RQLQ) [Juniper EF 1989] and the Rhinoconjunctivitis Total Symptom Score (RTSS) [Pfaar O et al., 2014], which is calculated using diary data of daily symptoms. The correlations between all three scores were investigated in a DBPC trial.

Method: Within the framework of a DBPC trial conducted during the German grass pollen season 2014, the symptom scores of 33 grass pollen allergic patients were analyzed. Patients were treated with a sublingual placebo preparation, as well as with anti-symptomatic medication. Rhinitis symptoms were evaluated by means of the RCAT (6–30), RTSS (0–18) and RQLQ global score (0–6) assessed during the peak pollen season. Both the RQLQ and the RCAT scores were assessed retrospectively for the 14 (RQLQ) and 7 days (RCAT) before the visits that took place during the pollen peak. All six separate questions of the RTSS were answered on a daily basis in a 30-day patient diary completed throughout the pollen peak.

Results: The evaluation resulted in a mean RCAT score of 19.18 (± 3.618), a mean RTSS of 0.92 (± 2.764), and a mean RQLQ global score of 1.84 (± 1.153). Overall, 30.3% of all patients had RCAT score values >21 which is the cut-off for symptom control. A strong correlation was shown between the RCAT score and RQLQ global score ($R = -0.871$), the RCAT score and RTSS ($R = -0.811$), and the RQLQ global score and RTSS ($R = 0.746$). In a further study with 94 tree pollen allergic patients, the evaluation of the RQLQ global score and the RCAT score showed a similarly strong correlation between both scores ($R = -0.795$).

Conclusion: Consisting of only six items, the RCAT is a less complex and therefore user-friendly tool for assessing allergic rhinitis symptoms, which shows high correlations to the results of more complex scores.

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The effects on ECP, MCT and clinical symptoms in allergic rhinitis guinea pigs with Fluticasone furoate/ vilanterol treatment

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Background: Allergic rhinitis (AR) is a global health problem. The effectiveness of currently available medications is limited and therefore investigation for more effective drugs is essential. Intranasal corticosteroid sprays (INCSs) are commonly used for therapy of AR. Fluticasone furoate (FF)/vilanterol (VI) is a once daily (OD) inhaled corticosteroid/long-acting β2-agonist combination asthma therapy approved.

Method: Used toluene 2, 4 – diisocyanate (TDI) and ovalbumin (OVA) preparation of guinea pig model of allergic rhinitis, after the success of the model, it is divided into OVA group, OVA drug treatment group, TDI group, TDI drug treatment group, set up healthy controls (brine intranasal), each group 20 cases, observing animals clinical symptoms (nose, sneezing, nasal discharge), used ELISA method to detect ECP, MCT, IgE, IL - 5 protein expression levels in nasal mucosa tissue homogenate and serum .

Results: In clinical symptom: scores of OVA group and TDI group was obviously higher than that of OVA drug treatment group, TDI drug treatment group and saline group ($P < 0.01$), OVA drug treatment group and TDI drug treatment group scores higher than healthy controls ($P < 0.01$). In OVA group and TDI group, the expression of ECP, MCT, IL-5, total IgE were higher than OVA/TDI drug treatment groups and healthy control group ($P < 0.05$) in tissue and serum. ECP, MCT, IL - 5, IgE, content were significantly higher than that of healthy controls ($P < 0.05$), but the OVA/TDI group had no significant difference with OVA/TDI drug treatment group ($P > 0.05$).

Conclusion: FF/VI can effectively improve the high reactivity symptoms of allergic rhinitis animal model. At the same time can effectively reduce the guinea pig nasal mucosa inflammation action. Therefore local application of FF/VI can relieve inflammation of the nasal mucosa reaction.

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An increased prevalence of self-reported allergic rhinitis in major Chinese cities from 2005 to 2011

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Background: The prevalence of allergic rhinitis (AR) has increased worldwide in recent decades. The present study was conducted to investigate the prevalence of self-reported AR and profiles of AR-related comorbidities in the adult population of China over time.

Method: This study surveyed residents of 18 major cities representing seven geographic regions in mainland China.Telephone interviews were conducted with study participants after sampling target telephone numbers by random digital dialing. The questions asked during telephone interviews were based on those included in validated questionnaires used by the ISAAC and ECRHS, and focused on topics regarding allergic rhinitis, non-allergic rhinitis (NAR), acute/chronic rhinosinusitis (ARS/CRS), asthma, and atopic dermatitis (AD).

Results: During 2011, a total of 47 216 telephone interviews were conducted, and the overall response rate was 77.5%. When compared with the AR prevalence found in 11 cities surveyed in 2005, there was a significant increase in self-reported adult AR in eight of those cities ($P < 0.01$). In 2011, the overall prevalence of self-reported adult AR in the 18 cities surveyed was 17.7%.The overall prevalences of NAR, ARS, CRS, asthma, and AD in the general population were 16.4%, 5.4%, 2.1%, 5.8%, and 14%, respectively; while the

prevalences of ARS, CRS, asthma, and AD in the AR population were 30.4%, 10.1%, 28%, and 24.2%, respectively. Moreover, the prevalence of AR was only positively correlated with the concentration of atmospheric SO2 in multiple factors including air pollution, meteorological conditions and socioeconomic status in 2011.

Conclusion: During a 6-year period, there was a significant increase in the prevalence of self-reported AR in the general Chinese adult population which suggests that the prevalence of AR in mainland China has not yet reached a plateau. The prevalence of AR being accompanied by rhinosinusitis, asthma or AD was significantly higher among individuals having self-reported AR when compared with the general population.

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Monosensitized and polysensitized patients with respiratory allergy: are two different phenotypes?

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Background: The prevalence of polysensitization is greater than monosensitization. Polysensitization may be associated with a different clinical picture with respect to severity of disease and immune response to allergens.

Objective: To investigate the immune reactivity and clinical features of monosensitized and polysensitized patients who have rhinitis with or without asthma.

Methods: A total of 44 adult patients participated in this prospective study. Among them, twenty patients were mono- [sensitive to only house dust mite (HDM)] and 24 were polysensitized (to at least 1 additional allergen beyond HDM). Immunological parameters including CD203c expression on basophils induced by *Der p* allergen stimulation (NAVIOS FC, Beckman Coulter, Miami, USA), total IgE, specific IgE and IgG4 (Pharmacia, Uppsala, Sweden) antibodies were evaluated. Clinical features were assessed using monthly symptom-medication scores [(Total rhinitis symptom score (TRSS), total asthma symptom score (TASS), total symptom score (TSS) and total medication score (TMS)], visual analogue scale (VAS), quality of life questionnaire (Mini-RQLQ and AQLQ), and nasal allergen provocation test (NPT).

Results: There was no difference between two groups in terms of age, gender, duration of disease and the number of patients with asthma. In polysensitized group, skin test reactivity to *Der P* (mean oedema diameter) was found to be higher ($P < 0.05$). CD203c expression on basophils after stimulation with 1.6 μg/ml and 0.16 μg/ml *Der P* increased in the polysensitized patients when compared to monosensitized ones ($P < 0.05$). Total IgE, specific IgE and IgG4 levels were not different between the two groups ($P > 0.05$). Monosensitized allergic rhinitis patients accompanying asthma had higher TRSS than in polysensitized ones ($P < 0.05$). AQLQ scores of 'environmental stimuli' domain were better in monosensitized patients than in polysensitized ($P < 0.05$). Nasal response to allergen provocation was similar in both groups. No significant difference was found between the mono- and polysensitized groups in terms of TSS, TMS, VAS, Mini-RQLQ and other AQLQ scores ($P > 0.05$).

Conclusion: This study provides evidence that mono- and polysensitized patients exhibit distinct basophil behavior but not antibody production. In addition, mono- and polysensitized patients seems to have different clinical characteristics. However, further studies are required in larger populations.

Clinical trial registration:NCT01795846.

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Serum vitamin D levels in patients with allergic rhinitis and its association with quality of life

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Background: There is evidence that vitamin D levels are lower in allergic rhinitis (AR) and chronic rhinosinusitis (CRS). However, its impact on quality of life (QoL) in these patients is yet to be assessed.

Method: The study comprised 118 consecutive patients with AR (males/females, 63/55; 18–60 years). 32 healthy volunteers functioned as controls. IRB approval and informed consent was obtained. AR was diagnosed as per the ARIA guidelines. All patients had a positive skin prick test and normal spirometry without significant reversibility and underwent CT-PNS documenting CRS as per EPOS guidelines. Serum vitamin D level was estimated with automated ELISA readers. Patients were divided into three groups: AR (Group1), AR with CRS (Group2) and controls (Group3). Patients in Groups1 and 2 were also categorised as 'Sneezers-Runners' and 'Blockers' as per predominant symptoms. Disease severity and impact on QoL was assessed with Sinonasal Outcome Test 22 (SNOT-22), Visual Analogue Scale (VAS) and Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ).

Results: Group1 comprised 40 (33.9%) patients, Group2:78 (66.1%) and Group3:32 controls. Mean serum vitamin D levels (ng/ml) were significantly lower in patients with AR as compared to controls (15.05 ± 9.43 v/s 27.25 ± 24.26; $P = 0.0001$). Levels of vitamin D were significantly lower in patients of Group1 as compared to Group2 (17.75 ± 10.12 vs 13.66 ± 8.90; $P = 0.04$). 'Blockers' too had a significantly lower vitamin D levels as compared to 'Sneezers-Runners' (12.04 ± 5.35 vs 16.84 ± 10.92; $P = 0.013$). There was a significant difference in mean SNOT-22 scores (1.56 v/s 1.98; $P = 0.03$) and mean global VAS scores (6.13 vs 7.33; $P = 0.028$) between Group1 and 2. RQLQ scores were higher in Group2 compared to Group1, however difference was not significant (1.56 vs 1.62; $P = 0.50$). The correlation-coefficient between vitamin D levels and SNOT-22, global VAS scores and RQLQ was $r = -0.30$, -0.11 and -0.15 respectively.

Conclusion: Vitamin D levels were significantly lower in patients with AR as compared to controls. Similarly, patients with AR and concomitant CRS had significantly low levels of vitamin D as compared to AR. Compared to 'Sneezers-Runners','-Blockers' had significantly lower vitamin D levels. Sinusitis significantly increased the severity of the disease and negatively impacted the QoL. There was a weak correlation between vitamin D levels and QoL scores implying that patients with lower vitamin D levels had worse QoL.

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Effectiveness of MP-AzeFlu* for the treatment of allergic rhinitis in real-life according to phenotype, severity and patient age: meta-analysis of data from 5 European countries

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Background: The importance of allergic rhinitis (AR) control has been prioritized

at the EU level.[1] More effective disease management and control assessment tools have been identified as key to success. The visual analogue scale (VAS) has been endorsed as the new language of AR control, and incorporated into the most recent MACVIA ARIA (Fighting chronic diseases for active and healthy aging Allergic Rhinitis and its Impact on Asthma) guideline. The aim of this analysis was to assess the effectiveness of MP-AzeFlu* (a novel intranasal formulation of azelastine hydrochloride and fluticasone propionate in a single spray) in routine clinical practice in several European countries using a VAS, in line with EU and MACVIA-ARIA initiatives, according to severity, phenotype and patients' age.

Method: 2795 patients (≥12 years old) with moderate/severe SAR were enrolled into 5 multi-centre, prospective, non-interventional studies in Germany (n = 1781), Sweden (n = 431), Romania (n = 253), Denmark (n = 170) and Norway (n = 160). Patients assessed symptom severity using a VAS from 0 mm (not at all bothersome) to 100 mm (very bothersome) on Days 0, 1, 3, 7, and the last visit (~Day 14) in the morning prior to MP-AzeFlu* use. A VAS score of ≤38 mm was defined by patients as the 'well-controlled' VAS score cut-off. Data are presented as a meta-analysis (whole population) and according to disease severity (less severe: 50–74 mm baseline VAS; more severe: 75–100 mm baseline VAS), phenotype (i.e. SAR, PAR or SAR + PAR) and patient age (i.e. 12–17 years, 18–65 years, >65 years).

Results: MP-AzeFlu* reduced the VAS score from 73.7 mm (SD 17.3) at baseline to 23.4 mm (SD 20.3) by the last visit, a shift of 50.3 mm (SD 26.1). MP-AzeFlu*-patients experienced a rapid symptom reduction from the first day of treatment, with effectiveness sustained for the whole study period. On average, patients achieved the 'well-controlled' VAS score cut-off (i.e. 38 mm) by Day 7, irrespective of disease severity, phenotype or patient age, with consistent results noted across the countries.

Conclusion: MP-AzeFlu* provided effective and rapid AR symptom control in a matter of days across all age groups, severities and phenotypes assessed in a real-life pan-European setting, aligning with EU and MACVIA ARIA aims for improved disease control. These data support MP-AzeFlu*'s position as the drug of choice for the treatment of AR.

***Dymista:** (1) Bousquet J, et al. J Am Med Dir Assoc 2015;16:1020-6.

Poster Discussion Session PDS 20

Air pollution and environmental allergies

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Aeromycological study on the fungal spore diversity in Kolkata and study on allergens of *Aspergillus oryzae:* a major sensitizer of asthma patients

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Background: Airborne fungal spores constitute a significant fraction of atmospheric bioparticles and most of them act as an important source of aeroallergens causing hypersensitivity, respiratory disorder and allergic diseases. The present study was conducted in an urban megacity Kolkata to determine the airborne fungal spore diversity, their seasonal variation, the impact of their allergenicity on the health of local population and to detect the allergenicity of one of the most predominant fungi *Aspergillus oryzae*.
Method: Environmental Biomonitoring of viable and non-viable fungal spores was performed in different indoor and outdoor environments by using Andersen sampler and Burkard personal sampler. Spore concentration was correlated with different meteorological parameters. One of the most predominant fungi *Aspergillus oryzae* was chosen for immuno-proteomic study as very few works have been performed on this particular species so far. After Skin Prick Test allergenic potential of *Aspergillus oryzae* was further confirmed by ELISA. Total protein was resolved in SDS-PAGE and 2-Dimensional gel electrophoresis. Allergenicity was investigated by 1D and 2D Immunoblotting. Glycoproteins in the allergen profile were tested by Periodic Acid Schiff's staining and the role of sugar moiety in IgE-binding was assessed b meta-periodate modification. Mass spectrometry based identification of allergens from IgE reactive spots was done by MALDI-TOF-TOF.
Result: Maximum spore concentration was observed in outdoor environments and was greatly influenced by meteorological parameters. Ascospores, *Periconia* sp., *Curvularia* sp. were found to be dominant non-viable fungi whereas *Aspergillus* sp. was the most predominant viable fungus. The survey and hospitalization records were found to observe positively associated with spore load. For *Aspergillus oryzae*, 4 IgE reactive bands were found as glycoprotein and some of them have glycol moiety as a major contributor of IgE-epitope. From 2D immuno-blot 16 allergens were detected of which 4 IgE reactive spots were identified by MALDI-TOF-TOF. These were α amylase A (55.288 kDa), glycosyl hydrolase (47.837 kDa), glucan 1, 3-beta-glucosidase A (45.434 kDa) and Reductase like protein (37.080 kDa) of which last two were not reported yet as allergen.
Conclusion: Maximum spore concentration was observed in outdoor environments. *Aspergillus* sp. was found to be the dominant fungus. In case of *Aspergillus oryzae* 4 allergens were identified till now.

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Molecular components involved in the IgE cross-reactivity between *Aedes aegypti* and arthropods

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Background: Several mosquito species, including *Aedes aegypti*, may induce skin and respiratory allergic reactions. A high degree of cross-reactivity between *A. aegypti* and arthropods has been suggested. Besides tropomyosin, other potentially involved molecular components have not been fully described and it remains unclear whether mosquitoes may act as a primary sensitizers. The main objective of this study was to identify and characterize IgE cross-reactive proteins and to establish the relevance that cross-reactivity may play in the allergenic properties of *A. aegypti*.
Methods: Sera from thirty-four patients were obtained from allergic individuals with asthma and/or rhinitis and specific IgE to mosquito residing on the tropical Caribbean island of Martinique. Specific IgE levels were determined by ELISA. Cross-reactivity among *A. aegypti*, *D. pteronyssinus*, *D. farinae*, *B. tropicalis*, *P. americana* and *L. vannamei*, and the recombinant tropomyosins (Lit v 1, Der p 10, Blo t 10 and Per a 7) were analyzed by competitive ELISA. *A. aegypti* derived allergens were identified by immunoblotting and the cross-reactive bands by immunoblotting inhibition experiments. Four cross-reactive bands were further characterized by MALDI-TOF/TOF.
Results: In these sera, the frequency of positive IgE reactivity to at least one mite species was 82.35%; to *A. aegypti* 64.7%; to *P. americana* 29.41% and to *L. vannamei* 23.53%. High degree of inhibition of mosquito specific IgE binding (>64%) was achieved when a pool of sera was adsorbed with *L. vannamei*; moderate (39.60%) when adsorbed with *D. pteronyssinus*, *P. americana* and *B. tropicalis* and low (<20%) when adsorbed with *D. farinae*. Eleven bands of *A. aegypti* cross-reacted with arthropod extracts. Four of them were further identified: Odorant binding protein, Mitochrondial cytochrome C, Peptidyl-Prolyl cis-trans isomerase, and Protein with hypothetical magnesium ion binding function. Accordingly, pre-incubation of the sera with recombinant tropomyosin from mosquito, mite, shrimp and cockroach partially inhibited IgE reactivity against allergenic extracts.
Conclusion: We demonstrated that in addition to tropomyosin, several proteins including 4 novel allergens, participate in the cross-reactivity between *A. aegypti* and other arthropods. Further studies are necessary to clarify the role of these molecules in the pathophysiology of allergies in the tropics and other geographical locations where mosquitoes are abundant.

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Allergenic profile of *Quercus rotundifolia* pollen in Alentejo, Portugal

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Background: Grasses and olive are the most relevant allergenic species in the

Alentejo region. However, aggravation of allergic symptoms has been reported in the early spring, before grass and olive pollen seasons. Quercus pollen is the most abundant pollen type in the early spring in Alentejo, nonetheless its allergen profile has not yet been evaluated. The aim of this work was to characterize the allergen profile of pollen from *Quercus rotundifolia* among the most representative species showing pollination in April, prior to the main pollen season in Alentejo.

Method: Pollen from *Quercus rotundifolia*, *Olea europaea* and *Dactylis glomerata* was extracted with ammonium bicarbonate buffer, lyophilized and stored at −80°C until analysis. Extract from *Quercus ilex* pollen was kindly offered by Bial. Protein content was determined by the Bradford method. SDS-PAGE followed by western blot, using allergic patient sera (obtained from the Hospital do Espírito Santo de Évora - HESE), were performed to evaluate the allergenic profile of the pollen. Sensitization and cross-reactivity was assessed by solid phase immunoblot.

Results: Most of the patient evidenced sensitization to pollen extracts of *Q. rotundifolia*. Protein profile of *Q. rotundifolia* has shown several bands in the Mr 10–90 kDa, mostly overlapping with *Q. ilex*. Western blot have shown several immunoreactive bands. Immunoreactive bands were also observed in the protein profile according to the pI in the range 4.0–6.1. Cross-reactivity between *Q. rotundifolia* with *O. europaea* and *D. glomerata* was found.

Conclusion: These results evidenced allergens found in *Q. rotundifolia* pollen. It also shows that protein profile of *Q. rotundifolia* and *Q. ilex* are mostly alike suggesting that similarities in allergen profile are expected. Moreover, cross-reactivity between *Q. rotundifolia* and highly allergenic species such as *O. europaea* and *D. glomerata* was found which probably contributes to the aggravation of pollinosis in the early spring.

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Mus m 1 personal exposures in laboratory animal workers in facilities where mice are housed in open or individually ventilated cages

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Background: Laboratory animal workers face the risk of developing an IgE-associated respiratory allergy to airborne proteins, such as Mus m 1 (mouse urinary protein). Historically, approximately 15% of exposed employees developed IgE sensitisation and 10% clinically apparent disease. We have embarked on a large study ('SPIRAL': Safe Practice In Reduction of Allergy in Laboratories) to gain a greater understanding of laboratory animal allergy and to determine whether we can devise a code of safe practice to prevent, as far as possible, the future occurrence of the disease.

Aim: To determine personal exposure to Mus m 1 within animal facilities where mice are housed in open cages or individually ventilated cages (IVCs).

Methods: Full shift and task-specific samples were collected from 5 animal units. Selected employees wore Casella Apex pumps (2 l/min) to collect inhalable particulate onto fluoropore membrane (1 μm), 25 mm filters using IOM sampling heads. 215 full shift samples and 91 task-specific samples from 2 IVC units, 1 open cage unit and 1 mixed cage unit (open cage & IVC) were collected and analysed for Mus m 1 using a commercial sandwich enzyme linked immunoassay (Indoor Biotechnology).

Results: Full shift samples

Animal unit	Geometric mean (ng/m$^{3)}$	% of samples >5 ng/m^3
Unit 1	0.69	11.4% (n = 70)
Unit 2	7.89	86.4% (n = 44)
Unit 3	2.66	30.4% (n = 56)
Unit 4	21.99	100% (n = 26)
Unit 5	3.80	47.4% (n = 19)

Table 1. Mus m 1 (ng/m3) (full shift samples)

Task-specific samples

Performing minor procedures on mice and removing dirty bedding from cages were tasks associated with high levels of exposure. Mus m 1 levels from Unit 3 for 'removing dirty bedding from cages 'ranged from 16.93 to 55.96 ng/m^3. Mus m 1 levels from Unit 4 for 'dosing/injecting mice' ranged from 27.37 to 254.62 ng/m^3.

Conclusions: The majority of samples from non-IVC units were above 5 ng/m^3, a figure previously suggested to limit the incidence of LAA. The mean Mus m 1 levels from the 3 IVC-only units were significantly lower than levels found in non IVC units. There was significant variation in Mus m 1 levels for the same tasks performed in the same units. Exposure to high allergen levels will be influenced by cage type, variation in individual working practice and carrying out of specific 'high-risk' tasks; some of these factors may be modifiable and these results may be used to change practice.

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Exposure chamber: a novel technology to assess the efficacy of air purifiers

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Background: Pollen exposure is mostly seen as an out-door exposure but studies have shown the presence of different pollen species including birch and grass pollen in houses, schools and shops leading to long-lasting symptoms even after the pollen season. The objective of this study was to determine the efficacy of an available air purifier in removing airborne grass pollen with the aim of preventing allergic nasal symptoms in persons with grass pollen-induced allergic rhinoconjunctivitis.

Method: A Philips AC4012 Air Purifier was tested in a mobile exposure chamber (www.mcxperts.com) in two steps.

1 Testing the potential influence of the airflow at the outlet on triggering symptoms. The test subjects sat in the pollen chamber at a distance of 150 cm from the outlet of the air purifier, which was equipped with a filter. No pollen was released.
2 Pollen exposure without and with filter in the air purifier: four non-smoking, adult subjects suffering on grass pollen-induced allergic rhinitis without asthma were exposed twice on two different days to a concentration of 4.000 grass pollen/m3 air per 90-minute observation period. Symptoms of the eyes, nose and bronchia (scale of 0 - 3) were recorded before, every 10 min. during and after the exposure, peak nasal inspiratory flow (PNIF) and peak expiratory flow every 30 min, spirometry before and after challenge. The TSS scale allows a maximal symptom score

of 12 points per organ. Since nasal symptoms account for over 80% of total symptoms, the Total Nasal Symptom Score (TNSS) is the critical parameter for determining the results of intervention in allergic rhinitis.

Results: The airflow at the outlet without airborne pollen in the chamber induced symptoms scored under 1 point. The use of the air cleaner reduced the pollen-induced TNSS significantly from 6 and 4 points (1st and 2nd exposure without filter) to less than 1 point when air cleaner was activated.

Conclusion: The novel study protocol in the mobile exposure chamber is suitable for testing efficacy of air cleaners and the tested air cleaner is effective in reducing clinical symptoms due to grass pollen in an indoor environment.

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Regression models for predicting total pollen concentration in Central Lagos State, Nigeria

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Background: The significance of pollen forecast in aerobiology cannot be overemphasized since the goal is to provide accurate information on pollen in the air. Sensitive individuals use this information to help them optimize the management process in their treatment. This paper presents an investigation of the meteorological variables which influence pollen counts in Central Lagos, from 2013 to 2014, as a basis for the development of predictive models for forecasting.

Method: The analysis of total pollen concentrations, and dominant pollen such as *Alchornea cordifolia,* Amaranthaceae, *Casuarina equisetifolia,* Cyperceae and Poaceae were determined using three data sets - 2013 only, 2014 only and 2013 to 2014; and *stepwise* regression.

Results: No meteorological parameter was significantly useful in modelling pollen forecast in the 2013 data set. *Casuarina equisetifolia* results showed that no parameter could be used to model a possible forecast in all data sets. Data set 2013 to 2014 showed that, relative humidity was the best for modelling total pollen forecast as well as in individual pollen groups. In the 2014 data set, Poaceae pollen recorded the best modelling variable of 85.8% using a combination of wind and relative humidity parameters, while other pollen groups produced good results with relative humidity modelling only.

Conclusion: This shows that when considering forecasting, individual pollen or pollen groups such as Poaceae or Cyperaceae should be considered. This study is the first in Nigeria to highlight the need to monitor changes in aeropalynology and it provides a framework for targeting the most important meteorological parameters in terms of modelling pollen forecast. It also recognizes the need for continuous aerobiological study to obtain sufficient data for accurate pollen forecast.

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Predicting the Poaceae pollen concentration in the air using time series models

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Background: Poaceae pollen is the major cause of respiratory allergies in Europe. This fact is due to the great allergenic potential of this pollen type and the wide distribution of grasses in many parts of the world. On the other hand, the Poaceae pollen season is one of the most complex because pollen comes from a great diversity of species. The aim of this study was to predict the Poaceae pollen concentration in the atmosphere using time series models to define the days of greatest risk of allergy.

Method: Airborne Poaceae pollen was monitored daily using a Hirst volumetric spore trap. The pollen time series for the period 2006–2013 was decomposed using seasonal-trend decomposition procedure based on LOESS smoothing, and different components were obtained. The seasonal component showed the periodic behavior of the pollen curve and the residual component was modeled through partial least square regression using variables from the pollen concentration of the previous days and meteorological variables such as temperature and rainfall. The model was validated from independent data (year 2014).

Results: The calibration of the model showed that the 58% of the variance was explained in the pre-peak period (i.e. prior to the day of greatest concentration) and the 69% of the variance in the post-peak period. The most important variables are the pollen concentration of the previous days and the maximum temperature, and both showed positive influence on pollen concentrations. Otherwise, other meteorological variables such as minimum temperature and rainfall, influence negatively. With respect to the validation of the model, Spearman's correlation test indicated a high degree of association between observed and predicted values ($R = 0.79$ for the pre-peak period and $R = 0.63$ for the post-peak period). Furthermore, no significant differences were revealed according to the Wilcoxon signed-rank test.

Conclusion: The seasonality of the pollen time series summarize the behavior of the pollen curve in relation to the phenological cycle of the *Poaceae* species. This component was isolated from the time series and was used in order to predict the daily concentration of *Poaceae* pollen in conjunction with the modeling of the residual which is partly response of the short-term meteorological variations. This methodology has been suitable in order to predict a pollen season as complex as the one of *Poaceae* and it can be used to predict other complex pollen seasons.

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Atmospheric concentrations of pollen grain and real time information

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Background: Currently in Europe, the method to detect and quantify the pollen grain in the atmosphere is based on a pollen trap 'Hirst-Type' and microscopy analysis. The methodology is robust and efficient to provide exact information but the results are available only a week after the sampling. Recently lots of efforts have been done by the scientific community to build new automated instruments for pollen detection and identification. All these instruments are size segregated optical particle counters. Some of them are combining two analytical processes which are the single particle counting associated to the fluorescence of each particle. In this study we are focusing on some of them: the KH3000, the FIDAS 200 and the WIBS.

Method: The KH3000 is a particle counter and has been developed by the University of Kyoto (Japan). The operation of this device is based on the diffraction of the light due to the pollens which pass in front of a laser beam. This mechanism allows getting the pollens concentration after a calibration factor. The FIDAS 200 is a size segregated particle counter. It has been certified by the TÜV for the measurement of Particulate Matter below 10, 2.5 and 1 μm (PM10, PM2.5, PM1). It allows getting the granulometric distribution of the particles in the two modes number and mass. The Wideband Integrated Bioerosol Sensor (WIBS) measure the size and fluorescence of each particle entering in the detection chamber. These instruments can detect biological particle like pollen, fungal spores

and bacteria. Today, all these devices provide real time data but there are still some artefacts.

Conclusion: The information is not specific and needs to be constrained by the HIRST method. All these devices are, for the moment, complementary to the HIRST method and provide real time information. They can be easily connected to internet and send the data. Today the historical data obtained by the RNSA network combined with one these instruments is useful to get an alert of pollen burst every 1 h.

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Change of major allergens after large-scaled annual mowing of ragweed for twelve years

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Background: Ragweed pollen is an important aeroallergen in North American and Europe. Sensitization rate to ragweed pollen was reported to be as high as 60% in Hungary. Kinmen is an island outside of Taiwan. In 1997, Tsai and his colleagues reported that in Kinmen, sensitization rate to ragweed pollen in patients with allergic rhinitis and/or bronchial asthma was up to 78.9%, comparing to 6.8% in Taipei, the capital of Taiwan. At the same time, the sensitization rate to *Dermatophagoides pteronyssinus* were also different in Kinmen and Taipei (25.7% and 90.6%, respectively). This result let Kinmen government initiate massive ragweed mowing program since 1998. This study is aim to investigate any change of allergen sensitization in Kinmen after massive ragweed mowing for 12 years.

Method: This retrospective study was performed by chart reviews. From January to October in 2011, patients confirmed to have allergic diseases by physicians in Kinmen hospital were included. Serum levels of allergen-specific immunoglobulin E were measured by an automated microfluidic-based multiplexed immunoassay. We compared the sensitization class between patients older and younger than 12 years old by Mann-Whitney *U* test.

Results: One hundred and thirty eligible patients were included. Age ranged from 2 to 79 years old. Patients with allergic rhinitis, allergic conjunctivitis, asthma, and atopic dermatitis were 73.8%, 26.2%, 28.5%, and 26.9% respectively. There were only 13.1% patient sensitized to ragweed pollen. In study population born before massive mowing (older than 13 year-of-age), the sensitization class was significant higher than the younger population ($P < 0.05$). However, the sensitization class to *Dermatophagoides pteronyssinus* was significantly higher in younger population than the older one ($P < 0.05$).

Conclusion: After 12 years of large-scaled ragweed annual mowing program, major allergens in Kinmen changed. Ragweed pollen sensitization rate decreased. *Dermatophagoides pteronyssinus* became the major allergen.

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Development of a method to evaluate cat dander levels by light microscopy

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Background: The cat allergen challenge model using live cats housed in a challenge chamber creates levels of Fel d 1 that generate symptoms in allergic subjects. This model has the advantage that it replicates the exposure subjects receive in their homes and it has been used in several studies. However this approach is complicated by specific disadvantages. Fel d 1 levels can vary substantially over time so that subjects are not exposed to comparable allergen levels on different days and the delay in completing the ELISA assay for Fel d 1 means that the actual levels are only known after the fact. Our purpose in the proposed study is to determine whether it is possible to assess cat dander levels using light microscopy and correlate these levels to Fel d 1 measured by ELISA. This would allow more rapid measurements and tighter control on the allergen levels.

Method: Calibrated air sampling pumps (Gillian 5000, Sensidyne) with attached filters (25 mm, 0.4 μm pore size, Millipore) will be used to measure both Fel d 1 and dander levels. The pump draws air through the filter for a specified period of time at a known flow rate. Cat dander in the air impacts on the filter which is eluted and analysed for Fel d 1. Dander levels can also be measured directly from filters using light microscopy. Our aim is to determine whether parallel samples, one processed for ELISA and the other used for counting dander, can provide comparable information so that the visual count can be used for immediate results and validated by later ELISA measurements. To obtain an initial understanding of the feasibility of this method, measurements will be taken from several locations in a collaborating cat-only hospital. Sampling times will be varied to determine what the optimal duration is for counting dander. In addition, standard histopathological stains will be evaluated for better visualization and counting of dander. In parallel, samples taken in the same location under the same conditions will be sent for Fel d 1 analysis using commercial ELISA. The counts obtained from the microscopic analysis will be correlated with the ELISA results to see if there is a simple and predictable relation between the two.

Conclusion: The results of this study may prove useful in ensuring more stable allergen concentrations in cat challenge chambers.

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Differences in Der p 2 measurements by immunoassays that recognize different isoforms

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Background: The major dust mite allergens from Group 2 account for the largest proportion of IgE reactivity to mite. Fifteen Der p 2 isoforms are listed in the WHO/IUIS Allergen Nomenclature database, which differ in amino acids located in 9 positions. Five of the isoforms (including Der p 2.0101 and Der p 2.0105-0108), have the N114D substitution that impairs binding of mAb 1D8. The goal was to develop an assay that would detect most isoforms.

Methods: Recombinant Der p 2.0101 was expressed in *E. coli*, purified from inclusion bodies and refolded by dialysis. Der p 2.0103 was expressed in *P. pastoris* and purified from culture supernatant by affinity chromatography. Antibody reactivity and allergen content in commercially available extracts were measured by ELISA. Allergen content was measured using either 1D8 or DpX as coating monoclonal antibodies (mAb), and biotinylated 7A1 as detection mAb.

Results: Five anti-Der p 2 mAb were tested for binding to isoforms Der p 2.0101 and Der p 2.0103. Three mAb (5H7, 7A1 and DpX) detected both isoforms at similar levels, whereas two showed low detection of Der p 2.0101 (1D8 and 10E11). An assay that used 1D8 as a coating mAb measured 75% of the amount of natural Der p 2 measured by an assay that detects

Der p 2.0101 (DpX as coating antibody). This value was in line with the composition by mass spectrometry of the natural Der p 2 preparation used as a reference. However, the average ratio between allergen levels in mite extracts measured by the 1D8 assay vs levels measured by the Dpx assay that does recognize Der p 2.0101 was 1.9 ± 0.6 (range 1.2–3.0 for $n = 5$ extracts). This unexpected result showed that the Der p 2 levels were higher with the 1D8 assay than with the DpX assay, if Der p 2.0103 was used as standard, and suggested assay differences in detection of different isoforms in the extracts. The 1D8 assay might also detect additional isoforms not recognized by DpX. The ratio range indicates differences in isoform composition among *D. pteronyssinus* mite extracts.

Conclusions: Allergen isoform composition is a source of variability in assessment of environmental exposure when amino acid substitutions are involved in recognition of the antibodies used for the assay. Analysis of antigenic determinants in allergens can contribute to improve tests for assessment of allergen exposure.

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Cat and dog allergen concentrations in day-care centres and dwellings on electrostatic dust collectors

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Background: Young children are not exclusively exposed to indoor allergens in their homes but also in day-care centres. Aim of the study was to compare pet allergen concentrations in airborne settled dust from day-care centres and dwellings and to analyse the parameters which influence exposure.

Method: In 20 day-care centres in Germany (168 rooms) and in parallel in the children's and day-care workers' homes (227 rooms) electrostatic dust collectors (EDC) were used four times a year for 14 days to collect settled airborne dust (620 samples in day care centres, 602 in homes). The samples were extracted and analysed with fluorescence enzyme-immunoassays based on monoclonal antibodies (Indoor Biotechnologies, Charlottesville, US) to Fel d 1 (major cat allergen) and Can f 1 (major dog allergen).The influences on pet allergen concentrations were analysed in mixed linear models considering values below detection limit (LOD: 7 ng/m²).

Results: Pet allergen concentrations on EDC were on average much higher in day-care centres than in homes although lower than in homes with pets. In day-care centres, 90% of samples were positive for Fel d 1 (median 55.4 ng/m²) and 95% for Can f 1 (median 52 ng/m²). In homes without a cat (91.7% of samples) 27% contained Fel d 1, and in homes without a dog (84.2% of samples) 39% contained Can f 1. In homes with cats, the median Fel d 1 was 486 ng/m²; in homes with dogs, the median Can f 1 level was 578 ng/m². Thus, the most significant factor for pet allergens in homes was the presence of the respective animal, increasing levels more than 100-fold. Whereas the room type had no significant influence on pet allergen levels in homes, carpets covering more than 50% of the floor gave significantly lower levels of Can f 1 on EDC. In day-care centres, in group rooms the highest pet allergen levels were found followed by side rooms and corridors. The levels in these rooms were significantly higher than in staff and sleeping rooms. Both, in homes and day-care centres, significantly higher allergen levels were found in autumn (and for Can f 1 also in winter) than in summer and spring. In addition, less frequent room usage in day-care centres gave lower allergen levels.

Conclusion: On average, higher concentrations of cat and dog allergens in settled airborne dust were found in day-care centres than in homes. Pet allergen levels were dependent on the season, room type and frequency of usage in day-care centres, and animal presence in homes.

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Grass pollen season 2015 in Vienna (Austria), Berlin (Germany) and Turku (Finland): spatial and temporal variation in pollination of different grass species and their impact on pollen allergy sufferers

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Background: Grasses are one of the largest plant families with nearly ubiquitous distribution. Moreover, grasses are the most important aeroallergens worldwide with sensitization rates up to 30%. The grass pollen season is not homogenous and thus more than one peak can occur during the pollen season, since it is composed of the flowering periods of many different grasses. Furthermore, different grass taxa are represented in different regions, allergenicity of grass pollen varies from species to species and sensitization to allergens within a single grass species might differ individually.

Method: Several grasses, that are most prevalent in the respective areas of Austria (Vienna), Germany (Berlin) and Finland (Turku) were included in this study and evaluated by use of phenology, pollen monitoring and symptom data. Phenological observations were performed at various locations in the respective cities and compared with local pollen measurements as well as to data entries from the Patient′s Hayfever Diary (PHD; www.pollendiary.-com), including exclusively symptom data from people suffering from allergic symptoms in the area of the respective cities and surroundings.

Results: Preliminary results indicate that in all three European observation sites Kentucky bluegrass (Poa pratensis) and fescue grasses (Festuca sp.) are important contributors in the grass pollen season. In Berlin (Germany) and Vienna (Austria) the flowering of orchard grass (Dactylis glomerata) and false-oat grass (Arrhenatherum elatius) indicate a greater importance whereas in Turku (Finland) a broader spectrum of varying grass species contributed to the main grass pollen season. Thus, depending on the locality different grass species show regional distinctions and unique grass compositions.

Conclusion: This study represents an unique approach combining phenological observations, pollen monitoring and symptom data in order to provide an insight into the contribution of various grass species in different European regions to the main grass pollen season and the impact on the allergic burden of grass pollen allergy sufferers.

Poster Discussion Session PDS 21

Non IgE-mediated food allergy

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In adult eosinophilic esophagitis patients, amino acid-based diet induces histological remission, reduces clinical symptoms and restores esophageal mucosal integrity whereas it does not affect duodenal integrity

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Background: The pathophysiology of eosinophilic esophagitis (EoE) is mainly driven by food allergy, whereby an increase in esophageal mucosal permeability might facilitate transepithelial allergen flux. Duodenal permeability has also been suggested to be impaired in EoE, implying that this could serve as another entry port of allergens. Studies on the effect of amino acid-based diets in adults are scarce and adherence is challenging. The aim of this study was to assess the effect of a ready-to-drink amino acid-based formula (Neocate, Nutricia) on eosinophilic inflammation and to study its effect on the integrity of esophageal and duodenal mucosa.
Method: In this prospective study 21 adult patients with active EoE were included. In order to obtain reference values, 8 healthy subjects (HS) were included. Patients underwent endoscopy before and 4 weeks after treatment. Clinical, endoscopic and histological responses, along with the esophageal and duodenal mucosal integrity were evaluated, using electrical tissue impedance *in vivo* and transepithelial electrical resistance (TER) and molecule flux through esophageal and duodenal biopsies in Ussing chambers. Additionally a validated sugar absorption method was performed using lactulose: mannitol (L/M) ratios as a measure of duodenal permeability.
Results: Peak eosinophil count decreased significantly after the diet from 40 to 9 per high power field (hpf) ($P < 0.001$). In total, 17 (81%) patients completed the diet, 12 (71%) patients showed complete histological response (≤15 eosinophils) and 4 (24%) patients showed partial histological response (≥50% decrease of eosinophils). Symptoms decreased substantially and 15 (88%) patients became asymptomatic ($P < 0.001$). Additionally a strong improvement of endoscopic signs was observed ($P < 0.001$). Esophageal permeability decreased and mucosal impedance and TER increased significantly after diet ($P < 0.05$). All parameters, except from TER, reached values comparable to those of HS. Duodenal permeability and impedance did not significantly differ between patients and HS and values seem not to be affected by treatment. Additionally, there was no significant difference between L/M ratios of HS and EoE patients.
Conclusion: This study strongly indicates that in adults with EoE, an amino acid-based diet reduces eosinophilic inflammation, induces clinical remission and restores the esophageal mucosal integrity, whereas the duodenal mucosal integrity seems not to be affected.

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Polyphenol-enriched plant extracts differently modulate skin, lung and esophageal allergic inflammation

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Background: Polyphenols are naturally derived bioactive compounds with numerous reported health benefits. We have previously reported on the beneficial effect of polyphenol-derived apple extract in a murine model of food allergy.
Aim: Here we aimed at comparing the potential beneficial effect of different polyphenol-enriched plant extracts in an atopic dermatitis model also displaying eosinophilic esophagitis, and allergic airway inflammation. We selected based on polyphenol contents, polyphenol-enriched extracts from cocoa and apple (containing epicatechin and/or procyanidins), from thyme (rosmarinic acid), from green coffee (chlorogenic acid) and from pomegranate (ellagic acid).
Methods: We used a murine model of atopic dermatitis induced by two epicutaneous sensitizations with *Aspergillus fumigatus,* followed by an intranasal allergen challenge to induce allergic airway and esophageal inflammation. Polyphenol-enriched plant extracts were supplemented in the diet. Atopic dermatitis symptoms and transepidermal water loss were assessed as well as eosinophilic infiltration in the esophagus and the lungs.
Results: All the plant extracts, but polyphenol-enriched apple extract, showed a significant decrease in eosinophilic esophageal inflammation and transepidermal water loss. This effect on skin and esophageal inflammation was only observed when the food extracts enriched in polyphenols were supplemented for treatment (during and after the allergen challenge). No effect was observed in the skin, the esophagus or the lungs, when the polyphenols were supplemented for the prevention of the allergic inflammation. Total and specific IgE levels were never significantly decreased with any of the extracts. Polyphenol-enriched green coffee and cocoa extracts were also potent at significantly reducing eosinophilic infiltration in the bronchoalveolar lavage.
Conclusion: Polyphenol-enriched plant extracts may not all display beneficial effect in allergic models suggesting that different polyphenols may be potent at decreasing only specific allergic manifestations. Clinical relevance: Polyphenols may confer benefit for the treatment of allergic features and symptoms. Human clinical trials confirming this effect are warranted.

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Eosinophilic esophagitis in adults: results from our follow-up

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Background: Eosinophilic esophagitis (EoE) is a chronic inflammatory disease of the esophagus consequent to an inadequate response of the esophageal mucosa to an allergic/immuno-mediated stimuli, characterized by eosinophilic infiltration and clinical alternation of acute and remission phases. The EoE affects 4 persons every

10 000 inhabitants, with higher incidence in males (M: F = 3:1). Outset can interest any age.We present our data regarding the clinical follow up of a population of 31 adult patients aged between 18 and 62 (24 M, 7 F, mean age 34.9 years). Our aim is to analyze the rate of clinical relapse after a year since cessation of dietetic and pharmacologic therapies.

Method: 31 patients with a diagnosis of EoE according to the current guidelines were clinically revaluated after 12 months from therapy interruption. All the patients undergone a 8 weeks-long therapy with oral PPI and swallowed fluticasone propionate with clinical benefit. A dietetic therapy was associated: 4 patients followed the six-food elimination diet (SFED), 19 eliminated the foods towards they were sensitized according to skin prick test and IgE assay, while 8 did not followed any diet. We assessed the clinical situation of the patients with a questionnaire (Mayo Clinic ®).

Results: After 12 months from therapeutic interruption, 5 patients (16%) present a clinical and histological recurrence of EoE, while only 2 had signs of macroscopic lesions at gastroscopy. Of these patients previously 3 followed SFED, 2 specific IgE-targeted diet, 1 did not undergone any dietetic regimen. The other 26 patients were clinically free of symptoms and did not repeat endoscopic exams. 75% of patients submitted to SFED presents a relapse within 12 months, while only 10.5% of those who followed a IgE-targeted diet and 12.2% of who did not undergo any diet presents a recurrence of EoE.

Conclusion: Our results seems to support the prescription of a IgE-targeted diet and discourage SFED. Our data are derived from a small group of patients and must be confirmed in larger studies. In fact, while the efficacy of IgE targeted diet in children affected by EoE is demonstrated, its role in adults is still under debate.

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Filaggrin and periostin in eosinophilic esophagitis

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Background: Eosinophilic esophagitis (EoE) is a chronic inflammatory esophageal disease resulting from a complex interplay between environmental and genetic factors. Limited previous data suggest that the expression of filaggrin (FLG) and periostin (POSTN) proteins may be dysregulated in the inflamed mucosa of EoE patients, but the exact role of these candidate biomarkers remains poorly investigated.

Method: A total of 61 prospectively collected paediatric cases, including 40 children with EoE and 21 children with GERD, as well as a control group of 14 sex and age-matched healthy children, were included in the study. The immunohistochemical expression of FLG and POSTN was evaluated in esophageal biopsies obtained from patients and archived esophageal tissue samples from controls. The immunohistochemistry results were correlated with EoE-related clinicopathological parameters, including severity of symptoms, maximum number of eosinophils (EOS)/high power microscopic field (HPF) and treatment data.

Results: (+) FLG and (−) POSTN staining were observed in all esophageal biopsies from normal controls. In contrast, FLG and POSTN stained (−) and (+), respectively, in all pretreatment biopsies obtained from patients with EoE, while FLG and POSTN stained (+) in 57.1% and 95.2% of GERD cases, respectively ($P < 0.001$). A statistically significant decrease of the proportion of cases with (−) FLG and (+) POSTN staining was observed from the pretreatment to the post-treatment biopsy in the subgroup of patients with EoE ($P < 0.001$).

Conclusion: Our results suggest that FLG and POSTN expression may be downregulated and upregulated, respectively, in the esophageal mucosa of patients with active EoE, and these changes may be restored with treatment in a significant percentage of patients.

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A phenotypical approach of Greek pediatric population with FPIES

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Background: Food protein-induced enterocolitis syndrome (FPIES) is a non-IgE-mediated food allergy, often unrecognized, while its diagnosis is mainly based on clinical features and is not usually straightforward.

Objective: To characterize clinical features and triggers of children with FPIES in a tertiary pediatric Allergy clinic in Greece.

Method: This is a retrospective study on records between October 2010 and October 2015. Diagnosis was based on self-reported clinical history as well as in conducting oral food challenges (OFCs) to children with unclear clinical reaction in implicated food. Age at diagnosis, offending foods, symptoms at reaction were recorded. OFCs were performed either to confirm diagnosis (in case of atypical history) or at a later time point, at least 12 months, in order to evaluate potential tolerance.

Results: Fifty one patients (26 males, 25 females) were diagnosed with FPIES (mean age at diagnosis 13.14 months). The predominant symptom at presentation was vomiting (100%), followed by lethargy (7.84%) and diarrhea (3.9%). We performed OFCs to 44 of 51 patients (7 to confirm diagnosis, 37 to evaluate potential tolerance). The most prevalent implicated foods were cow's milk (52.94%) and fish (27.45%). In children with 2 confirmed episodes, the result of OFC was independent from both 1st and 2nd episode's age. OFCs have been conducted 12–36 months after diagnosis. No statistical significant difference in relation to the positive or the negative outcome of OFC was observed, when this interval was smaller or bigger ($P = 0.246$). OFC's outcome seemed not to be correlated with type of food ($P = 0.085$), neither with personal history of atopy ($P = 0.402$). Even there was no correlation ($P = 0.362$) between food product and OFC's outcome after diagnosis, we noticed that 50% of patient with FPIES to fish had positive challenge, compared to other food products (cow's milk 16.66% and 0% the other foods).

Conclusion: The main symptom in children with FPIES is vomiting while the main offending foods are cow's milk and fish in our setting. We didn't identify any relationship between FPIES and other known allergic history. It seems that the syndrome caused by fish has slower remission. Our decision to challenge our patients in shorter interval (12 months) didn't seem to affect the result of OFC.

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Food protein-induced enterocolitis syndrome (FPIES) for Razor Shell (Ensis sp.)

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Background: Food Protein-Induced Enterocolitis Syndrome (FPIES) is a non-IgE-mediated gastrointestinal food hypersensitivity disorder thought to be cell-mediated, although the exact pathophysiologic

mechanism requires further study. It predominantly occurs in childhood but could also affect adults. Cow's milk and soy are the most common causes of FPIES, but cereal grains (rice, oat, and barley), fish, shellfish, poultry and vegetables may also cause it. We present a case of FPIES for Razor Shell (scientific name Ensis) which diagnosis is based on clinical history.

Method: The patient is a 47 years old woman, with no previous history of food allergy and no other significant comorbilities, who suffered two episodes in different occasions of profuse vomiting, diarrhea and pre-syncopal symptoms approximately 1 h after eating Razor Shell. No one else who ate the same food, in both occasions, experimented such clinic. Actually, she tolerates the rest of shellfish. After taking a detailed clinical history, we performed a standard Skin Prick-test, expanded with shellfish and a Prick by Prick with cooked and raw Ensis. We also measured the IgEs blood level of Anisakis and of the shellfish available in our laboratory. According to the severity of the clinic and the possibility of easily avoiding such food, we considered not indicated performing an oral food challenge (OFG).

Results: At the Skin Prick Test we obtained only a slight positivity to Anisakis, the rest was negative. Total IgE were 14.40 IU/ml, IgEs to Anisakis were 0.47 KU/L and to Clam, Crab, Shrimp, Oyster, Mussel and Lobster were 0.00 KU/l.

Conclusion: Our patient presented a case of FPIES caused by a specific kind of shellfish with tolerance to the rest. Nowadays we haven't methods different from the OFG to confirm this syndrome and it remarks the extreme importance of taking a detailed clinical history.

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Food protein-induced enterocolitis syndrome caused by fish: clinical features of a children population in Madrid

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Background: Food Protein-Induced Enterocolitis Syndrome (FPIES), is an uncommon non IgE mediated gastrointestinal food hypersensitivity, typically appears in the first year of life, usually due to cow's milk or soy. Among solid foods, rice is the most frequent cause, but other foods have been reported to be responsible of FPIES.

Method: A retrospective study including all the children diagnosed of FPIES by fish since 1996 was performed. The diagnosis was made based on clinical criteria. Skin Prick Test (SPT) were performed with commercial extracts to fish. Serum fish specific IgE antibodies were measured by CAP System. Oral Food Challenge (OFC) were performed to verify if tolerance to trigger food was achieved. Relation with other atopic diseases were also collected.

Results: Fifty-seven children were included [34 male (54.6%) and 23 female (40.4%)], mean age of debut 13 months (range 6–96). Most common symptoms were vomiting (63.2%), vomiting with diarrhea (28.1%) and diarrhea (3.5%). Fish more frecuently involved were hake (87.5%), sole (28.6%) and megrim (26.8%). Ninety-four OFC were performed in 38 children, and 41 of those were positive with this main symptoms: vomiting (56%), vomiting with diarrhea (16%) and vomiting with other symptoms (12%) as pallor, cyanosis and abdominal pain. Twenty-two patients achieved complete tolerance to fish (38.6%) median of age of 4 (range 2.25–7), eleven accomplish partial tolerance to some fishes, such as tuna and swordfish, median age of 4.5 (range 2–9.9). Relation with other allergic diseases were counted. Atopic Dermatitis and rhino-conjunctivitis were the most common, in 29.8% and 22.8% respectively. Twenty children had IgE mediated allergy to other food and two had FPIES to cereal and cow milk proteins.

Conclusion: In our patients 38.6% achieved tolerance to the offending fish and 19.3% partial tolerance. More than a half of the children reached tolerance to some type of fish. Tuna and swordfish are the better tolerated fish in those children that did not tolerate the offending fish.

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Establishing the prevalence of low vitamin D levels in children with non-IgE mediated gastrointestinal food allergy

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Background: There is evidence that food allergic children are at risk of developing vitamin D deficiency. There is no data on the prevalence of vitamin D deficiency in non-IgE mediated gastrointestinal food allergy. The aims of our study were to understand the prevalence of vitamin D insufficiency and deficiency in children with non-IgE mediated gastrointestinal food allergy and identify predisposing factors.

Method: A retrospective study was performed at Great Ormond Street Hospital from January 2002 to September 2015. Children 0–18 years old with a confirmed diagnosis of non-IgE mediated gastrointestinal food allergy who had a vitamin D level measured during the course of their disease were included. Vitamin D levels were defined as normal (≥ 50 nmol/l) or low (<50 nmol/l), more specifically, insufficient levels were defined as 25–50 nmol/l and deficient levels as <25 nmol/l. Patient characteristics (i.e. gender, age, ethnicity) and clinical factors (i.e. gastrointestinal symptoms, food elimination diets) were also recorded.

Results: Ninety-two patients met the study criteria; 49% were female and median age was 10 years 2 months [IQR: 4 years 8 months to 13 years 7 months]. Of the cohort, 26% (24/92) had low vitamin D levels; 16% had insufficient vitamin D levels and 10% had vitamin D deficiency. Gender (being female) ($P = 0.043$) and age ($P = 0.035$) were significantly associated with low vitamin D levels. Twelve percent of children who were on an amino acid formula (AAF) had low vitamin D compared to 31% of children who were not on an AAF ($P = 0.06$). No other clinical factors were found to be significantly associated with low vitamin D levels.

Conclusion: Children with non-IgE mediated gastrointestinal food allergy seem to be at risk of vitamin D insufficiency and deficiency, especially if they are older and female. Further prospective studies need to be performed in all children with non-IgE mediated gastrointestinal food allergies.

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Presenting symptoms of Non-IgE mediated milk allergy in a cohort of infants in a UK allergy clinic

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Background: The clinical presentation of cow's milk allergy varies widely in both severity and symptomatology. Milk allergy can be either immediate onset IgE mediated with symptoms involving the skin, gut and respiratory and cardiovascular systems, or delayed onset non-IgE mediated where gastrointestinal symptoms predominate. IgE mediated milk allergy is better recognised and described as early onset of symptoms and confirmatory allergy tests allow for easier recognition. Whilst non-IgE mediated milk allergy is well recognised, there is little information of the

symptom profile of affected patients. We audited infants attending our service with milk allergy to describe the presentation of non-IgE milk allergy.

Method: We prospectively collected clinical data on children under 12 months of age presenting to our children's allergy service over a two-year period. Cases were identified by clinical presentation with confirmation by improvement or resolution of symptoms with dietary exclusion of all dairy, and negative skin prick tests (0 mm).

Results: We recruited 152 patients (79 male) of whom 76 had non IgE-mediated milk allergy. 27 patients did not have skin prick tests performed in view of young age (most <16 weeks) and have not been included in the analysis. In the non-IgE cohort, 62% of patients were breastfed, 45% of patients had eczema and 73% had a family history of atopy. Mean age at clinic presentation was 24 weeks. The mean age of symptom onset was 8 weeks and time to diagnosis was 13 weeks. 54.8% of the presenting symptoms were gastrointestinal with vomiting, diarrhoea and back arching being the most prevalent symptoms. The most common cutaneous symptom was eczema and 4% of the presenting symptoms were respiratory in nature. The ratio of IgE:non IgE milk allergy in this cohort was 1:1.6.

Conclusion: This is a novel set of data which describes the presenting symptoms of non-IgE mediated milk allergy in a current cohort of infants in an allergy centre in the UK. The ratio of IgE mediated to non-IgE mediated allergy was similar to that encountered a recent pan-European study. There was no incidence of FPIES in this cohort of patients which has been encountered at this allergy centre previously. The results are concordant with the hypothesis that non-IgE mediated milk allergy tends to present with gastrointestinal symptoms.

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Characteristics of single and multiple food allergic infants with proctocolitis

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Background: Food protein induced allergic proctocolitis is a frequent cause of rectal bleeding and considered as one of the major causes of colitis in infants. Our aim was to identify characteristics of infants with proctocolitis, determine whether multiple food allergies affect the presentation or outcomes and to compare colonoscopy performed infants with infants who had no colonoscopy.

Method: Consequtively selected 132 infants with proctocolitis were evaluated. All the infants were diagnosed by a pediatric allergist and/or a pediatric gastroenterologist according to guidelines. Endoscopic evaluation was performed to the infants whose proctocolitis symptoms continued or when clinically indicated. We compared single with multiple food allergic infants and analysed sIgE/SPT positive infants, and colonoscopy performed infants.

Results: Cow's milk (97.7%) was the most common allergen, followed by egg (22%). Forty-five (34.1%) infants had allergy to more than one food. Infants with multiple food allergy had higher eosinophil counts (613 ± 631.2 vs 375 ± 291.9) and had higher frequency of sIgE and/or SPT positive results than patients with single food allergy. Symptoms persisted in 14 (10.6%) patients after age 2. Time from symptoms to diagnosis was longer in colonoscopy (+) infants compared to colonoscopy (-) infants (2.3 ± 2.8 vs 1.3 ± 1.4) ($P < 0.01$). Leucocyte counts ($12\,357.7 \pm 4859.1$ vs 9993.7 ± 3201.1) were higher in infants who underwent colonoscopy.

Conclusion: High serum eosinophil levels and positive sIgE and/or skin prick test results may support multiple food allergy at the time of diagnosis. If the clinician thinks that infant may have multiple food allergy performing SPT/sIgE should be considered earlier.

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Partially hydrolysates in food protein-induced Allergic proctocolitis (FPIAP)

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Background: FPIAP is a benign transient condition with overt rectal bleeding in otherwise healthy infants. In formula-fed infants the reaction is mostly caused by cow's milk (CM) and a highly hydrolysed infant formula is supplied as a substitute to induce tolerance. It was hypothesized that partially hydrolyzed protein formulas (PHF), considerably less expensive, could become an alternative. This study aimed to evaluate the effectiveness of PHF in FPIAP.

Method: Sixty one formula-fedinfants (aged 3–13 weeks, median 6) with mucoid, bloody stools, negative sIgE to CM and highly suspicious for FPIAP, participated in the study. Atopy patch tests were performed in 30 patients, 13 were (43.3%) positive. They received a partially hydrolysate formula (60/40 whey-to-casein ratio, 850 to 1200 Daltons). If the condition did not subside within 1 week, an extensively hydrolysed protein formula was introduced and3 months later another attempt was made. In order to investigate the statistical associationsbetween sex and week of age of intervention and success of our approach,we modeled our data for survival analyses with a Cox proportional hazards model. Results are presented as hazard ratios (HR) with 95% confidence intervals (CI).

Results: In 33/61 (54.1%) infants symptoms resolved within the first few days on PHF, whereas 16/28 (57.1%) tolerated the PHF 3 months later.In total, 49/61 (80.3%) children were successfully fed with PHF after a 3 months period. Sex, and age of intervention, were not related with the time symptoms resolved (HR: 1.11, CI: 0.32–5.89, and HR: 0.93, CI: 0.52–4.99, respectively).

Conclusion: PHF could be considered as an alternative treatment in formula-fed infants diagnosed with FPIAP. In this mild phenotype of non-IgE-mediated food allergic disorder, PHF could be supplied not only as a much less expensive formula, but hopefully as a favorable tolerance induction. The follow up of children participated in the study is continued.

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Severity grading of gastrointestinal allergy in infants

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Background: Non-immunoglobulin E (IgE)-mediated gastrointestinal (GI) allergies are classified according to clinical manifestations such as food protein-induced enterocolitis syndrome, allergic proctocolitis, and enteropathy. As infantile GI allergies do not necessarily fall into such categories, the term 'gastrointestinal allergy in infants' is used to refer to infantile GI allergies in Japan. Although infantile GI allergy is classified considering the symptoms, attempts to grade it according to severity and to understand the relevance of severity grading are few. Hence, we propose a severity grading system for infantile GI allergy and compare the clinical features.

Method: We retrospectively assessed 16 neonates or infants with GI allergy (9 boys; 0 days to 9 months old) treated in Gunma University Hospital since 2003. We classified all the patients according to the severity as follows: GI symptoms only

(Group 1), GI symptoms and poor weight gain (Group 2), and additional systemic symptoms (Group 3). We investigated the blood test results, endoscopic findings, treatments, and prognoses.

Results: Seven patients were classified into Group 1; 3, into Group 2; and 6, into Group 3. In Group 3, the peak peripheral neutrophil count, platelet count, alanine aminotransferase level, and C-reactive protein level were significantly higher, and the lowest levels of hemoglobin, serum total protein and albumin were significantly lower. No significant differences were observed in the total IgE and milk-specific IgE antibody levels. The allergen-specific lymphocyte stimulation test results with milk protein antigens were positive for all the cases and did not significantly differ between the groups, although the severer group showed a trend of the higher stimulation index values. The endoscopic findings in Groups 1 and 2 are erosion and erythema of the mucosa and eosinophil infiltration, while a marked increase in neutrophil infiltration was observed in Group 3. All the cases in Groups 3 switched to a single nutrition, with either extensively hydrolyzed milk or amino acid milk. All the patients tolerated milk proteins, with a trend of later resolution in the more severe cases.

Conclusion: Classification and analysis of infantile GI allergy according to severity may be useful in therapeutic milk type selection and clinical course prediction. Furthermore, it may provide insight about infantile GI allergy pathogenesis.

Poster Discussion Session PDS 22

Effector mechanisms

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A novel disruptive IgE inhibitor: efficacy assessment in non-human primate and human precision-cut lung slices

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Background: Asthma is one of the most common chronic diseases worldwide. An estimated number of 334 million people are affected. The new disruptive inhibitor DARPin bi53_79 was developed to counteract immunoglobulin E (IgE)-dependent allergic symptoms capturing free and dissociating receptor-bound IgE. Efficacy of the inhibitor was evaluated in passively sensitized and allergen-challenged non-human primate (NHP) and human precision-cut lung slices (PCLS). Early airway reaction (EAR) was assessed by bronchoconstriction measurement and histamine release. It was hypothesized that bi53_79 prevents EAR in PCLS by dissociating IgE from primate mast cells.
Method: PCLS with cross airway sections were generated from lung tissue of humans ($n = 3$), common marmosets (*Callithrix jacchus*, $n = 3$), and cynomolgus macaques (*Macaca fascicularis*, $n = 4$). Allergic human plasma containing anti-house dust mite (HDM)-IgE was used to passively sensitize PCLS duplicates overnight. Controls were incubated in non-allergic human plasma. On the next day, the non-sensitized PCLS as well as a sensitized PCLS duplicate remained untreated. Other sensitized PCLS were incubated in increasing concentrations of bi53_79 for 2 h. All PCLS were stimulated with HDM, and the airway area was recorded for 20 minutes. Histamine release was measured from corresponding PCLS supernatants.
Results: Incubation in allergic plasma resulted in HDM-induced bronchoconstriction, which reduced the initial airway area to 31.9 ± 8.0% in human, 74.0 ± 7.8% in marmoset, and 70.7 ± 7.9% in cynomolgus PCLS, respectively. Compared to non-sensitized PCLS, histamine levels were increased (human: 30.8 ± 18.0 ng/ml; marmoset: 2.0 ± 0.7 ng/ml; cynomolgus: 21.4 ± 5.6 ng/ml). Pretreatment of passively sensitized PCLS with bi53_79 prevented EAR by a concentration of 0.5 μM in marmoset and 5 μM in human and cynomolgus PCLS.
Conclusion: In conclusion, allergic asthma symptoms were efficiently prevented by the novel IgE inhibitor DARPin bi53_79, both in human and NHP PCLS. Cynomolgus PCLS showed the highest similarity to the situation in human PCLS, indicating a high potential of this species for future efficacy and risk assessments. Funded by German Research Foundation (DFG)

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Characterization of NET response to adjuvants used in allergy vaccines

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Background: Most subcutaneous allergy vaccines in Europe contain alum as adjuvant, and a few monophosphoryl lipid A (MPL), a TLR-4 agonist. For alum it has been shown in mice that neutrophil-derived DNA mediates adjuvant activity. Neutrophils are the most abundant white blood cell population in humans and part of the innate immune system. As a first line of immune defense their repertoire includes the ability to trap, kill and phagocytose pathogens extracellularly by releasing DNA and granular material, so-called neutrophil extracellular traps (NETs). We intend to characterize the NET response of human neutrophils to alum and MPL and their possible role in the immune response induced by allergen-specific immunotherapy.
Method: Freshly isolated human neutrophils are seeded on coverslips and stimulated with NET-inducing factors including PMA and LPS in comparison to alum and MPL. Formation of NETs is evaluated by staining of DNA or granular proteins and fluorescence microscopy. Time course experiments to assess the amount of extracellular DNA are performed and elastase activity in supernatants determined.
Results: The response to MPL showed expected similarity to the LPS-triggered NET-formation with DNA-fibers, granular myeloperoxidase, elastase or LL-37 sticking to them and intact nuclei. In contrast, alum induced cloud-like NETs, also associated with vital nuclei and granular proteins. None of the adjuvants caused cell death after 3 h, as it was observed with PMA. In time course experiments increased amounts of extracellular DNA was observed with alum. In supernatants increased extracellular elastase activity upon stimulation with both adjuvants was observed.
Conclusion: The two adjuvants induce different distributions of extracellular DNA, which shows the typical features of NETs, DNA associated with granular proteins. In order to further investigate the stimulatory capacities of these NETs, co-cultivation experiments with APCs will be performed.

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Searching for drug targets - Are Solute Carrier (SLC) transport proteins a promising target on human naive and activated CD4+ T cells

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Background: The naive CD4+ T cell forms the basis of the immunologic memory, gives rise to all Th cell subsets and the differentiation process of this central immunological cell must be strictly regulated. Particularly, since we know that a misled T cell differentiation is one of the causes for allergy and asthma. Furthermore it was shown that an imbalance in the ion homeostasis could promote pathologic T cell differentiation. Some members of the large family of solute carrier (SLC) transporters, including ion transport proteins, are already described as targets for approved drugs. Our aim was to map the present SLC members on human naive CD4+ T cells, describe their expression changes during T cell activation and evaluate their potential as T cell drug target.
Methods: Human naive CD4+ T cells were isolated from healthy blood donors and stimulated with aCD3/aCD28 (time

course). Genome-wide microarray-based transcriptomic expression analysis was performed and coupled to bioinformatics localization analysis. On proteomic level, cell surface SLCs were identified and quantified via PAL-qLC-MS/MS technique. Both datasets were scaled to combine and correlate them. All detected SLCs were examined about their status as drug target via a DrugBank search.

Results: 220 SLC-coding transcripts were detected in naive and/or activated CD4+ T cells and for 85 of them, the bioinformatics analysis revealed cell surface localization of the corresponding proteins. On the proteomic level, we measured the expression of 28 proteins belonging to the family of SLCs, 15 of them registered as targets for approved drugs. The comparison of transcriptomic and proteomic data mainly reflects early transcriptomic in later proteomic events.

Conclusion: The correlation analysis of transcriptomic and proteomic level showed, that both levels need to be investigated to decide on a favorable time point to target SLC transporters. The expression of most members of the SLC protein family on protein level is only detected in lower levels on naive CD4+ T cells, but increases after 24 h of activation at the latest. All detected SLC members, besides SLC44A2, show their highest abundance after 48 h of stimulation, which might indicate them as a valuable drug target for interference into later T cell activation.

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Altered presentation of IgE epitopes on allergens plays a critical role in allergenic activity

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Background: Usually a reduction of the allergenic activity (i.e. ability to cross-link mast cell and basophil-bound IgE) of an allergen can only be achieved by a reduction of its IgE reactivity by denaturation, fragmentation, mutation or structural reassembly. So far, only one example of a trimeric form of the major birch pollen allergen Bet v 1 has been reported which despite maintained IgE reactivity exhibited reduced allergenic activity.

Methods: A hybrid molecule consisting of the major grass pollen allergen Phl p 5 and major birch pollen allergen Bet v 1 was expressed as his tagged protein. IgE reactivity and basophil activation of the purified hybrid allergen were studied by ELISA and basophil activation tests and compared to results obtained with the equimolar mixture of Phl p 5 and Bet v 1. Secondary and tertiary structures of the hybrid were investigated by circular dichroism (CD), size exclusion chromatography (SEC) and dynamic light scattering (DLS).

Results: The hybrid exhibited stronger IgE reactivity than the equimolar allergen mixture but unexpectedly showed a reduced allergenic activity. SEC and DLS showed that the hybrid formed stable and soluble high molecular weight aggregates which according to CD preserved alpha helical fold to a large extent.

Conclusion: The Phl p 5/Bet v 1 hybrid is the second known example of a protein which despite maintained IgE reactivity exhibited reduced allergenic activity due to aggregation and thus due to altered presentation and/or orientation of IgE epitopes.

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Alterations in cross-epithelial barrier integrity and inhalant allergen sensitivity in children

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Background: Adenoid tissue plays a role in the innate immune system by the barrier function of the tight junctions in its lymphoepithelial structure. The aim of this study was to evaluate the relationship between status of cross-epithelial barrier elements in adenoid tissue lymphoepithelium and inhalant allergen sensitization.

Method: Children aged 5–15 years who underwent adenotonsillectomy were enrolled consecutively to this study. Adenotonsillectomy tissues were obtained. Occludin, ZO1, e-cadherin, β-catenin, desmoglein, desmoplakin, connexon-43 were stained immunohistochemically in the sections and scored by H-score.

Results: Among the zonula occludens proteins, median for occludin was 84 (40–108) and 304 (280–350) in atopic and nonatopic groups ($P < 0.001$). Median for the other zonula occludens proteins, claudin and ZO-1, were 84 (40–108) vs 304 (280–350) and 100 (84–192) vs 280 (266–320) in atopic and nonatopic groups respectively (<0.001 for all). Similarly, median e-cadherin and β catenin proteins of the zonula adherens were significantly lower in the atopic group compared to the nonatopic group [84 (56–120) vs 280(280–300), $P < 0.001$ and 84 (56–120) vs 280 (280–300), $P < 0.001$ respectively]. Among the desmosomal proteins both desmoglein and desmoplakin H-scores were significantly lower in the atopic group [60 (50–100) vs 280 (260–300), $P < 0.001$ and 105 (87.5–120) vs 280 (67.25–300), $P < 0.001$ respectively]. Moreover, connexin-43 protein of the gap junction was significantly lower in the atopic group compared to the nonatopic group [120 (87.5–150) vs 322.5 (284–355) respectively, $P < 0.001$].

Conclusion: Adenoid tissue that is the main lymphoid tissue of the upper respiratory tract and the initial point of contact with inhalant allergens demonstrates epithelial barrier junctional protein changes in children with inhalant allergen sensitization without clinical allergic disease symptoms. Therefore, it may be concluded that epithelial barrier function and dysfunction play an important role in development of allergen sensitization vs tolerance. Future treatment modalities that target epithelium may need to be centralized in our effort to treat allergic diseases.

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Isolation and characterization of an IgG-derived ScFv specific for the major birch pollen allergen Bet v 1 from a healthy donor immunized with hypoallergenic Bet v 1 fragments: high affinity binding despite germline configuration - challenging the principle of affinity maturation

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Background: Allergen-specific blocking IgG, as induced in the course of specific immunotherapy (SIT) is important for successful SIT outcome by blocking allergen-IgE interactions. The current dogma is that somatic hypermutation is important for high affinity binding of antibodies to antigens.

Methods: We have constructed a combinatorial ScFv library from lymphocytes of a healthy donor who was immunized with hypoallergenic derivatives of the major

birch pollen allergen Bet v 1 to analyse Bet v 1-specific IgG antibodies induced by the immunization. Isolated ScFvs were tested for specificity and cross-reactivity to Bet v 1 and homologous pollen and food allergens and epitope mapping was performed. Possible germline ancestor genes were determined with the ImMunoGeneTics (IMGT) database and mutations were revealed. The affinity to cross-reactive allergens was determined by Surface Plasmon Resonance (SPR) measurements. The ability to inhibit patients' IgE binding to ELISA plate-bound allergens and allergen-induced basophil activation was assessed.

Results: Screening of our ScFv library led to the identification of a Bet v 1-specific ScFv (clone H3-1) directed to the C-terminus of Bet v 1, which cross-reacts with homologous allergens. Although IMGT analysis revealed that H3-1 hardly deviates from germline configuration and that diversity was mostly induced by P- and N-nucleotide insertions, H3-1 binds to Bet v 1 and its homologues with high affinities.

Conclusion: Our results demonstrate that an allergen-specific IgG antibody developed in the course of immunization and it exhibits high affinity binding without showing extensive signs of somatic hypermutation.

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Human rhinovirus 1B infection enhances IL4-induced IgE synthesis by PMBCs

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Background: Infections with respiratory viruses, including rhinoviruses, have been implicated in elicitation of atopic allergy and are considered the major cause of asthma exacerbations. However, cellular and molecular mechanisms of virus interaction with the allergic immune response have not been fully revealed. The aim of the study was to assess the effect of human rhinovirus 1B (Rv1B) on IL-4-induced IgE synthesis by peripheral blood mononuclear cells (PBMCs) from allergic patients and healthy individuals.

Method: PBMCs from 9 patients (3 patients with allergic rhinitis and/or asthma, mean age 35.0 ± 3.5) and 6 healthy, non-allergic individuals (mean age 46.3 ± 5.9) were infected with Rv1B (MOI: 0.1; 0.5 or 1.0) at the 2nd or the 7th day of the cell culture. After 11 days of culture, cell-free supernatants were collected and frozen at −70°C. IgE concentration in cell supernatants were assessed with the fluoroimmunoenzymatic method (UniCAP, Sweden).

Results: In allergic patients, IL-4 added to PBMCs not significantly affected IgE synthesis during 11 days of culture (change from 0.43 ± 0.07 to 0.61 ± 0.11 kU/l, $P > 0.05$). However, infection with Rv1B (MOI 0.5) at the 2nd day of culture increased IgE synthesis in the presence of IL-4 from 0.6 ± 0.11 to 1.44 ± 0.26 kU/l ($P < 0.05$). Infection of PBMCs from allergic patients with Rv1B at the 7th day of the culture did not affect IgE synthesis at any virus MOI tested ($P > 0.05$). In PBMCs from non-atopic individuals IL-4 significantly increased IgE synthesis (from 0.17 ± 0.06 to 0.59 ± 0.22 kU/l, $P < 0.01$) during 11 days of culture. Infection with Rv1B neither at the 2nd nor at the 7th day of the culture affected IL-4-induced IgE synthesis at any rhinovirus concentration ($P > 0.05$). Rv1B infection without the presence of IL-4 did not affect IgE synthesis by PBMCs ($P > 0.05$). Stimulation of PBMCs with mixture of TLR agonists (TLR3, TLR5, TLR9) did not affect IL-4-induced IgE synthesis either in allergic or non-allergic subjects ($P > 0.05$).

Conclusion: Rv1B infection may enhance IL-4-induced IgE synthesis by PBMCs from allergic patients, but not from healthy individuals.

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The role of proteases in allergic sensitisation to birch pollen

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Background: Proteases in allergen sources have been suggested to contribute to primary sensitisation to allergens and exacerbate allergic disorders. Major allergens, for example the house dust mite allergen Der p 1, have long been identified as key players in allergic diseases, showing a defined role of protease activity. However, patients also become sensitised and develop allergy to non-protease allergens such as Der p 2 from dust mites or the major birch pollen allergen, Bet v 1.

Objectives: Hence, using birch pollen as model system we are keen to investigate the role of pollen-derived proteases in sensitisation and allergy development to non-protease allergens.

Methods: Focussing on birch pollen extract, we aim to characterise and purify pollen-derived proteases (PDPs), which might be involved in the process of allergic sensitisation. Preliminary experiments using zymography show existing gelatinase activity, further elucidation of which was done using mass spectrometry and transcriptome analysis.

Results: By using mass spectrometry, we were able to identify non-allergenic proteases in birch pollen. We could cluster the protease into distinct families, which will provide the basis for in detail characterisation of the effects of proteases on the early steps of allergic sensitisation.

Conclusion: It has been reported that allergenic pollen has a substantial proteolytic activity, which will eventually even lead to the degradation of tight junction proteins of epithelial cells. However, none of the known birch pollen allergens have been identified as having protease activity. Through identification of such pollen-derived proteases, we therefore hope to be able to investigate the role of proteolytic activity on immune-polarisation and the onset of allergic sensitisation.

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Rise in total IgE levels during omalizumab therapy is not due to induction of IgE production

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Background: Omalizumab targets free IgE and inhibits its binding to FcεRI on mast cells and basophils. Interestingly during omalizumab therapy an increase in total serum IgE levels has been observed. In the present study we investigated whether the latter is caused by enhanced IgE production via activation of IgE^+ memory B cells upon crosslinking of their B cell receptor by omalizumab.

Method: To investigate this, we intranasally challenged patients (5/group) with omalizumab, placebo or Bet v 1 and

measured total and allergen-specific IgE before and 8 weeks after the challenge.
Results: Intranasal challenge with omalizumab did not induce a change in total or allergen-specific serum IgE levels however challenge with Bet v 1 induced a rise of birch-specific serum IgE as previously reported. Furthermore we tested the effect of omalizumab on IgE production by B cells *in vitro*. Omalizumab did not augment IgE production induced by IL-4 and anti-CD40 in culture. In addition we determined total and allergen-specific serum IgE in patients before and after subcutaneous treatment with omalizumab ($n = 15$) or placebo ($n = 6$).Omalizumab treated patients showed a 2–6 fold increase of total IgE and a polyclonal rise in specific IgE.
Conclusion: In summary, we observed no effect of omalizumab on IgE production by B cells. However a polyclonal rise in allergen specific IgE levels was observed upon subcutaneous treatment. Thus the total IgE increase observed during omalizumab therapy is most likely caused by complex formation of omalizumab with IgE in the blood, thereby prolonging the half-life of IgE.

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Regulation of allergen-specific immune responses through the human members of the T cell immunoglobulin and mucin domain (TIM) family

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Background: The T cell immunoglobulin and mucin domain (TIM) genes have been implicated as allergy and asthma susceptibility genes. In humans this family comprises three molecules, TIM-1, 3 and 4. Human TIM-1 was demonstrated to be associated with protection against atopic diseases and able to mediate increased T cell proliferation and IL-4 production in Th2 cells. TIM-3 has been shown to down-regulate Th1 and Th17 cytokines, indicating that TIMs play a crucial role in T cell immunity particularly Th2 responses. Furthermore, TIMs have been shown to mediate uptake of apoptotic cells via binding of phosphatidyl serine. Moreover, TIMs act as genuine immunomodulatory receptors: TIM-1 has been reported to bind several ligands, namely TIM-4, CD300b and P-selectin and TIM-3 was proposed to act as cellular receptor for CEACAM1.
Method: We analyze the interaction of human TIM-molecules with their ligands and their role in T cell stimulation and regulation. We stimulate Jurkat T-cell reporter lines that stably express TIM-molecules to determine their activation in response to TIM-ligands. Our next steps will be to study the effects of TIM-ligation on proliferation and cytokine production of allergen-specific T cells receiving Signal 1 in the context of TIM-signals.
Results: Our results confirm interaction of TIM-molecules with some of the proposed ligands and point to a weak interaction between TIM-1 and TIM-3. Stimulation of TIM-1 expressing Jurkat in presence of their ligands did not affect reporter activation.
Conclusion: Our reporter assays do not give evidence for a role of TIM-1 in T cell activation.

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Effect of sublingual bacterial immunostimulation on the proliferative capacity and cytokine production of splenocytes from immunized mice

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Background: Sublingual polybacterial immunostimulation is successfully used in the clinical practice for the prophylaxis of recurrent urinary tract infections. MV140 is a preparation that contains a mixture of equal amounts of inactivated selected strains of *Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris* and *Enterococcus faecalis*. This study investigated the systemic response of the sublingual immunostimulation with MV140 in mice, evaluating the *in vitro* proliferative capacity of splenocytes and the production of cytokines to specific and unspecific stimuli.
Method: 32 Balb/C mice, divided in 4 groups of 8 animals each (4 females and 4 males), were immunized sublingually with different concentrations of MV140 (group 1: 10^{10} bacteria/ml, group 2: 10^9 b/ml and group 3: 10^8 b/ml) or with sham. Anesthetized mice received 10 µl of each dose or sham weekly for 1 month and afterwards were sacrificed. Isolated splenocytes were cultured with phytohemagglutinin (PHA), 2 concentrations of MV140 (10^7 b/ml and 10^6 b/ml), sham and culture medium. The proliferation of the splenocytes was measured with Carboxy Fluorescein Succinimidyl Ester (CFSE) after 6 days. Cytokine levels (IFN-γ, TNF-α, IL-10 and IL-2) in supernatants were determined after 48 h with the Cytometric Bead Array.
Results: The proliferative response was dose dependent, being higher in mice immunized with the highest concentration of MV140 (10^{10} b/ml). This proliferation was significantly higher than the sham group in response to specific (MV140) ($P = 0.01$) and to unspecific (PHA) stimuli ($P < 0.001$). The production of IFN-γ, TNF-α and IL-2 was also dose dependent. Levels of IFN-γ and TNF-α were significantly higher in the supernatants from group 1 splenocytes in response to MV140 (10^6 b/ml) ($P < 0.05$). In response to PHA, IFN-γ levels were highest in group 1 splenocytes ($P < 0.05$) and were similar for IL-2 and TNF-α in splenocytes from groups 1 and 2 ($P < 0.05$). IL-10 production was only specific with MV140 stimulation. IL-10 levels were higher in splenocytes from groups 1 and 2 ($P < 0.05$), in response to MV140 (10^6 b/ml), being highest in group 2, whose immunization concentration dose was the same used in the normal clinical practice in humans (10^9 b/ml).
Conclusion: *In vivo* sublingual immunization of mice with MV140 elicits a systemic and strong proliferation of splenocytes in response to specific and non-specific stimuli with a Th1 lymphocytes polarization and high levels of IL-10.

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Regulatory impact of probiotic bacteria on immune system gene expression

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Background: Structural and functional genomics are rapidly developing areas of modern immunology assessing interactions between patterns of bacteria with receptors and signaling pathways of the immunocompetent cells and alteration of the level of gene expression.
Methods: For activation and growth of peripheral blood mononuclear cells and dendritic cells LPS from E.coli, inactivated cells of probiotic bacteria bifidobacteria, and Neisseria and Streptococcus along with their cell walls were used. Gene expression in PBM was assessed under the influence of E.coli LPS ($n = 9$), neisseria ($n = 3$), biofidobacteria ($n = 3$) and streptococcus ($n = 3$) and their cell walls ($n = 9$). Total RNA was isolated by Tri-Reagent. cDNA was synthesized using Superscript cDNA Synthesis Kit (Invitrogen, CA). Hybridization of labeled cDNA was

carried out on a biochip Arrayit Dendritic & Antigen Presenting Cell Pathways Microarrays and scan to Innoscan 700 (Carbon, France). For statistical analysis Expander 6 and Statistica 10.0 were used.

Results: Comparative analysis of gene expression under influence of LPS demonstrated increasing activity of SSL7 gene (chemokine ligand, an attractant for monocytes and macrophages) and CCL7 gene (chemoattractant for monocytes and macrophages) and down regulated expression of CD44 gene (cell adhesion, migration). Bifidobacteria cells and cell walls increased expression of CD4, CD8, CD28, CD86, CD1c and ILR8 genes. Streptococcus and their components increased activity of genes such as TAPBP, TRAP1, CDKN1A, CD1D, IL8, IL8RA, CCL11, CCL13, CCL16, CCL19, CCL3L1, CCL4, CCL5,CCR1, ICAM1,IFNG, IL12B, CD40LG, and CDC42. Regulatory potential of Neisseria emerged with increased expression of CD40L, IL8 and TLR2 genes.

Conclusion: Regulatory effects of probiotic bacteria on immunocompetent cells gene expression was seen. Micro-array technology is a useful approach for molecular-genetic characterization isolates of probiotic bacteria.

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Modulatory capacities and possible implications of soluble Fc-epsilon RI in the IgE-mediated immune response

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Background: IgE-mediated allergies are potentially life threatening immunologic reactions towards otherwise harmless environmental antigens and serum IgE is the common marker for allergy diagnosis. However, allergen-specific IgE levels not always correlate with allergic reactions. The recently discovered soluble form of the high affinity receptor for IgE (sFcepsilonRI) present in serum may interfere with IgE levels. Currently, the humanized monoclonal anti-IgE antibody Omalizumab (OmAb) is the accepted treatment for some IgE-mediated diseases. However, its mechanism of actions is not clearly understood.

Method: The modulatory capacities of sFcepsilonRI were analysed using a Mel-JuSo cell line stably transfected with functional FcepsilonRI. Cells were stimulated with chimeric IgE (cIgE) plus its specific ovalbumin (NP-OVAL). To measure the blocking capacity, FAB-like (Facilitated Antigen Binding) tests were performed with sFcepsilonRI collected from the supernatants, and this blocking effect was compared with the effect OmAb. The capacity of sFcepsilonRI to remove already bound-cIgE from the cell surface was also measured and compared with OmAb. Change on FcepsilonRI surface expression and bound-cIgE were measured by flow cytometry. Besides, serum sFcepsilonRI levels from defined food allergic patients from different regions were measured by ELISA.

Results: sFcepsilonRI expression in the supernatant increased in a dose-dependent manner upon cIgE (5–30 μg/ml) plus NP-OVAL (1–100 μg/ml) stimulation. The blocking capacity of sFcepsilonRI reached 57–63% of inhibition in cIgE-FcepsilonRI binding, showing a similar effect to OmAb with a higher efficiency. Moreover, preliminary data suggest that the effect of sFcepsilonRI to remove already bound-cIgE is comparable to OmAb. After analysing the sFcepsilonRI content in serum from different patients groups, we found a high positive correlation between total and complexed-sFcepsilonRI, confirming that sFcepsilonRI maintains its binding affinity in the serum and it is capable of binding free IgE.

Conclusion: sFcepsilonRI showed to have a similar effect as compared with Omalizumab in the IgE-mediated response. It also maintains the high IgE-binding affinity and inhibits IgE-FcepsilonRI binding. Thus sFcepsilonRI could be an important player in the complex signalling pathway of the allergic response.

Poster Discussion Session PDS 23

Experimental studies in asthma

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The mixture of siRNAs targeted to IL-4 and IL-13 genes effectively reduces of the airway hyperresponseveness and allergic inflammation in a mouse model of asthma

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Background: Allergic asthma (AA) is one of the most common chronic inflammatory disease of airways. IL-4 and IL-13 play a key role in AA pathogenesis. They induce IgE synthesis, eosinophil infiltration into the lungs and increase of airway hyperreactivity, that make these cytokines the promising targets for treatment. RNA interference provides a novel approach for regulation of gene expression by siRNA molecules. The aim of this study was to evaluate whether siRNAs targeted to IL-4 and IL-13 reduce AA symptoms in a mouse model of asthma (MMA).
Method: BALB/c mice were divided into 6 groups. Groups 1-5 were sensitized with i.p. injections of ovalbumin (OVA) (20 μg/mouse) emulsified in $Al(OH)_3$ (2 mg/mouse) on days 0, 14, 28 and challenged by intranasal applications (INA) with 50 μl/mouse of 1% OVA on days 42–44. Group 3 received INA with siRNA targeted to IL-4 (siIL4) on days 41–44 in a total dose of 120 μg/mouse. Group 4 received the same dose of siRNA targeted to IL-13 (siIL13) and group 5 - the mixture of siIL4 and siIL13. The group 2 (negative control) received siRNA targeted to GFP gene (siGFP). Group 1 was not treated with any kind of siRNAs. Group 6 included naïve mice. Serum levels of anti-OVA IgE, IgG1, IgG2a were measured by ELISA. Airway hyperresponsiveness (AHR) to methacholine was measured by whole body plethysmography on day 45. Bronchoalveolar lavage fluid (BALF) was collected on day 46 and analyzed by light microscopy. Left lungs were removed for histological analysis.
Results: The levels of anti-OVA IgE, IgG1, IgG2a in mice treated with siIL4, siIL13 and siIL4/siIL13 mixture were not changed compared to mice treated with siGFP. AHR in mice received siIL4/siIL13 mixture was improved in 21% compared to mice treated with siGFP. Treatment of mice with siIL4 led to decrease of eosinophil cell count in BALF by 25% compared to mice treated with siGFP. INA with siIL13 or siIL4/siIL13 mixture led to even more significant reduction of eosinophil count by 63% and 62%, respectively. The histological analysis of lung tissues demonstrated that treatment with siIL13 or siIL4/siIL13 mixture decreased of allergic inflammation compared to treatment with siGFP.
Conclusion: The treatment of mice with the mixture of siRNAs targeted to both IL-4 and IL-13 is more effective in decreasing of AHR and allergic inflammation in MMA in compare to treatment with siIL4 or siIL13 only that provides a promising approach for the development of novel drugs.

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Novel nanoparticles blocking IL4Rα signaling efficiently control lung inflammation

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Background: Drug resistance and harmful side effects accompanying prolonged corticosteroid treatment of chronic pulmonary diseases prompted the development of more specific anti-inflammatory approaches. Several strategies aiming to block IL4Rα, the receptor for a key pro-inflammatory pathway, were investigated. Their efficiency, however, was limited mostly due to the systematic or subcutaneous route of administrations. To control lung inflammation of OVA sensitized mice via intranasal treatment with biocompatible nanoparticles targeting IL4Rα.
Method: OVA sensitized mice were treated with anti-IL4Rα conjugated nanoparticles. Levels of pro-inflammatory cytokines in lungs and broncho-alveolar lavage fluid (BALF) were determined using cytokine array assay. Effect of nanoparticle treatment on activation of lung inflammatory cells as well as their ability to proliferate and produce cytokines were determined using FACS analysis. Lung inflammation was also monitored using immunohistochemistry staining.
Results: Treatment of mice with anti-IL4Rα nanoparticles significantly decreased pro-inflammatory cytokine expression and release in BALF fluid and airway lung tissue. Levels of lung tissue lymphocytes, neutrophil and eosinophil were also dropped. Interestingly, anti-IL4Rα nanoparticles deactivated lung tissue CD4 and CD8 T cells and paralyzed their ability to produce pro-inflammatory cytokines to a significantly lower level than treating with free anti-IL4Rα. Moreover, they induced a sustained low level of lung inflammation for one week following last instillation, contrary to the case with free anti-IL4Rα antibodies.
Conclusion: Together, these data suggested that the enhanced tissue penetrability and sustainability of these nanoparticles improved strength and durability of anti-IL4Rα immunosuppressive effects.

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Role of protease inhibitor in allergic sensitization and effector phase response in mouse

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Background: Proteases play a crucial role in allergic sensitization by activation of both structural and immune cells to release inflammatory mediators. The present study was aimed to elucidate the effect of cockroach protease in allergen sensitization and effector phase response.
Method: The effect of protease inhibitor 4-(2-Aminoethyl) benzenesulfonyl fluoride (AEBSF) in allergen uptake was evaluated in bone marrow derived dendritic cells (BMDCs) pulsed with cockroach extract (CE) / heat inactivated CE (iCE) and Alexa Fluor 647-conjugated ovalbumin. *In vivo* effect of protease in DCs migration and sensitization was observed by administration of CE/ iCE and AEBSF to mice

during sensitization phase and the effector phase of allergic response.

Results: AEBSF reduced the allergen uptake by BMDCs. AEBSF also reduced the DCs activation by lowing CD86 expression on CE exposure. AEBSF given to CE exposed mice prior to ovalbumin-alexa administration reduced DCs migration to the lung draining lymph nodes (LNs). CE exposure increases cellular infiltration, neutrophils and DCs migration to LNs. It also elevated IL-6, TNFα, in BALF in protease dependent manner whereas, TSLP IL-12 in CE protease independent manner. AEBSF administration reduced IL-4, IL-6 and TSLP in BALF of mice during sensitization phase. During effector phase mice showed increased cellular infiltration including eosinophils and neutrophils and DCs migration to LNs. Immunized mice also showed increased IL-13, GM-CSF, TNFα in CE protease dependent and IL-4, IL-6, IL-33, TSLP in protease independent way in BALF. AEBSF administration during effector phase of allergy reduced IL-4, IL-6, IL-13 and GM-CSF. Furthermore, AEBSF given during sensitization reduced IL-13 and GM-CSF more effectively during effector phase than challenge. However cellular infiltration, airway resistance and IgG1 was more effectively reduced when protease inhibitor given during challenge in comparison to given during sensitization.

Conclusion: Serine protease inhibitor reduced allergen uptake and Th2 skewing during allergen sensitization. The inhibition of serine protease reduced allergic sensitization and work different manner than challenge.

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The effect of a single exposure to house dust mite allergens on gene expression in the airways of wild type and 12/15-lipoxygenase knockout mice

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Background: In allergic asthma patients eicosanoids participate in the regulation of inflammatory response to allergen challenge. The aim of this study was to evaluate the role of 12/15-lipoxygenase (12/15-LOX) in expression of selected genes in the lungs of mice exposed to Dermatophagoides pteronyssinus allergens (Dp).

Method: Allergic airway inflammation was induced in wild type C57Bl and 12/15-LOX knockout (12/15-KO) mice by sensitization and subsequent exposure to Dp. Lung samples were obtained 6 (T6) and 24 (T24) hours after a single nebulization of Dp extract. Sham challenged mice were used as controls. Expression of 84 genes was evaluated using SYBR Green-based microarray. Serum concentration of Dp-specific IgE (Dp-IgE) was evaluated using ELISA.

Results: In all mice elevated levels of Dp-IgE was demonstrated in serum. Six hours after Dp challenge in C57Bl mice significant up-regulation of *Arg1, Ccl12, Ccl24, Ear11, IL-25, IL-5Ra, MMP9* and *Retnlg* expression was demonstrated. At T6 in 12/15-KO mice significant up-regulation of *Ccl12, Ear11, IL-17A, MMP9, PD-1* and *Retnlg* expression was demonstrated. The up-regulation of *IL-17A, PD-1* and *Retnlg* was greater in 12/15-KO than in wild type mice. At T24 in C57Bl mice the strongest up-regulation was demonstrated for *Arg1, CCL17, CCL22, Clca3, Ear11, IL-13, IL17A, IL-9, Muc5, Retnlg*. In 12/15-KO mice greater up-regulation of *Arg1, Clca3, MMP-9,Muc5,and Retnlg*, than in wild type mice was demonstrated. Both at T6 and T24 the expression of *Retnlg* in 12/15-KO mice was more than 2-fold greater than in wild type counterparts.

Conclusion: In the airways, 12/15-LOX may regulate the response to Dp challenge by affecting expression of several genes participating in inflammation and tissue remodeling. The modulatory effect of 12/15-LOX on selected gene expression is seen already 6 h after the allergen challenge.

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Development of experimental allergic asthma model using birch pollen allergenic extract

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Background: The purpose of this study was to compare different modeling variants for development of mouse model of asthma (MMA) using extract from birch (*Betula verrucosa)* pollen *(Bet v)*.

Method: BALB/c mice were i.p. immunized three times (interval between first and second immunizations was 4 weeks and between second and third immunizations – 2 weeks) with different doses of lyophilized *Bet v* extract with or without $Al(OH)_3$. One week after the last immunization mice were pulmonary challenged with *Bet v* extract in a protein equivalent of 100 μg/mouse for 5 consecutive days by intranasal applications (INA). Mice were divided into 9 groups: group 1 was immunized with 5 μg/mouse of *Bet v* extract; group 2 – with 10 μg/mouse of *Bet v* extract; group 3 – 20 μg/mouse of *Bet v* extract; group 4 – 50 μg/mouse of *Bet v* extract; mice of groups 5, 6, 7 and 8 were immunized with the same doses of *Bet v* extract, respectively, but together with 2 mg/mouse of $Al(OH)_3$. Mice in group 9 (negative control) were sham immunized and challenged. Airway hyperresponsiveness (AHR) to methacholine was measured by whole-body plethysmography in 24 h after the last challenge. 48 h after the last challenge bronchoalveolarlavage fluid (BALF) was collected for differential cell count and left lungs were removed for histological examination. 1 week after the last immunization and 48 h after the last challenge serum IgE, IgG1 and IgG2a antibodies to *Bet v* allergens were measured by ELISA.

Results: The highest levels of serum *Bet v*-specific IgE were observed in groups 5, 6, 7 and 8 (immunized together with $Al(OH)_3$), both before and after the challenge. Serum levels of *Bet v*-specific IgG1 and IgG2a in groups 5, 6, 7 and 8 demonstrated the same trend of increasing. AHR in all model groups was significantly higher than that of group 9 (negative control). Elevated eosinophil and lymphocyte counts in BALF were observed in all model groups in comparison with group 9 (negative control). Histological picture of allergic inflammation in lungs (peribronchial and perivascular infiltration with pro-inflammatory cells) was most expressed in groups 5, 6, 7 and 8.

Conclusion: These data indicate that i.p. immunization and INA challenge with birch extract is acceptable approach for obtaining (dose dependent) of mild/moderate sings of MMA that may be useful for preclinical studies of safety and efficacy of new anti-allergic (anti-asthmatic) drugs including allergen-specific immunotherapy.

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Regulatory T cell depletion abolishes the protective effect of dietary galacto-oligosaccharides on eosinophilic airway inflammation in house dust mite-induced asthma

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Background: In a murine model for house dust mite (HDM)-induced asthma, dietary non-digestible galacto-oligosaccharides (GOS) have been shown to suppress allergic symptoms. Previously, $CD25^+$ regulatory T-cells (Treg) were found to

contribute to allergy protection induced by non-digestible oligosaccharides.
Aims: To examine the effect of Treg depletion in HDM-induced asthma and to study the contribution of Treg in the protective effect of dietary intervention with GOS.
Methods: BALB/c mice were intranasally sensitized and challenged with HDM or PBS while being fed a control or a 1 w/w % GOS diet. Treg were depleted with anti-mouse CD25 antibody (PC61). T-helper (Th) cell subtypes in lung and spleen of control diet fed anti-CD25-treated mice were analyzed by flow cytometry and cytokines were measured. In all mice, leukocyte subtypes were analysed in the bronchoalveolar lavage fluid (BALF) and IL-33 and CCL5 measured in lung homogenate supernatants.
Results: Anti-CD25 depleted $CD25^+Foxp3^+$Treg in lung and spleen of control and HDM-allergic mice, while increasing activated Th2 cells and cytokine secretion upon *ex vivo* lung cell restimulation. BALF leukocyte numbers and the percentage of eosinophils increased in HDM-allergic mice and remained unaffected by the anti-CD25 treatment. The GOS diet decreased airway eosinophilia and IL-33 concentrations which was abrogated by anti-CD25 treatment, CCL5 showed the same tendency.
Conclusion: Dietary GOS reduces airway eosinophilia which was abrogated by Treg depletion, indicating regulatory T-cells to contribute to the protective effect of GOS in the prevention of HDM-induced allergic asthma.

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Galectin-9 enhances the effect of allergen-specific sublingual immunotherapy in a *Dermatophagoides farinae*-induced mouse model of chronic asthma

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Background: Allergen-specific sublingual immunotherapy is a potential treatment for allergic asthma. Its effectiveness and underlying mechanism remain to be explored. Galectin-9 (Gal-9) is a β-galactoside-binding protein that exhibits various biologic reactions, such as chemoattraction, cell aggregation, and apoptosis. Recent studies demonstrated that Gal-9 has acts an immunomodulator in excessive immunologic reactions by expanding regulatory T cells (Tregs) and enhancing transforming growth factor-βsignaling. We investigated the efficacy of sublingually administered Gal-9 as an adjuvant to a specific allergen in a Dermatophagoides farinae (Derf)-induced mouse model of chronic asthma.
Method: BALB/c mice were intranasally sensitized with Derf extract every 5 days for 5 weeks, followed by sublingual treatment with Derf-allergen extract for 2 weeks, 5 days a week. Three days after the final sensitization, mice were intranasally challenged with Derf extract. The early asthmatic response (EAR) was evaluated 10 min after the last challenge. Airway hyperreactivity (AHR) was assayed and bronchoalveolar lavage (BAL) was performed 24 h after the last challenge. We analyzed the serum IgE and cytokines levels, as well as the number of inflammatory cells, in the BAL fluid.
Results: Sublingual Derf treatment alone significantly reduced AHR, but not EAR. Sublingual Derf treatment in the presence of Gal-9 significantly reduced both AHR and EAR. The number of eosinophils and the IL-5 levels in the BALF were significantly reduced by sublingual Derf treatment in the presence and absence of Gal-9. Furthermore, IL-13 levels in the BALF and serum IgE levels were significantly reduced by sublingual Derf treatment in the presence of Gal-9, but not in the absence of Gal-9.
Conclusion: Our finding indicated that Gal-9 enhanced the effects of sublingual Derf allergen-specific immunotherapy in a Derf-induced mouse model of chronic asthma. Further studies are required clarify the mechanisms of Gal-9 in sublingual Derf immunotherapy.

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Epicutaneous immunotherapy with a hypoallergenic Bet v 1 suppresses asthmatic features in a murine model of birch pollen allergy

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Background: Due to reduced allergic potency, hypoallergenic variants have been suggested as safer and potentially more efficacious alternative to the corresponding wild-type allergens in allergen-specific immunotherapy. Here, we aimed at investigating the efficacy of recombinant Bet v 1B2, a hypoallergenic folding variant of Bet v 1, in epicutaneous immunotherapy (EPIT) to suppress asthmatic features using a murine model of birch pollen allergy.
Methods: After sensitization with rBet v 1 plus ALUM and intranasal challenges with birch pollen extract, BALB/c mice received EPIT with rBet v 1, or rBet v 1B2 on their depilated back and subsequent intranasal re-challenges with birch pollen extract. After EPIT, the serum levels of Bet v 1-specific IgE antibodies were measured by ELISA and basophil mediator release assay. After the final challenge, lung inflammation in the mice was assessed by histological analysis and counting the number of eosinophils in bronchoalveolar lavage, whereas their lung function was measured by whole body plethysmography.
Results: EPIT with rBet v 1, or rBet v 1B2 did not suppress the serum levels of Bet v 1-specific IgE antibodies. However, EPIT with rBet v 1, or rBet v 1B2 tended to reduce the concentrations of Th2 cytokines (e.g. IL-4 and IL-5) and the number of eosinophils in bronchoalveolar lavage. Remarkably, EPIT with rBet v 1B2, but not with rBet v 1 significantly suppressed development of airway inflammation and impairment of lung function.
Conclusion: This study is the first to show the effect of EPIT with a recombinant form of a hypoallergenic folding variant on suppression of asthmatic features. Our results suggest that rBet v 1B2 along with its reduced IgE-binding capacity could be a preferred therapeutic allergen than wild-type rBet v 1 in EPIT of birch pollen-induced allergic asthma, in particular due to a lower risk of allergic side effect.

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Choline chloride attenuates the allergic airway disease by inhibiting the lysophosphatidylcholine induced allergic manifestation

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Background: Lysophosphatidylcholine (LPC) is involved in allergic airway disease manifestation *via* CD1d restricted NKT cells. Choline chloride (ChCl) and LPC have 'choline' moiety in the structure and we hypothesized that ChCl might inhibit effect of LPC.
Method: To test the hypothesis, mice were sensitized with cockroach extract (CE), challenged with CE or exposed to LPC and 1 h after the challenge or exposure, were given ChCl. Airway hyper response (AHR) was recorded and TLC, DLC, Th2 type cytokines- IL-4 and IL-5, oxidative stress marker- 8-isoprostanes in BALF and lung histology were evaluated. Further, mice were exposed to LPC without sensitizing with CE, and were given ChCl 1 h later and inflammatory parameters were measured.

Results: AHR, TLC, DLC, Th2 type cytokines-IL-4 and IL-5, 8-isoprostanes level in BALF and inflammation score based on lung histology was significantly increased on challenge with CE or exposure to LPC ($P < 0.05$). All the above mentioned parameters were reduced significantly after administering the mice with ChCl 1 h after CE challenge or LPC exposure ($P < 0.05$). The inflammatory parameters were significantly increased in mice exposed to LPC and not sensitized with CE. A significant decrease was observed in the parameters when mice were administered with ChCl after LPC exposure demonstrating the role of ChCl in inhibition of LPC induced allergic airway disease manifestation.
Conclusion: ChCl attenuates the allergic airway disease by inhibiting the LPC induced allergic manifestation.

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Lung expression of IL-33 is not increased in ovalbumin-induced murine asthma model

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Background: Recent studies have shown that IL-33 regulates the expression of IL-5 and IL-13 and can be involved in the bronchial asthma (BA) pathogenesis. The aim of this study was to evaluate the IL-33 expression in different cell types in the mouse model of BA.
Method: In order to induce allergic inflammation and thereby develop BA phenotype the 1st group of female BALB/c mice was sensitized with 20 μg ovalbumin (OVA) mixed with 2 mg aluminum hydroxide on days 1, 14, 27 followed by i.n. challenged with 500 μg OVA on days 40, 41, 42. The 2nd group was naïve. On day 43 sera of peripheral blood were assessed for OVA-specific-IgE and -IgG1 levels by ELISA. Airway hyperresponsiveness (AHR) was measured by plethysmography. On day 44 the bronchoalveolar lavage (BAL) was analyzed for total cell count. The lungs were taken for histological analysis, for mRNA-IL-33 evaluation by qPCR and for preparing of cell suspensions by collagenase digestion, which were stained by fluorophore labeled mAbs to determine IL-33 expression in different cell types by flow cytometry.
Results: After OVA sensitization and challenge the presence of OVA-specific-IgE and -IgG1 antibodies in sera, 2-fold increase in AHR and 1.8-fold increase of total leukocytes in BAL compared to naive mice were observed. Histological analysis of the lung tissues revealed the predominant eosinophil infiltration. These data indicate the induction of allergic inflammation of lungs and the development of BA phenotype in the 1st group. The levels of mRNA-IL-33 in the lung homogenates of mice with BA phenotype and naïve mice were similar. The flow cytometry analysis revealed that the percentage of IL-33$^+$ T-, B- and epithelium cells in mice subjected to BA modelling was similar to naïve animals: 29% vs 25%, 13.3% vs 14.2%, and 33% vs 37%, respectively; wherein the main IL-33 producers were epithelium cells. While, the percentage of IL-33$^+$ neutrophils and the mean of fluorescence intensity were significantly decreased in mice with BA phenotype compared to naïve mice: 39% vs 57% and 951 vs 1170, respectively.
Conclusion: The level of lung mRNA-IL-33 expression and IL-33 protein expression by T-, B- and epithelium cells did not changed after induction of allergic inflammation. However significant downregulation of IL-33 in neutrophils has been occurred. Supported by RSF No 14-15-00894.

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Time-dependent bone marrow neutrophil activation during induced allergic airway inflammation

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Background: Production of reactive oxygen species (ROS) by neutrophils of different stages of maturation can suppress Th2 cell proliferation. At the same time uncontrolled ROS production leads to formation of neutrophil extracellular traps (NETs) that are known to enhance the inflammation. The aim of the present study was to trace alterations of ROS production amplitude and of extracellular nucleotide levels in the population of bone marrow neutrophils of mice with different stages of induced allergic airway inflammation.
Method: Allergic airway inflammation was induced in BALB/c mice using standard protocol of ovalbumin (OVA)-induced asthma model. Neutrophils were obtained from bone marrow cells by negative selection using magnetic separation. ROS production was detected by luminol-based chemiluminescence. Extracellular nucleotide level was measured according to Sytox Green fluorescence.
Results: *In vitro* experiments revealed that OVA did not alter spontaneous and induced ROS production in population of bone marrow neutrophils of intact mice. At the same time the level of extracellular nucleotide that was detected after 4 h of incubation of neutrophils with nonspecific activator phorbol myristate acetate (PMA) significantly increased in presence of OVA. In mice with induced allergic airway inflammation spontaneous ROS production was decreased both at 24 and at 48 h after the last allergen challenge compare to control mice. However amplitude of PMA-induced ROS secretion was significantly increased in mice at acute phase of inflammation and significantly decrease at 48 h after the last allergen challenge compare to control animals. When bone marrow neutrophils of mice with induce allergic inflammation were incubated with PMA significantly reduced extracellular nucleotide level was detected compare to the level in samples of neutrophils from control mice.
Conclusions: Ovalbumin can promote NET formation or necrosis in activated neutrophils. Continuous exposure of sensitized mice to ovalbumin suppress the activity of bone marrow neutrophils.

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PKR activation can induce endoplasmic reticulum stress in neutrophilic severe asthma

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Background: The double-stranded RNA (dsRNA)-activated serine/threonine kinase R (PKR) is well characterized as an essential component of the innate antiviral response. In fact, PKR is implicated in TLR signal transduction in response to bacterial cell wall components. Furthermore, PKR activation is associated with IgE class switching and subsequent induction of IgE-mediated disorders such as allergy and asthma. As well known, PKR phosphorylates e-IF2a, one of branches for unfoled protein response (UPR). Conversely, endoplasmic reticulum (ER) stress activates PKR which stimulates various inflammatory signaling pathways. However, its contribution to the asthmatic inflammation and airway hyperresponsiveness, especially steroid-resistant severe asthma has not yet been elucidated.
Method: In this study, we investigated whether PKR activation is involved in the pathogenic symptoms of severe asthma and which molecular mechanism is associated with the role of PKR in the pathogenesis

of asthma focusing on ER stress with using mice sensitized with ovalbumin (OVA) and lipopolysaccharide (LPS) and challenged with OVA (OVA_{LPS}-OVA mice).

Results: We found that PKR inhibition using 2-AP decreased severe asthmatic features; the number of airway inflammatory cells in bronchioalveolar lavage (BAL) fluids, airway hyperresponsiveness, and the expression of Th2 cytokines, IL-17 and KC in lung tissues. Interestingly, the PKR expression was increased in lung tissues from OVA_{LPS}-OVA mice. Moreover, the phosphorylation of PKR and the expression of ER stress marker, CHOP and GRP78 in primary cultured tracheal epithelial cells from mice.

Conclusion: This study indicates that PKR activation contributes to the pathogenesis of the severe neutrophilic asthma through the induction of ER stress in bronchial epithelium, highlighting the therapeutic potential of PKR inhibitor as well as the novel role of PKR as an immune modulator in allergic airway inflammation.

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Roflumilast ameliorates airway hyperresponsiveness caused by diet-induced obesity in a murine model

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Background: Obese asthma patients respond poorly to conventional asthma medications, resulting in severe symptoms and poor prognosis. Roflumilast, a phosphodiesterase-4 inhibitor that lowers the levels of various substances which are implicated in obese asthmatics, may be effective to treat obese asthmatics. We evaluated the potential of roflumilast as a novel therapeutic agent for obese asthmatics.

Method: We designed three models (diet-induced obesity [DIO], DIO with ovalbumin [OVA], and OVA). We fed C57BL/6J mice a high-fat diet for 3 months with or without OVA sensitization and challenge. Roflumilast or dexamethasone was administered orally thrice at 2-day intervals at the last experimental week.

Results: Airway hyper-responsiveness (AHR) resulting from DIO significantly improved in the roflumilast-treated group, compared with dexamethasone-treated groups. Although DIO did not affect the cell proliferation in bronchoalveolar fluid (BALF), increased fibrosis was seen in the DIO group, which significantly improved by the treatment of roflumilast. DIO-induced changes in adiponectin and leptin levels were improved by roflumilast, while dexamethasone aggravated them. Messenger RNA levels and proteins of tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β, interleukin (IL)-1β, and interferon (IFN)-γ increased in the DIO group, and decreased by roflumilast. The ROS levels was also increased in the DIO group, and decreased by roflumilast. In the DIO-with-OVA and OVA models, roflumilast improved Th1 and Th2 cell activation to a greater extent than dexamethasone.

Conclusion: Roflumilast is significantly more effective than dexamethasone against AHR caused by DIO in the murine model. Roflumilast may represent a promising therapeutic agent for the treatment of obese asthma patients.

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Changes in epithelial barrier components E-cadherin, beta-catenin, EGF with steroid treatment in murine model

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Background: Airway epithelium has become the new therapeutic target in asthma as its central role in asthma pathogenesis is revealed. E-cadherin a transmembrane protein, provides essential architecture and immunological function to the airway epithelium, a barrier structure that plays an essential role in asthma pathogenesis. Epidermal growth factor (EGF) is likely to be important regulators of epithelial restitution by virtue of their ability to stimulate cell migration, proliferation, differentiation and survival. Therefore, we aimed to investigate the influence of steroid treatment on epithelial barrier components e-cadherin and beta-catenin and assess the association of change in EGF with these molecules.

Method: Six-8 week old BALB/c mice weighing 18–20 g kept in hygienic macrolene cages in air-conditioned rooms on a 12 h light/dark cycle. We developed experimental asthma model using intraperitoneal (IP) and inhaled ovalbumin in 16 BALB/c mice.Mice enrolled in the study were grouped into two groups of eight mice each.

Group 1: Received IP saline ($n = 8$).
Group 2: Received IPdexamethasone ($n = 8$).
Group 3: Six mice in which asthma model was not developed was enrolled as the control healthy group.

Samples obtained from the middle zone of the left lung of mice. Sections were stained with Hemotoxylin&Eosin, Toluidin-Blue and Periodic Acid Schiff. Tissues were postfixed with osmium tetroxide.The thin (60–90 nm) sections were obtained and stained with uranyl acetate and lead citrate. E-cadherin, Beta-katenin, Epidermal Growth Factor were stained on lung samples with immunohistochemical indirect avidin-peroxidase method and semi-quantified with H-score.

Results: Median H-scores for groups were 125, 138 and 225 for e-cadherin; 120, 135 and 191 for beta-catenin; in the untreated asthma, steroid treatment and healthy control groups respectively ($P = 0.001$ and $P < 0.001$ respectively). On the other hand EGF H-scores were not significantly different among the groups ($P = 0.13$).

Conclusion: Tight junction proteins E-cadherin and Beta-catenin are significantly lower in asthmatic epithelium as consistent with previous articles that reported impaired physical barrier properties in asthma. Steroid treatment improves levels of these proteins suggesting an epithelial barrier enhancing influence. However, this change in epithelial barrier proteins is not associated with a similar change in EGF.

Poster Discussion Session PDS 24

Atopic and contact dermatitis

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The effect of cord serum 25-hydroxyvitamin D (25(OH)D) on the development of atopic dermatitis(AD) in first 3 years of life: COCOA study

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Background: Prenatal and early-life dietary exposures of vitamin D have been suggested to play an important role in the development of atopic dermatitis (AD) in childhood. But, the association between serum vitamin D deficiency at birth and AD is uncertain. The aim of this study was to investigate the relationship of cord serum 25(OH)D on the development of AD from a prospective birth cohort study (COCOA).

Method: Children aged 0 through 3 years from a birth cohort in the Cohort for Childhood Origin of Asthma and allergic diseases (COCOA) study were enrolled. The cord blood obtained from 831 at birth. 555 of them had complete data by the child's age of 1 year. Also 420 of them had complete data by the child's age of 2 years. And then, 331 of them had complete data by the child's age of 3 years. Therefore, 331 children were included in this study. The 25(OH)D from their cord blood are measured in a chemiluminescence immunoassay (Roche, Indianapolis, IN, USA). And specific IgE antibodies against egg and milk by CAP were performed at age of 1, 3 years. Also skin prick test were conducted at age of 3 years.

Results: The median cord serum 25(OH)D was 17.79 ng/ml. Low cord serum 25(OH)D (<20 ng/ml) is associated with milk sensitization at 1 year of age (aOR, 2.079; 95% CI, 1.038–4.166; P -value, 0.039). Severe cord serum 25(OH)D deficiency (<10 ng/ml) increased the risk of AD at age 2 (aOR, 2.308; 95% CI, 1.034–5.152; P -value, 0.041). And cord serum 25(OH)D is relationship with total IgE at cord blood ($y = -0.073x + 4.583$, $R^2 = 0.025$, $P = 0.069$) and 1 year ($y = -0.284x + 3.8291$, $R^2 = 0.0178$, $P = 0.022$). We also found that cord serum 25(OH)D may affect outcome of AD. Low cord serum 25(OH)D (<20 ng/ml) reduced the remission of AD (aOR, 0.555; 95% CI, 0.302–1.021; P-value, 0.058). And we replicated the associations between cord serum 25(OH)D levels and probability of remission in AD using a cox proportional hazard analysis (HR, 0.492; 95% CI, 0.272–0.889; P -value, 0.019).

Conclusion: Cord serum 25(OH)D were associated with AD, milk sensitization and food allergy. And cord serum 25(OH)D is relationship with total IgE at cord blood and 1 year. Also, low cord serum 25(OH)D reduce the remission of AD. These data suggest that low cord serum 25(OH)D affect the development and prognosis of AD.

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Genetic variants in the epidermal differentiation complex (EDC) genes on chromosome 1q21 are associated with atopic dermatitis. An effect independent of filaggrin mutations?

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Background: Evidence exists that genetic variants in the epidermal differentiation complex (EDC) genes on chromosome 1q21 may be involved in the pathogenesis of atopic dermatitis similar to the well-known FLG mutations. The aim of the study was to investigate the association of SNPs in hornerin (HRNR), small proline rich protein 2B (SPRR2B) and filaggrin-2 (FLG2) genes with the course and risk of atopic dermatitis.

Method: Genotyping for HRNR, SPRR2B and FLG2 was performed in 188 children younger than 2 years of age, previously screened for the 4 common filaggrin (FLG) null mutations. All subjects were selected using a detailed questionnaire and specific IgE measurement were obtained. Atopic dermatitis cases were diagnosed according to the criteria of Hanifin and Rajka and skin examination. Atopic dermatitis severity was assessed by using the SCORing Atopic Dermatitis index (SCORAD). All SNPs were genotyped by real-time PCR assays with subsequent melting curve analysis using a SimpleProbe® probes.

Results: The HRNR rs877776[C] was associated with significantly increased risk for atopic dermatitis (OR 1.99; 95% CI; 1.12–3.56; $P = 0.013$) in both allergic and non-allergic group. Concerning SPRR2B rs6693927 polymorphism, the presence of at least one allele [A] increased the risk for atopic dermatitis (OR = 3.02; 95% CI 1.17, 8.00; $P = 0.011$), but only in non-allergic group. Interestingly, the rs877776 and rs6693927 associations were independent of the well-established FLG risk alleles. There was no significant association between tested rs877776 and rs6693927 variants and atopic dermatitis severity. We also did not find a significant effect of rs877776 and rs6693927 variants on allergic sensitization and allergic sensitization in subjects with atopic dermatitis. For FLG2 (rs12568784) polymorphism, we observed an association of the risk allele [T] with atopic dermatitis (OR = 1.91; 95% CI 1.01, 3.65; $P = 0.033$), severity of the disease (OR = 3.37; 95% CI 1.52, 7.56; $P = 0.001$) and allergic sensitization (OR = 3.76; 95% CI 1.74, 8.19; $P = 0.000$). However, the effect of FLG2 polymorphism didn't remain significant after adjusting for the combined FLG null mutations.

Conclusion: Our results indicate that the HRNR rs877776, SPRR2B rs6693927 and FLG2 rs12568784 variants on the chromosome 1q21, in addition to the FLG gene,

may play a role in the development of atopic dermatitis. Moreover, the HRNR rs877776 and SPRR2B rs6693927 variants are likely to be independent risk factors.

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Filaggrin-independent development of allergic skin lesions in the mouse model for human atopic dermatitis

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Background: Deficiency in filaggrin (FLG) due to loss-of-function mutations in *FLG* gene was previously proposed as the major cause of skin barrier destruction, resulting in development atopic dermatitis (AD). However, FLG deficiency is only part of the explanation why patients can develop AD, as many patients with severe AD have no *FLG* gene mutations. In this study, we measured FLG levels in the skin of NC/Tnd mice, a model for human AD, each time point during development of AD, and evaluated roles of FLG in severity of AD.
Method: FLG levels were examined using western blotting and immunostaining during spontaneous development of AD. Simultaneously, barrier function of the affected skins were monitored by measurement of trans epidermal water loss and skin surface conductance. We also analyzed natural moisturizing factors (NMF), break down products of FLG, by a HPLC procedure.
Results: Compared with the skin of age-matched SPF NC/Tnd mice without AD, the levels of proteolytically cleaved FLG proteins increased in the epidermis of conventional NC/Tnd mice as dermatitis progressed. Immunostaining for FLG was more widespread within the proliferating layer of the epidermis in conventional NC/Tnd mice with AD-like skin lesions. Surprisingly, the concentrations of NMF were significantly higher in the stratum corneum of conventional NC/Tnd mice with AD than in those of SPF NC/Tnd mice without AD.
Conclusion: FLG production was not impaired in NC/Tnd mice; however, development of AD was never prevented. Results indicate that FLG deficiency is not sufficient for the onset of AD. To investigate further mechanisms of AD, NC/Tnd mice would be a suitable model for FLG-independent AD.

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Implicated role of neonatal skin toward atopic dermatitis development in capsaicin-induced AD rat model

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Background: Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease, characterized by severe itching and dysfunction of skin homeostasis. Previously, new AD model was established through capsaicin injection to neonatal rats, which displayed itching behavior and skin inflammation from 3 weeks after injection.
Method: Rats were injected with capsaicin and AD skins were analyzed using immunohistochemistry, RT-PCR, and immunoblot analysis.
Results: Capsaicin-injected AD rats displayed altered proteolytic pattern of filaggrin and corneodesmosin (CDSN), suggesting of underlying alterlation of proteolytic systems.

New-borne rat shows well-developed epidermis, which became thinner till 2 week-age when hair began to grow. After that, epidermal thickness gradually increased, which was co-related with expression of epidermal differentiation markers, suggesting of U-shape epidermal development. To investigate relationship between AD and epidermal development, neonate, 2 and 4 week-age rats were injected with capsaicin and AD symptoms were monitored. A more late injection produced earlier development of AD but AD symptoms were less severe and shorter duration, suggesting of stimulation in neonatal period potentiated AD symptoms. Subsequent immunohistochemical staining for Lgr6 which is epidermal stem cell maker showed persistent expression in AD rat skin in contrast to diminishing pattern in control rat epidermis after hair growth.
Conclusion: These results suggested that neotnatal epidermal development may influence on AD development.

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Altered gut microbial composition at 6 months associated with atopic dermatitis in 12 months old infants

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Background: Microbial colonization of the infant gut is unstable and shows a wide range of diversity between individuals. Increasing evidence suggests that perturbations in the gut microbial composition of infants are implicated in the pathogenesis of atopic dermatitis (AD), while the actual composition of the gut microbiome begins with AD remain unclear. We hypothesized that atopic dermatitis at 12 months could be linked to specific gut microbiome at 6 months. The aim of our study was to evaluate differences in gut microbial composition according atopy and atopic dermatitis.
Method: Twenty-nine infants were enrolled (14 non-atopic healthy, 10 infants with atopic AD, and 5 infants with non-atopic AD in 12 months). All enrolled children were born by vaginal delivery and had no antibiotics or probiotics during the first 6 months of life. All subjects were fed with breastmilk feeding only or a combination of breastmilk and formula during the first 6 months of life. Fecal samples were collected at 6 month. The microbial compositions were analyzed using 16S rRNA gene pyrosequencing.
Results: The proportion of abundant Clostridium g6 was reduced ($P = 0.07$), while the Streptococcus was highly enriched in the AD group at phylum level ($P = 0.01$). The Shannon index was significantly increased in non-atopic AD and atopic-AD subjects compared with non-atopic healthy. Infants with atopic AD had increased levels of Streptococcus and Veillonella and decreased levels of Clostridium g4, Clostridium and Akkermansia.
Conclusion: Gut microbiome at 6 months of age may be linked with the development of AD and atopy in 12 months old infants.

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miRNA changes after probiotics supplement in atopic dermatitis

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Background: Interest is emerging on the role of the skin and gut microbiome in atopic dermatitis (AD). However, the mechanisms underlying these effects remains to be addressed. Epigenetic changes may shed light on these pathways. We want to assess epigenetic changes of genes after probiotics exposures in AD children.
Method: Children with AD who received probiotics (*Lactobacillus)* in a clinical trial were recruited. Initially, 11 AD children with and without prominent SCORAD score decrease after taking probiotics supplement for 3 months were selected for miRNA microarray analysis. The expression changes of differential expressed miRNAs in the samples used for microarray were validated by quantitative PCR. Correlations between the microRNA fold change of target genes before and after probiotics exposure were assessed by pair t test.
Results: By microarray for gene expression profiling, we identified 2 miRNAs (miR-125 and miR-424) that were 2-fold up-or down-regulated after probiotics treatment. After further testing in a larger sample of children, quantitative PCR results confirmed that miR-125 was particularly strongly induced ($P = 0.002$). Then, we applied bioinformatics tools to predict the target genes of miR-125. -language: AR-SA′>Lactobacillus) in a clinical trial were recruited. Initially, 11 AD children with and without prominent SCORAD score decrease after taking probiotics supplement for 3 months were selected for miRNA microarray analysis. The expression changes of differential expressed miRNAs in the samples used for microarray were validated by quantitative PCR. Correlations between the microRNA fold change of target genes before and after probiotics exposure were assessed by pair t test.
Conclusion: Significant miR-125 change was found after taking probiotics. These results suggest that probiotics can alter miRNA expression, a potentially novel mode of probiotics effect.

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miR-432 suppressed inflammation in atopic dermatitis-like animal model

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Background: MicroRNAs (miRNAs) are involved in many diverse biological processes and they may potentially regulate the functions of thousands of gene. And they are well known not only as important modulator of inflammation but also as key factor for AD pathogenesis. However, little is known about the differences in miRNA expression in cell with inflammation. So we trying to find out correlation between miRNAs and inflammation in human mast cell line.
Method: To explore the potential involvement of miRNAs in the inflammation, we analyzed miRNA expression in normal mast cell and activated mast cell using miRNA arrays. We further investigated the function of microRNAs in atopic-like murine model.
Results: We identified 80 miRNAs that were significantly differentially expressed in inflammatory mast cell relative to normal mast cell. We found 55 up regulated miRNAs and 25 down regulated miRNAs among the 5200 miRNAs. One of the highest-ranked up regulated miRNAs in inflammatory mast cell was miR-432, this mircoRNA suppressed inflammation in atopic like murine model.
Conclusion: These results suggest that miR-432 are correlated the inflammation in mast cells, suppressed inflammation in atopic like animal model.

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Staphylococci and acute / chronic form of atopic dermatitis in children

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It is known that *S. aureus* (SA)infects skin lesions in AD.SA strains take the place of coagulazo-negative strains(CNS)and after inflammation easing-off and eradication the bacterium new strains with other properties appear.
Method: The study enrolled119patients with acute AD (averagely aged 4.69 (2 month–17 years). We mostly observed children with acute forms among which erythematosquamous forms-56.3% ($n = 67$), erythematosquamous forms with lichenization 30.3% ($n = 36$), and16 patients (13.4%) had a lichenoid form. We identified SCORAD as follows: in group1 -37.02 ± 5.4; in group2 -51.97 ± 6.1 и in group3 -80.9 ± 7.9. The skin smear seeding was performed on blood agar. The isolates obtained were identified according to the conventional microbiological tests. The staph.types were identified was tested by means of the automated microbiology analyzer 'Walka-way96plus', Siemens Healthcare Diagnostics, Germany.
Results: We detected the staph. presence on skin lesions in the majority of our patients with AD(74%).The study proved that SA and S.epidermidis (SE) prevailed over the other strains (53 and 54 patients respectively). We isolated the following CNS- *S. haemolyticus* (SH), *S. warneri*, *S. hominis*; *S. saprophyticus* (SS),S.sciuri и S.capitis.26% cases showed no bacterial growth. We obtained no evidence for more frequent skin contamination with SA and AD aggravation ($P = 0.49$). 17 patients demonstrated associations of different staph.strains: 9 cases of SE and SA, 2-SH and SA, 1-SH and SE, SS and SA, SE and S.hominis, SE and SW and 1-a triple association of SE, SA and S.hominis. The staph.associations neither accompanied AD more severe aggravation ($P = 0.33$) nor showed a relationship with the different clinical forms of the disease.There was no significant breakdown of the patients by AD exacerbation and different staph.-skin isolation ($P = 0.5$). We noted that SA more frequently contaminated skin in patients with chronic AD.And while comparing the frequency rate among *SA, SE and CNS or SA and SE* we reveled the only tendency ($P = 0.38$, $P = 0.076$), however with all CNS being separated as a single group we positively determined the more frequent detection of SA and the increased lichenization area ($P = 0.051$).
Conclusion: *SA* more frequently contaminated skin in the patients with prolonged AD and chronic inflammation, thus displacing CNS.It is also quite probable that the association of different staph.types on skin points at a certain period of local inflammation progress while skin still retains its ability of controlling SA skin colonization.

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Reliability and validity of the atopic dermatitis symptom score

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Background: We developed Atopic Dermatitis Symptom Score (ADSS) for assessing daily skin symptoms in children with AD. The objective of the present study was to validate its clinical utility.
Method: We enrolled children with the age of 18 years or younger who were diagnosed as AD at Samsung Medical Center ($N = 308$). The patients or caregivers were asked to record daily symptom score by using ADSS in which 6 symptoms (itching, sleep disturbance, erythema, dryness, oozing, and edema) were measured with a scale of 0 to 4. For comparison, the severity of AD was evaluated using the SCORAD index (severity scoring of atopic dermatitis) by pediatric allergist when the patients visited our clinic. Test-retest reliability, discriminant validity, and screening accuracy between the ADSS and SCORAD were assessed. Test-retest reliability was assessed by calculating the intraclass correlation (ICC) between day and night ADSS score. Generalized estimating equation analysis model was used for discriminant validity analysis the association between the ADSS and SCORAD. Receiver operating characteristic (ROC) analyses were conducted to evaluate the cut points of ADSS total score in predicting severe AD (the SCORAD total score of the day ≥ 40). The following statistics were reported for potential cut point: sensitivity, specificity, positive predictive value, negative predictive value, and area under the ROC curve.
Results: Test-retest reliability of the 6 items of daily symptom score for the subset of patients was good (ICC $\geq$ 0.8) between day and night ($n = 138$). Discriminant validity demonstrated a strong association between increased SCORAD index and increased ADSS of the day ($P < 0.0001$). For screening accuracy of ADSS, a cut point of 7 accounted for severe AD. It demonstrated high sensitivity (90.0%), good specificity (78.6%), low positive predictive value (21.1%), high negative predictive value (99.1%), and high area under the ROC curve (0.90).
Conclusion: ADSS is a valid and reliable tool for monitoring daily skin symptom changes in children with AD.

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How reliable is atopic dermatitis on the internet?

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Background: Internet is a very widely used information source and frequently used to obtain information about health, in particular. However, the reliability of the information provided via the Internet is controversial. The aim of this study; is to investigate, with reference to the international guidelines, the contents of Turkish web pages, prepared to provide information about atopic dermatitis (AD).
Method: The web sites, are obtained through the search engine http://www.google.com.tr/, using the keywords atopic dermatitis and eczema. The first 100 pages in the database were examined. Sites are classified according to their source and were examined in terms of definition, diagnostic methods, and treatment recommendations with reference to the international guidelines. Analysis of data was performed as number and a percentage.
Results: In the analysis according to source, most of the pages (29%) were constituted by blog/forum pages, prepared by unknown persons and personal doctor pages (22%). While the proportion of sites with video information was 6%, the proportion of social networking sites was 2%. In the analysis according to their content, the complete and accurate information in terms of diagnosis and treatment of AD; were found in child health and disease and child allergy & immunology specialist personal pages, the page of associations and the Internet encyclopedia Wikipedia. However, the content of the sites in terms of diagnostic approaches were found to be quite low (5%). Similar results are available for the treatment content (10%). While the most accurate treatment approaches were non-pharmacological treatment (17%) and prophylactic treatment (25%), topical medication have been defined in quite insufficient number of sites. The number of sites with proposal of alternative medicine was found to be quite high (28%).
Conclusion: Although the excess of internet sites providing information on atopic dermatitis; it was seen that it is not based on medical resources, and the content of incomplete or incorrect information, mainly involving the review of users, was too much. Incorrect or incomplete information may lead to negative results in the treatment of AD. It is believed that to avoid this situation Internet sites need to be arranged for the content, the existing information needs to be improved in terms of reliability and timeliness.

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Omalizumab treatment in severe atopic dermatitis

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Background: Atopic dermatitis (AD) is a chronic skin disease.A significant percentage of AD patients (pts) have severe forms.Treatment with the antibody monoclonal *omalizumab* induces clinical benefit with few side effects in selected pts with AD refractory to other therapeutics.The objective of this study is to evaluate the efficacy of *omalizumab* in pts with severe AD.
Method: Pts with severe AD refractory to standard therapy were proposed to off-label treatment with *omalizumab*. Laboratory parameters were monitorized before starting *omalizumab* and every 6 months. Clinical response was evaluated using the SCORAD (Scoring Atopic Dermatitis) index and calculating the daily/rescue medication before and after treatment.
Results: 23 atopic pts (13 male) with severe AD, mean age 30.4 years.Average total IgE 8835 (2296–21691)KU/L.Before treatment all pts were medicated with montelukast 10 mg/day, topical/oral steroids (average dose 20 mg/prednisolone/day), topical calcineurin inhibitor and anti-H1/H2 antihistamines (maximum dose).

7 mite-sensitized pts had received specific immunotherapy (SIT) during 16 months (6–36 months) before the treatment.SIT induced a worsening of atopic dermatitis in all the pts.12 pts were also medicated with cyclosporine and 1 of them was previously medicated with intravenous immunoglobulin G (1000 mg/kg/month) with no response and with hepatic toxicity.2 pts were also medicated with azathioprin with no response.

Omalizumab was subcutaneously administrated at doses range 150–600 mg every 2 weeks for average 19.6 months of treatment.

The daily/rescue medication decreased in both dose and number of drugs.Systemic steroids were stopped with no relapse of symptoms.SCORAD index was 63.8

average at the beginning and 28.1 after treatment.

After treatment,all except one patient improved,although the medium time from the beginning of the treatment until the initial improvement was variable.Overall,15 of 23 pts had a complete response (65.2%),6 (26.1%) pts had a partial response and 1 (4.3%) did not improve at all.

5 patients stopped *omalizumab*: 3 pts had a complete answer, 1 patient stopped for inefficacy and only 1 patient stopped the treatment for exuberant local reaction.

Conclusion: *Omalizumab* was effective in atopic dermatitis and may provide a safer alternative when treating pts with severe atopy that is refractory to other therapeutic measures.The treatment with *omalizumab* was associated to a optimal safety profile.

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Sensitive skin and their allergy frequencies in Shanghai women

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Background: Sensitive skin is characterized by subjective complaints of discomforts such as burning, stinging and itching with use of everyday products such as cosmetics or toiletries. It is found in more than 50% of women and 40% of men, creating a sizable demand for products designed to minimize skin discomforts. With suitable guide for sensitive skin individuals, we identified their physiological and biological aspects with self-diagnosed signs in China.

Methods: 153 Shanghai women were recruited for patch test using cosmetic ingredients, sting test using 5% lactic acid in D.W. With these two factors, biophysical assessments (TEWL, pH, Sebum, and Water Contents) and self-diagnosed questionnaire were performed. Skin differences comparing sensitive skin to non-sensitive skin were detected with the parameter of cholesterol, fatty acids, ceramide and NMF using D-squame and LC-MS/MS.

Results: With objective irritation, sting sensation and self-questionnaire criteria, 28 sensitive and 29 non-sensitive skin women were selected. Comparing to non-sensitive skin, TEWL was significantly high on sensitive skin. However, total ceramides (significant), amino acids and fatty acids on sensitive skin were low. As a result of statistical analysis, ceramide NPs might be the important parameters for classifying the sensitive skin and non-sensitive skin. 16% of Shanghai women were characterized as sensitive skin on self-diagnosed questionnaire. Cosmetic uses, weather changes and UV light were the main causes for sensitive skin. They were allergic to metals (21%), pollens (43%), and foods (43%), however, only one person responded on pollen allergy in non-sensitive skin group.

Conclusions: We found sensitive skin has less abundant for lipids on stratum corneum than non-sensitive skin. Although the understanding of this phenomenon is as yet incomplete, these results now support biophysical aspects for sensitive skin on their skin lipid compositions. Furthermore, sensitive skin responded higher allergy frequencies than non-sensitive skin and these results may reflect suitable guidance for their skin problem when using daily products for sensitive skin.

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Time trends of contact allergy to the European baseline series in Lithuania

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Background: Monitoring trends of positive patch test reaction in consecutive patients is useful for epidemiological surveillance. The time trends of positive patch test reactions to standard contact allergens have not been previously reported in Lithuania.

Objectives: The aims of this study were to examine the prevalence of contact allergy to the European baseline series in patients with suspected allergic contact dermatitis (ACD) and to compare changes during 9 years.

Methods: In this retrospective study patch test results of 272 consecutive patients, tested with the European baseline series between 2014 and 2015, were analyzed.

Results: Positive patch test reaction to at least one allergen was observed in 157/272 (57.7%). The top ten most frequent allergens were as follows: nickel sulfate (31.6%), methylisothiazolinone (14.7%), methylchloroisothiazolinone/methylisothiazolinone (MI/MCI) (8.1%), potassium dichromate (6.6%), cobalt chloride (6.3%), *Myroxylon pereirae* resin (5.2%), *p*-phenylenediamine (5.2%), fragrance mix I (4.8%), formaldehyde (4.1%), methyldibromoglutaronitrile (3.3%). Sensitization rates to some allergens changed over 9 years. The increase of the sensitization to MI/MCI (2.3% in 2006–2008 and 8.1% in 2014–2015, $P < 0.0001$) nickel sulfate (16.4% in 2006–2008 and 31.6% in 2014–2015, $P < 0.0001$) and the decrease of sensitization to the paraben mix (3.2% in 2006–2008 and 0.37% in 2014–2015, $P < 0.006$) was observed. Sensitivity rates for the other allergens remained stable or showed only a trend (*Myroxylon pereirae* resin) towards a decrease ($P = 0.06$).

Conclusion: This study provides information on the prevalence of contact allergy in Lithuania. Changing trends in sensitivity rates for some contact allergens probably reflect increasing changes in exposure.

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Treatment management skills in pediatric atopic dermatitis

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Background: Children with atopic dermatitis (AD) and their parents need several treatment management skills. Knowledge of topical therapies, lifestyle adjustments and other treatment management skills are needed for successful treatment results. An important complication is reluctance to use corticosteroids, which negatively affects treatment results.

Method: We conducted a cross-sectional study including 579 parents of patients with AD who were registered at our outpatient clinic from 2009 to 2014. We assessed AD treatment management skills including knowledge and treatment management of topical therapies and reluctance to corticosteroids with a questionnaire. We used logistic regression analyses to identify factors that correlate with poor AD treatment management and reluctance to use corticosteroids.

Results: We found that poor knowledge of the basics of AD treatment (44%), poor AD treatment management skills (55%) and reluctance to use corticosteroids (48%) are very common in our study population. We also found that knowledge of topical therapy (knowing how much to apply, knowing frequency of application during a flare, knowing corticosteroid strength and knowing how to step up/phase out treatment), reluctance to corticosteroids, AD severity and age are important predictors for successful AD treatment management.

Conclusion: Health professionals involved in AD treatment should first address the basics of topical treatment and explore reluctance to corticosteroids before considering more potent treatment.

Poster Discussion Session PDS 25

Inflammatory mechanisms in asthma

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Exosomes from eosinophils of asthmatic subjects acts as an autoregulatory unit with functional capacity in asthma disease

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Background: Asthma is a chronic lung disease that affects millions of people worldwide. It's a disease of great importance given the high medical, social impact and the enormous health costs. Exosomes are nanovesicles that play a key role in intercellular signaling carrying diverse functional biomolecules (RNA, miRNAs, proteins and lipid mediators). Our group has reported that eosinophils from asthmatic patients release exosomes. The aim of the study is to determine the effect of the eosinophil exosomes over eosinophil function.
Method: Peripheral blood eosinophils from 18 asthmatic patients and 7 healthy subjects were purified and cultured with or without exosomes, previously purified from eosinophils from asthmatic patients. Different functional and activation studies were performed in eosinophils such as apoptosis, migration, quantification of total reactive oxygen species (ROS) and evaluation of nitric oxide production.
Results: When eosinophils from asthmatic subjects were cultured with exosomes, apoptosis, migration rate and production of ROS and nitric oxide showed changes compared to cells without exosomes (negative control). Apoptosis level of eosinophils from asthmatic and healthy individuals was higher in presence of exosomes than eosinophils without exosomes (23.8% vs 14.5%; and 47.6% vs 30.4%, respectively, $P > 0.05$). Furthermore the apoptosis percentage was significantly lower in asthmatic eosinophils than in healthy eosinophils cultured with exosomes (23.8% vs 47.6%, $P < 0.05$). Also, eosinophils showed a greater migration towards the medium where exosomes were present (1.65 fold, $P < 0.05$). Respect to ROS and nitric oxide production, we observed that eosinophils cultured in presence of exosomes produce a higher ROS and nitric oxide quantity compared to eosinophils cultured without exosomes ($P < 0.05$).
Conclusion: Exosomes from eosinophils of asthmatic patients exert a positive feedback regulation on inflammatory function of eosinophils present in local microenvironment (lung). These exosomes could develop an important role mediating and perpetuating inflammatory actions of eosinophils in asthma pathology.

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Procaterol suppresses epithelial to mesenchymal transition of bronchial epithelial cells induced by eosinophils

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Background: Bronchial asthma is a chronic airway inflammatory disease and often accompanied by structural changes in the airway, termed airway remodeling. Epithelial to mesenchymal transition (EMT) is currently recognized as an important mechanism by which eosinophils can induce airway remodeling. We previously reported that the direct contact of eosinophils with the BEAS-2B cells promotes increases in the expression of TGF-β1 leading to induction of EMT. Procaterol is a selective and full β2-agonist that is used as a rescue medicine for acute asthma as a potent bronchodilator. Studies *in vitro* have found thatβ2 selective-agonists also possess anti-inflammatory effect. In the present study, we hypothesized that procaterol suppresses EMT of airway epithelial cells induced by eosinophils.
Method: EMT is defined by changes in gene or protein expressions; during EMT epithelial markers (e.g. E-cadherin) decrease while mesenchymal markers (e.g. vimentin) increase. EMT was induced by using a co-culture system of human bronchial epithelial cells and human eosinophils or the eosinophilic leukemia cell lines, EoL-1. We analyzed the morphological change and measured vimentin and E-cadherin by RT-PCR, and TGF-β1 and GMCSF in the supernatant in the absence or presence of procaterol and/or Butoxamine, a specific β2-adrenergic receptor antagonist. Moreover we checked the expression of adhesion molecules on eosinophils by flow cytometry. Furthermore we examined whether the EMT in our co-culture system is the adhesion-dependent by using anti-integrin antibodies and/or anti-adhesion molecule antibody.
Results: Procaterol inhibited the morphological changes of BEAS-2B cells induced by eosinophils, decreased the expression of vimentin and increased E-cadherin. Butoxamine inhibited the effect of procaterol. In the view of the mechanism, ICAM-1 and VCAM-1 expression on BEAS-2B cells co-cultured with EoL-1 cells were enhanced in the absence of procaterol and inhibited when co-cultured with EoL-1 cells pre-treated by procaterol. Also we got the similar results for other integrin expression on eosinophils. Moreover EMT was suppressed by anti-integrin antibodies and/or anti-adhesion molecule antibody.
Conclusion: This study showed that procaterol suppressed eosinophils-induced EMT of airway epithelial cells. β2 agonist may ameliorate airway remodeling in bronchial asthma.

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Involvement of STAT6, SOCS and methylation in CCL26 production by bronchial epithelial cells: importance in asthma and its severity

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Background: High pulmonary eosinophil counts correlate with asthma severity and exacerbation. We recently showed that sputum CCL26 levels correlate with sputum eosinophils. We also found that among all CC chemokines, IL-13 selectively induced the expression of CCL26 by bronchial epithelial cells (BECs) and this phenomenon is significantly enhanced in BECs from severe eosinophilic asthmatics. We postulated that the superior CCL26 production that we observed in severe

asthmatics was the consequence of increased signaling events mediated by IL-13 and/or with a lower methylation level of the CCL26 promoter. We thus assessed (i) the expression and functional responses of the different signaling effectors linked to the IL-13 signaling and (ii) the importance of methylation in CCL26 expression by BECs from healthy subjects, mild asthmatics, and severe eosinophilic asthmatics.

Method: Human primary BECs were isolated and cultured from bronchial biopsies. BECs were treated with IL-13 or vehicle for different times. Inhibitors, demethylation agent or their vehicles were added to BECs 1 h before IL-13. CCL26 expression was assessed by qPCR and ELISA. STAT6 and phosphorylated (p)STAT6 were quantitated by ELISA in BEC lysates. SOCS3 level were assessed by immunoblotting.

Results: We confirmed the involvement of STAT6 in the induction of CCL26 expression by treating BECs with increasing STAT6 inhibitor. This led to a concentration-dependent inhibition of CCL26 expression in IL-13-stimulated BECs. In that regard, the pSTAT6/STAT6 ratios were increased in IL-13-stimulated BECs from severe eosinophilic asthmatics, compared to those from healthy subjects and mild asthmatics. This increased activation of STAT6 might be explained by decreased SOCS3 level in BECs from severe eosinophilic asthmatics. Using a demethylation agent, our preliminary data demonstrated that methylation is involved in CCL26 expression.

Conclusion: Our results show the importance of STAT6 and the possible implication of SOCS proteins and methylation in the enhanced CCL26 expression by BECs from severe eosinophilic asthmatics (Supported by the Fondation de l'IUCPQ and the JD-Bégin Research Chair).

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To study the role of T regulatory cells in developing asthma in a birth cohort of babies with family history of allergies

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Background: Asthma is known to be correlated with T_H2 immune response. There is evidence that T-regulatory cells are important to prevent allergic diseases like asthma. There are few studies based on the role of Treg cell in birth cohort babies with family history of allergy.

Method: Fifty children with asthma and respiratory allergy (study group), twenty children born with a positive family history and being followed up as a birth cohort (cohort group) and a group of 10 Healthy children with no history of allergy in the family were considered to be satisfactory (Control group) were recruited in this study. Total IgE level, pulmonary function test (PFT) was assessed. The levels of T_H1, T_H2, T_H17 and $CD4^+$ $CD25^{high}$ $FoxP3^+$ Treg cells expression were determined by Flow cytometry [FACSCanto (Becton Dickinson, Mountain View, CA, USA) with FACS Diva™ software]. The Ethics Committee of PGIMER approved this study (Micro / 2006 / 754 / 8th May 2006).

Results: The average level of total IgE in the studied group was 316.8 ± 189.8 IU/ml and significantly higher than cohort (124 ± 28.5 IU/ml, $P < 0.0001$) and control (50 ± 17.5 IU/ml, $P < 0.0001$). The frequency of T_H17 cells and culture supernatant level of IL-17 were 12.09 ± 8.67 pg/ml and significantly higher in study group than corresponding level in cohort [5.91 ± 3.56 pg/ml, $P < 0.001$] and control group [2.01 ± 1.27 pg/ml, $P < 0.001$], whereas the frequency of Foxp3 level were significantly lower in study group [(49.00 ± 13.47) %] and cohort group [(68.22 ± 8.55) %] compare to the control group [(95.91 ± 2.63) %]. The total serum IgE level is negatively correlated with FoxP3 level ($r = -0.5273$ $P < 0.0001$). The FoxP3 expression is negatively correlated with the IL-17 levels ($r = -0.5631$, $P < 0.0001$) and IL-4 levels ($r = -0.2836$ $P = 0.0460$).

Conclusion: Imbalance in T_H17/Treg was found in patients with allergic asthma. Elevated IL-17 and IL-4 cytokine response and down regulation of FoxP3 were associated with asthma. We also found that T regulatory cells expression decreases as asthma progression.

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Innate intranet communications in asthma: studies on dendritic cell interaction with airway epithelium

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Background: The interaction of various types of innate and adaptive immune cells during an ongoing allergic asthma is pivotal for the outcome and the resolve of the disease. Dendritic cells (DCs) are key regulators of this network, however, the airway epithelium is the first entry site for airborne allergens and signals derived from these cells can affect DC function decisively.

Method: A 3D co-culture model involving the airway epithelium cell line Calu-3, cultured under air-liquid interface conditions, and monocyte-derived DCs was used to enable studies on the interaction mode of these cell types when challenged with airborne allergens. In addition, we operated with a real-time cell analyzer (RTCA), an electronic system for sensing changes in cell morphology.

Results: While stimulation of Calu-3 cells with the allergens Der p1, Der p 2 and Bet v1 per se did not result in an altered release of immune-stimulatory cytokines, allergen-treatment of DCs induced diverging induction of cytokines such as IL-6, CXCL8 and CCL22. The 3D co-culture system further demonstrated that the cytokine release was enhanced even under conditions where direct epithelium/DC contact was prevented but it was at maximum when contact was enabled, indicating the importance of soluble factors together with direct cell interaction. Additionally, using the RTCA we see a modification of Calu-3 morphology when challenged with Bet v1 only in the presence of DCs.

Conclusion: Taken together, we established cell culture systems that enable allergen challenge studies on DC/airway epithelial cell co-cultures. First results underline the importance of the direct cell contact in this context and will provide further insights into the nature of the DC-epithelium interplay.

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Gender-specific effect of overweight and obesity on total serum IgE in adults with allergic asthma

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Background: Epidemiological data indicate that overweight and obesity increase the prevalence, incidence, and severity of asthma, which relationship is more often observed in women; there are also several reports describing a positive relationship between body mass index (BMI) and atopy in women. We examined the gender-specific impact of BMI on total serum IgE in steroid-naïve adults with mild-to-moderate allergic asthma.

Method: The subjects comprised 242 Japanese adults [105 men and 137 women, median (range) age 51 (20–92) years] with allergic asthma who were untreated with glucocorticosteroids and during attack-free periods. We retrospectively compared total serum IgE levels between overweight/obese [(BMI) (the weight in kilograms divided by

the square of the height in meters), ≥ 25] and normal weight patients (BMI, 18.5 to 24.9), separately for men and women.

Results: In 105 men, there was no significant difference in total serum IgE levels between 43 overweight/obese and 62 normal weight patients (798.6 ± 1850 and 648.7 ± 864.1 IU/ml, respectively; $P = 0.29$); in 137 women, 30 overweight/obese patients had significantly higher total serum IgE levels than 107 normal weight patients (530.3 ± 870.9 vs 299.1 ± 446.5 IU/ml, respectively; $P = 0.03$).

Conclusion: Our results show that overweight and obesity significantly increase total serum IgE levels in women, but not in men with mild-to-moderate allergic asthma. Atopy may mediate the relationship between overweight/obesity and allergic asthma in women.

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A metabolite of prostaglandin D2, 11β-prostaglandin $F_{2\alpha}$ (11β-$PGF_{2\alpha}$), in exhaled breath condensate and serum of asthmatics with airway hyperresponsiveness to distilled water

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Background: In asthmatics, PGD_2 effects the airways by causing bronchoconstriction, vasodilation, increasing capillary permeability and mucous production (Oguma et al. Allerg. Internat. 2008; 57: 307–312). Its role in osmotic airway hyperresponsiveness is ill-defined. PGD_2 is an unstable compound rapidly metabolized with 11β-$PGF_{2\alpha}$ being its primary plasma metabolite.

Methods: Exhaled breath condensate (EBC) was collected from 25 asthmatics and 5 healthy individuals before and after 3-min inhalation with ultrasonically nebulized distilled water (UNDW). Serum was obtained from 15 patients before the provocation. Content of 11β-$PGF_{2\alpha}$ was measured by EIA kit (No. 516521, Cayman Chemical) in EBC after freeze-drying and in serum after purification by solid phase extraction.

Results: The level of 11β-$PGF_{2\alpha}$ in EBC was below the detection limit (80% B/B_0) of the kit (5.5 pg/ml). Calibration curves were built using the 11β-$PGF_{2\alpha}$ standard in the range of 0–25.6 pg/ml with additional dilutions of the standard down to 0.64 and 0.256 pg/ml (obtained values for the dilutions were different from B_0). As a result, the calculated content of 11β-$PGF_{2\alpha}$ in EBC was in the range of 0–3.1 pg/ml. The total group of asthmatics ($n = 25$) had a lower basal level of 11β-$PGF_{2\alpha}$ (0.409 ± 0.144 pg/ml, ±SE) compared to the group of healthy controls (0.860 ± 0.307 pg/ml, $n = 5$), which further decreased following the UNDW challenge to 0.302 ± 0.092 and 0.534 ± 0.115 pg/ml, respectively. The group of asthmatics with airway hyperresponsiveness to UNDW (FEV_1 fall ≥10%, $n = 13$) was found to have a lower concentration of the metabolite (0.304 ± 0.144 pg/ml) as compared to the group without the hyperresponsiveness (0.522 ± 0.262 pg/ml, $n = 12$). The 11β-$PGF_{2\alpha}$ concentration decreased in the both groups after the challenge with a more pronounced changes in the first group (0.191 ± 0.039 vs 0.422 ± 0.189 pg/ml, respectively). The content of 11β-$PGF_{2\alpha}$ in serum was lower in the group with airway hyperresponsiveness to UNDW (9.4 ± 2.6 vs 16.9 ± 8.6 pg/ml).

Conclusion: The obtained results do not support an involvement of PGD_2 in the pathophysiology of airway hyperresponsiveness to hypoosmotic stimulus in asthmatics unless a specific conversation of the prostaglandin, apart from the formation of 11β-$PGF_{2\alpha}$, occurs in the airway under this condition. prostaglandin, apart from the formation of 11β-$PGF_{2\alpha}$, occurs in the airway under this condition.

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T cell-induced bronchoconstriction in the mice - a model for late asthmatic response

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Background: To investigate a role of helper T (Th) cells in asthma, T cell-transfer model was analyzed for immediate and late phase asthmatic responses after antigen challenge.

Method: Ovalbumin (OVA) specific Th clones were derived from either the regional lymphnodes of Balb/c mice immunized with OVA/CFA or splenocytes of DO11.10 transgenic mice expressing T cell receptor specific for OVA/H-2^d. Th clones were adoptively transferred into unprimed mice. After intranasal or inhalation challenge with OVA, airway resistance was continuously monitored by either unrestrained whole body plethysmography (BUXCO) or resistance/compliance analyzer under anesthetized condition. Bronchoalveolar lavage and analysis of airway hyperresponsiveness (AHR) were performed 48 h after OVA challenge. Supernatants of stimulated Th clones were analyzed for contractile activity using collagen gels embedded with murine primary bronchial smooth muscle cells. Effects of H_1R and LTR_1 antagonist were analyzed both *in vitro* and *in vivo*.

Results: When unprimed mice were transferred with Th clones, T5-1, T6-2, T6-4, and T6-7, Penh values were significantly increased 6 h after OVA challenge. In contrast, mice transferred with other Th clones, BF7, T6-1, or T6-10 did not show any change. Airflow limitation was confirmed by a direct measurement of airway resistance under anesthetized, restrained, and intubated conditions. The airflow limitation was also efficiently induced by the challenge with T cell epitope peptide, $OVA_{323–339}$. Contractile activity was detected in the supernatants of T6-2 stimulated with immobilized anti-CD3. T cell-induced contraction was not affected by H_1R or LTR_1 antagonist.

Conclusion: Activation of Th cells resulted in an airflow limitation besides eosinophilic inflammation, AHR, and mucous hyperplasia. T cell-derived bronchoconstrictor might be a target for treatment-resistant asthma.

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Concentrations of eosinophil mediators in nasal washes during experimental infections with rhinovirus in subjects with and without asthma

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Background: Rhinovirus (RV) infections frequently cause asthma exacerbations in children and young adults. Mechanisms, including the capacity of RV to stimulate Th2 related responses in the asthmatic and non-asthmatic, non-allergic host, remain unclear.

Method: Twelve subjects (ages 19–33 years) were inoculated with RV-strain 16 (dose = 300 $TCID_{50}$). They included 7 allergic asthmatics (AA); (total IgE levels 596–1989 IU/ml), and 5 non-atopic controls without asthma; (total IgE levels 5–42 IU/ml). Eosinophil mediators (ECP [Phadia AB] and EDN [MBL International Corporation]) were measured by ELISA in nasal washes (NW's) obtained before and during the infection. Results were analyzed in relation to symptoms and two Th2

associated chemokines (macrophage-derived chemokine [MDC] and TARC) measured in the same washes. All participants gave their written informed consent.

Results: Both ECP and EDN peaked by day 3, paralleling cold symptoms observed over the first 4 days of the infection. Cumulative values derived from morning NW′s during the first 4 days were significantly higher among AA subjects than controls (ECP: GM = 416 ng/ml and 16.8 ng/ml, respectively, $P < 0.05$; EDN: GM = 1320 ng/ml and 260.6 ng/ml, respectively, $P < 0.05$). Compared to baseline values measured before virus inoculation, mediator values in NW′s increased 20-fold for ECP and 16.6-fold for EDN by day 3 in the AA group. By comparison, ECP and EDN levels increased 2.6 fold and 4.7 fold, respectively, among the controls. Both ECP and EDN correlated positively with NW concentrations of MDC and TARC during peak symptoms.

Conclusion: The increase in eosinophil mediators (ECP and EDN) in nasal washes after RV inoculation was significantly greater during the infection in the allergic asthmatics than controls. The results also indicate that RV may have the capacity to stimulate a Th2 related eosinophil response in the non-allergic, non-asthmatic host.

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In vitro secretion of immunoregulatory cytokines by dendritic cells from elite athletes participating in endurance training

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Background: The immune system responds to increased physical activity. The impact of endurance exercise training on the immune system differs from that of not only non-athletes but also people with moderate physical activity.Prolonged strenuous endurance exercise may impact cytokine production by PBMC and dendritic cells (DC).

Methods: A comparative assessment of *in vitro* production of immunoregulatory cytokines (IFN-α, IL-31, TNF-β, IL-17A, IL-7, IL-1RA, IL-1α, IL-10, IL-15, IL-21 IL-22, IL-23, IL-27, IL-9) by dendritic cells (DC) derived from PBMC of professional athletes-skiers (7) and healthy volunteers (7) was performed to investigate the effect of prolonged intense exercise. DCs were obtained from peripheral blood mononuclear cells (BMD) in the presence of GM-CSF and IL-4. The concentrations of various cytokines in supernatants of 48-h cultures were assessed by Multiplex assays using Luminex xMAP technology.

Results: DC of athletes did not synthesize detectable concentrations of IL-17A and IL-7, whereas in the control group these the concentrations of cytokines were as follows: IL-17A – 16.46 ± 2.99 pg/ml and IL-7 – 5.81 ± 1 42 pg / ml. In both groups IL-1RA, IL-10, IL-1α were shown to be secreted, being more pronounced in athletes ($P < 0.05$). Secretion of IFN-α, IL-31, TNF-β was seen in the athletes, but not the control group. Detectable concentrations of IL-15, IL-21, IL-22, IL-23, IL-27, IL-9 were not seen in either group.

Conclusions: Prolonged endurance exercise impacts the secretion of cytokine by dendritic cells leading to immune modulation in endurance athletes.

Poster Discussion Session PDS 26

Angioedema

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The icatibant outcome survey: more than 2900 icatibant-treated attacks in patients with type I or II hereditary angioedema

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Background: Icatibant is a bradykinin B2 receptor antagonist used to treat attacks of hereditary angioedema due to C1-inhibitor deficiency (C1 INH-HAE) in adults. The Icatibant Outcome Survey (IOS; NCT01034969) is an international observational study monitoring the safety and effectiveness of icatibant in a real-world setting. Here we report data from over 2,900 icatibant-treated attacks in patients with type I or II C1 INH-HAE.
Method: IOS is currently conducted at 49 centers in 11 countries. Patient characteristics and icatibant treatment outcomes were recorded at clinic visits. Descriptive retrospective analyses were performed on data collected from July 2009-November 2015.
Results: Icatibant was used to treat 2,915 angioedema attacks in 463 patients with type I or II C1 INH-HAE. Mean age at enrollment was 40.6 years (range 16.5–81.2), and 57.9% of patients were female. Proportions of very mild/mild, moderate, and severe/very severe attacks were 9.2%, 34.0%, and 56.8%, respectively ($N = 2478$ attacks). Of attacks with anatomical location data ($N = 2821$), 58.4% of attacks affected the abdomen, 41.6% affected the skin, and 6.3% affected the larynx. Most icatibant injections were self-administered ($N = 2094/2688$ attacks; 77.9%). Median time to icatibant administration was 1.0 h ($N = 1351$ attacks). Median time to symptom resolution was 6.0 h ($N = 1292$ attacks). Median attack duration was 8.0 h ($N = 1127$ attacks). Of 2,915 icatibant-treated attacks, 309 (10.6%) also were treated with C1-INH rescue therapy. Icatibant was well tolerated, with no unexpected safety outcomes.
Conclusion: IOS has accumulated a large database of patients with C1 INH-HAE, providing insight into the characteristics of this rare disease. Treatment outcomes and safety profile of icatibant in the real world remain consistent with those from the Phase III studies.

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Improvement in hereditary angioedema diagnosis: findings from the Icatibant Outcome Survey

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Background: Hereditary angioedema due to C1-inhibitor deficiency (C1-INH-HAE) is a rare disease and often misdiagnosed with consequent delay in diagnosis. The Icatibant Outcome Survey (IOS) is an international observational study monitoring the safety and effectiveness of icatibant to treat angioedema attacks. Using IOS data from patients with C1-INH-HAE type I/II we investigated the relationship between patient age and both age of diagnosis and length of diagnostic delay.

Method: Patient's year of birth, age of diagnosis and delay in diagnosis (delay between first symptoms and diagnosis) were collected in 48 centers/10 countries from

Table 1

Country	*N*	Median (Q1,Q3) Age at Diagnosis (Years)	*N*	Median (Q1,Q3) Delay in Diagnosis (Years)
Israel	51	17.3 (10.4, 28.5)	48	1.1 (0.04, 13.6)
United Kingdom	46	18.3 (12.0, 32.0)	35	7.0 (0.04, 20.0)
Denmark	21	19.8 (16.7, 34.6)	20	12.2 (2.3, 21.1)
Italy	54	20.6 (14.2, 32.6)	51	10.5 (2.5, 21.2)
France	153	21.7 (15.5, 34.4)	130	5.7 (0.5, 15.7)
Spain	71	24.7 (16.9, 36.0)	68	10.8 (2.6, 20.9)
Greece	18	25.0 (9.4, 33.9)	16	13.5 (6.3, 21.5)
Germany	76	27.0 (14.0, 39.4)	71	5.0 (0.5, 21.9)
Austria	12	28.9 (21.4, 34.5)	12	16.0 (7.8, 19.8)
Brazi	15	29.5 (14.0, 38.6)	15	16.3 (0.1, 32.2)
All Countries	517	21.7 (14.0, 34.6)	466	7.3 (0.8, 18.5)

Table 2

Year of Birth	*N*	Median (Q1,Q3) Age at Diagnosis (Years)	*N*	Median (Q1,Q3) Delay in Diagnosis (Years)
<1950	53	45.9 (36.0, 62.6)	49	24.2 (5.9, 37.5)
1950–1959	78	34.1 (25.1, 51.4)	68	15.9 (6.1, 26.2)
1960–1969	103	24.5 (18.1, 35.6)	95	9.9 (1.7, 20.0)
1970–1979	124	21.7 (13.5, 32.9)	116	8.5 (1.5, 18.0)
1980–1989	111	16.0 (8.7, 21.0)	99	1.8 (0.0, 8.5)
≥1990	48	10.0 (4.9, 14.5)	39	1.4 (0.1, 6.5)
R^2; *P* value	517	0.4916; <0.0001	466	0.2493; <0.0001

July 2009 to April 2015. Patients diagnosed prior to their first attack, based on family history, were excluded. Regression analyses using year of birth (independent variable) and age of diagnosis and delay in diagnosis (dependent variables) was performed.
Results: Overall, the median age (years) at diagnosis and delay in diagnosis were variable across IOS countries (Table 1). When stratified by decade of birth there was a consistent reduction over successive decades in both median age at diagnosis ($r^2 = 0.49$, $P < 0.0001$) and median delay of diagnosis ($r^2 = 0.25$, $P < 0.0001$) (Table 2).
Conclusion: C1-INH-HAE patients, across all IOS countries, are being diagnosed at an apparently earlier age and experience shorter diagnostic delay. However there still remains a need to raise awareness of HAE.

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Hereditary angioedema presents in childhood but is diagnosed in adulthood - findings from the Icatibant outcome survey

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Background: Hereditary angioedema (HAE) is a rare inherited disease characterised by recurrent swelling of the skin and mucous membranes. We previously showed that in 171 patients, symptoms first presented at a median age of 12 (IQR 6, 19) years, but there was an 8.5-year delay in diagnosis (Zanichelli et al *Allergy Asthma Clin Immunol* 2013; 9:29). To help understand the reasons for the delay, we used data from the Icatibant Outcome Survey (IOS) to determine which physicians are diagnosing HAE and identify those to whom education and awareness efforts may be best directed.
Method: IOS (NCT01034969) is an international observational study monitoring the safety and effectiveness of icatibant for the treatment of HAE attacks. Data for this analysis were collected from sites in 11 countries between July 2009 and February 2015.
Results: The dataset included information on 583 patients with HAE type I or II, including 346 (59.4%) females. The median (IQR) age at first symptom presentation was 13.0 (5.0, 18.5) years and the median age at diagnosis was 20.8 (13.4, 34.1) years. Of patients with available data ($n = 383$), most were diagnosed with HAE by a specialist (316, 82.5%) or a non-ER hospital physician (68, 17.8%). Specialists who most frequently diagnosed HAE were allergologists (120, 31.3%), clinical immunologists (63, 16.4%), and dermatologists (56, 14.6%). A total of 10 (2.6%) patients were diagnosed by a paediatrician, and 10 (2.6%) patients were diagnosed by a paediatrician-immunologist.
Conclusion: Although most patients present with symptoms of HAE during childhood or adolescence, few were diagnosed by a paediatrician. Increased awareness of this rare disease among paediatricians could further reduce the delay in diagnosis.

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Compound mutations in C1-INH gene aggravate its functional deficiency

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Backgroud: Hereditary angioedema (HAE) is a rare autosomal dominant disease,-caused by a deficiency of C1 inhibitor (C1-INH). It presents as remarkable heterogeneity in manifestations, even in the same family. However, the mechanism underlying such severity difference has not been well known. We aimed to explore whether the mixed heterozygous mutation would affect the severity of HAE.
Methods: We collected 4 HAE patients whose severity was obviously different from 2 families (two patients from family 1 and two patients from family 2), identified the mutations in C1-INH gene and measured their mRNA levels. Abnormal C1-INH genes were expressed in Hep G2 cell line and their function were tested.
Results: Four genotypes (genotypes of the first family, patient 1: c.1328A< G; patient 2: c.1328A< G+ c.167T< C; genotypes of the second family, patient 3: c.953C< G; patient 4: c.953C< G + c.49G< A) were identified and a reduced expression of C1-INH mRNA was detected in all of them. In Hep G2 cell line, all these genotypes led to a deficiency of C1-INH function (34.8%, 24.4%, 44.8% and 11.4% of normal standard). Compared with single mutation, genotypes of patient 2 and 4 induced a significant lower level of functional C1-INH.
Conclusions: *In vitro*, the mixed hereozygous mutation (genotypes of patient 2 and 4) contribnuted to more severe deficiency of functional C1-INH than single mutation (genotypes of patient 1 and 3), which may impact the severity of HAE in these patients.
Keywords: C1 inhibitor, clinical heterogeneity, genotype, hereditary angioedema.

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Concomitant diseases and their influence on the clinical course of hereditary angioedema due to C1-inhibitor deficiency (HAE-C1-INH) - A retrospective study in 152 adult patients

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Background: Hereditary angioedema due to C1-inhibitor deficiency (HAE-C1-INH) is a rare autosomal dominant inherited disease. The recurrent symptoms are subcutaneous edema and colic-like abdominal pain. Laryngeal edema are rare, but life-threatening if untreated. It is well known that hormonal changes (i.e. during puberty, pregnancy) could have a negative influence of the clinical course of HAE-C1-INH. The aim of our study was to investigate the occurrence and the incidence of concomitant diseases and their potential influence on the clinical course in adult patients with HAE-C1-INH.
Method: The clinical course of HAE-C1-INH in 152 adult patients (i.e. triggers of angioedema attacks, first signs, localization of angioedema, attack severity) and the occurrence of any persisting concomitant diseases have been analyzed retrospectively over an observation period of 12 months in a single-center study. This analysis also included patient characteristics, relevant medical and surgical history and the individual management of HAE-C1-INH.
Results: A total of 152 patients with HAE-C1-INH could be enrolled in this study: 101 females (66.4%) and 51 males (33.6%). The mean patient age was 44.4 years (range: 18–86 years). The most frequent concomitant diseases in our patients' cohort were autoimmune thyroiditis, allergy, and hypertension. A correlation was found between age and hypertension, but no impact on the severity of HAE-C1-INH. In contrast, patients with immune deficiencies or an autoimmune disease seem to develop a more severe course of HAE-C1-INH independent from age and gender.
Conclusion: Concomitant diseases may have an impact on the clinical course of HAE-C1-INH and need to be considered for the individual management plan of HAE-C1-INH.

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Elevated plasma levels of vascular permeability factors in C1 inhibitor-deficient hereditary angioedema

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Background: Hereditary Angioedema with C1 inhibitor deficiency (C1-INH-HAE) is a rare inherited genetic disease characterized by recurrent swelling episodes of the skin, gastrointestinal tract and upper airways. Angioedema attacks result from increased vascular permeability due to the release of bradykinin from high molecular weight kininogen. Currently, there are no biomarkers predicting the frequency of angioedema attacks. Vascular permeability is modulated by several factors, including vascular endothelial growth factors (VEGFs) and angiopoietins (Angs).

Since increased circulating levels of VEGFs and Angs have been observed in diseases associated with higher vascular permeability (e.g. systemic capillary leak syndrome and sepsis), we sought to analyze plasma concentrations of VEGFs and Angs in C1-INH-HAE patients.

Methods: 68 healthy controls and 128 C1-INH-HAE patients were studied. Concentrations of angiogenic (VEGF-A, Ang1, Ang2), anti-angiogenic (VEGF-A_{165b}) and lymphangiogenic (VEGF-C) factors were evaluated by ELISA. C1-INH functional activity was assessed by EIA.

Results: Plasma concentrations of VEGF-A, VEGF-C, Ang1 and Ang2 were higher in C1-INH-HAE patients in remission than in healthy controls. Concentration of VEGF-A was further increased in patients with lower C1-INH functional activity. C1-INH-HAE patients experiencing more than 12 angioedema attacks per year were characterized by higher plasma levels VEGF-A, VEGF-C and Ang2 compared to the other patients.

Conclusions: We hypothesize that VEGFs and Angs induce a state of 'vascular preconditioning' that may predispose to angioedema attacks. In addition, the identification of increased plasma levels of VEGFs and Angs in C1-INH-HAE patients may prompt the investigation of VEGFs and Angs as biomarkers of C1-INH-HAE severity.

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Distribution of bradykinin-mediated angio-oedema conditions in paediatric patients admitted to a reference centre

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Background: Angio-oedema (AO) can be attributable to bradykinin (BK) accumulation, as is the case for the prototypical hereditary AO (HAO) due to C1 inhibitor (C1-INH) deficiency. However, our clinical experience in a reference centre has shown that some patients display a clinical history suggestive of HAO, but exhibit normal C1-INH function, have no mutation in the causative genes associated with HAO (SERPING1, F12), and report no intake of drugs known to promote AO. Accordingly, we have recently described clinical and pathophysiological characteristics of BK-mediated AO in an adult population and proposed a new classification based on BK metabolism assessment. We now report here a series of children with BK-mediated AO (BK-AO) followed in our reference centre.

Objectives: We sought to determine the frequency and distribution of different AO subtypes suspected to be (BK-AO) and defined by clinical, history and biological criteria (enzyme activities implicated in BK formation and catabolism) in paediatric patient.

Methods: The files of all children (aged 15 years or less at diagnosis) referred to our centre between September 2007 and November 2015 for suspicion of BK-AO were retrospectively analyzed.

Results: The distribution of patients ($n = 32$) was 44 and 9% with a mutation in SERPING1 or F12 gene, respectively, 6% with non-iatrogenic defective kininase activity and 28% with idiopathic increased kinin formation. Twelve percent of children had idiopathic BK-AO with no biologic anomalies. A family history of AO was found in 62.5% of patients ($n = 20$).

Conclusion: Our findings are original since children series of BK-AO reported so far are usually limited to C1 Inh deficit (HAO I and HAO II). As described previously in the adult population, BK-AO may be caused by multiple inherited or acquired factors triggering BK accumulation. Therefore, we assume that our novel typology based on the imbalance of production/catabolism of BK is valuable for pediatric BK-AO.

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Less angioedema, more quality of life and lower signs of depression in CSU during omalizumab treatment

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Introduction: Here we report an expanded analysis of the 'X-ACT' phase III-b, double-blind, placebo-controlled study in which patients with chronic spontaneous urticaria (CSU) who had angioedema at least 4 times in the last 6 months were enrolled. Patients also had to have an insufficient response to 4× up-dosing of 2nd generation H1-antihistamines and were treated either with omalizumab (OMA) 300 mg ($n = 44$) or placebo ($n = 47$) every 4 weeks for 28 weeks.

Methods: Angioedema episodes were assessed for their frequency and the size of swellings. Quality of life was measured via the Dermatology Quality of Life Index (DLQI) and the WHO-5 score, which specifically focusses on the domains mood, vitality and general interests. A WHO-5 score <13 indicates signs of depression.

Results: At screening, more acute swelling episodes were recorded in the placebo group (325) compared to the OMA (231) group. Most swellings had a diameter of <10 cm (OMA: 62.2%, placebo: 50.8%) followed by the category 10–20 cm (OMA: 23.4%, placebo: 30.5%). During the last 4 treatment weeks, all swellings in the OMA group ($n = 31$) had a diameter of <10 cm, whereas most swellings in the placebo group ($n = 206$) had a diameter between 10–20 cm (45.6%) or <10 cm (36.9%). DLQI total scores at baseline showed a very large (OMA: 59.1%, placebo: 43.5%) or extremely large effect (OMA: 18.2%, placebo: 32.6%) of CSU on patients' lives. OMA was significantly superior to placebo in reducing the DLQI total score over 28 weeks of treatment ($P < 0.001$). The difference (LS mean [95% CI]) between both treatment groups, OMA vs placebo, was −6.1 [−9.2 to −2.9]. Superiority of OMA to placebo was already reached after 4 weeks of treatment ($P < 0.001$). Similarly, the WHO-5 total score was significantly improved with OMA compared to placebo ($P < 0.001$), were 82.4% of the OMA group reached a meaningful response, i.e. a value below the 'depression threshold' of 13 points at week 28 compared to 41.9% in the placebo group. At the end of treatment, the OMA group had a 5.5 times higher chance to be free of signs of depression compared to the placebo group ($P = 0.005$ Wald t.). Overall,

OMA appeared to be safe and well tolerated and adverse events were in line with the established safety profile of OMA.

Conclusion: Omalizumab 300 mg every 4 weeks significantly reduced CSU related angioedema in size and frequency, and significantly improved of quality of life and reduced rates and signs of depression.

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Angioedema without urticaria

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Background: It is important to properly classify angioedema (AE) without urticaria because it brings therapeutic implications.

Method: A retrospective-observational study was performed, including patients with recurrent AE without wheals, referred to a monographic AE clinic of a third level Hospital, from Jan-2010 to Jun-2015.

Results: 151 patients were retrieved, 87 women (57.6%), mean age 57.65 years (SD = 19.22). They were categorized into 65 (43.1%) histaminergic AE (H-AE) and 65 (43.1%) non-histaminergic AE. 14 patients (9.27%) were not finally classified (undetermined angioedema) and 7 had irritative uvula edema (4.6%). Sixty out of the 65 patients with H-AE, were idiopathic and 5 had an allergic origin, being Anisakis simplex the ethiological factor in 4 (80%). Of the 65 patients with nH-AE, 22 (33.84%) had C1-INH deficiency (18 (81.8%) hereditary, 4 (18.2%) acquired) and 43 (66.15%) had normal C1-INH (36 (83.72%) related to ACE-inhibitors; 5 (11.62%) hereditary with a FXII gene mutation; 2 (4.65%) idiopathic).

The age of onset of symptoms was lower in H-AE compared to nH-AE (44.04 vs 6.71 years; $P < 0.001$). No differences were found related to gender (67.6%/51.5% women). Atopy was more frequent in H-AE patients (44.61% vs 17.18; $P < 0.001$) as well as sensitization to *Anisakis simplex* (36.9% vs 15.6%; $P = 0.01$), and food allergies (6% vs 0%; $P = 0.038$).

There were no differences between both groups regarding peripheral AE location (95.3% vs 78.12%; $P = 0.08$) except for facial AE, more present in H-AE (95.38% vs 73.43; $P = 0.003$). Oropharyngeal involvement was similar (66.15% vs 64.0%), whereas abdominal attacks were less frequent in H-AE (6.1% vs 28.12%; $P = 0.002$). Frequency of attacks at disease onset was lower in H-AE (9.7 vs 11.91; $P < 0.001$).

No differences were detected among triggers except for NSAIDs, which were more frequent in H-AE (13.8% vs 0%; $P < 0.001$).

Delay on diagnosis was shorter in H-AE (7.38 vs 13.12 years; $P < 0.001$).

Acute treatment and long term prophylaxis were similar in both groups before diagnosis. All received corticoids or antihistamines.

Conclusion: Differential diagnosis between H-AE and nH-AE is not easy based on clinical presentation, although a few differences may be helpful.

Diagnosis of nH-AE is mandatory in order to avoid long time incorrect treatment and to provide patients specific medication.

Poster Discussion Session PDS 27

Sublingual immunotherapy

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The SQ house dust mite (HDM) SLIT-tablet in respiratory allergic disease (RAD) - target patient profile

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Background: The SQ HDM SLIT-tablet (ALK/MERCK/Torii) was recently approved in 11 EU countries, with an indication in both HDM allergic rhinitis (AR) and allergic asthma (AA). Here we describe the target patient profile based on the completed phase III trials that form the basis for the indication in RAD.
Method: 2 RDBPC multi-centre, phase III clinical trials were conducted in adults, 1 trial in subjects with moderate-severe HDM AR despite use of pharmacotherapy (MT-06) and 1 trial in subjects with AA not well-controlled by ICS (including a subgroup with uncontrolled asthma according to GINA) (MT-04).
Results: The populations included in MT-04 and MT-06 had confirmed HDM sensitisation by skin prick test, positive specific IgE and a medical history consistent with persistent HDM RAD. ~70% of subjects were polysensitised. Furthermore, subjects had:

- MT-06: moderate-severe HDM AR despite use of allergy pharmacotherapy, ±mild asthma (GINA treatment steps 1–2). Subjects were required to have a high AR symptom level and frequent use of allergy pharmacotherapy and presence of at least 1 of the following ARIA quality of life items: sleep disturbance, impairment of daily activities or impairment of school/work, in the baseline period.
- MT-04: HDM AA not well controlled by ICS (GINA steps 2–4) and HDM AR (any severity). Not well-controlled AA was defined as asthma control questionnaire score between 1-1.5 at screening. For both trials, severe asthma exacerbation within 3 months prior to randomisation, ongoing viral infections and $FEV_1 < 70\%$ were exclusion criteria.

The data from the 2 trials demonstrated that the SQ HDM SLIT-tablet improved disease control in both AR and AA, resulting in fewer symptoms, less use of allergy pharmacotherapy, fewer AA and AR exacerbations and decreased impact of disease on patient's daily life. The trials also showed a favourable safety and tolerability profile, also in the MT-04 subgroup with uncontrolled asthma according to GINA.
Conclusion: The SQ HDM SLIT-tablet is indicated in adult patients (18–65 years) diagnosed by clinical history and a positive test of house dust mite sensitisation with at least one of the following conditions:

- Persistent moderate-severe HDM AR despite use of allergy pharmacotherapy.
- HDM AA not well controlled by ICS and associated with mild-severe HDM AR.

Initiation of treatment is contraindicated in patients with recent severe asthma exacerbation, ongoing viral infection and/or $FEV_1 < 70\%$.

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Quantitative benefit and risk assessment of SQ house dust mite (HDM) SLIT-tablet in allergic rhinitis; results from a randomised DBPC phase III trial

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Background: The SQ house dust mite (HDM) SLIT-tablet (ALK/Merck/Torii) has been shown to induce a clinically relevant reduction in symptoms and use of allergy pharmacotherapy in adults with moderate-severe HDM allergic rhinitis (AR). The safety profile is compatible with at-home administration. Here we present a quantitative assessment of benefits and risks using the numbers needed to treat (NNT) and needed to harm (NNH) to characterise the relative benefit and risk based on the efficacy and safety of treatment.
Method: The MT-06 trial (EudraCT 2011-002277-38) was a randomised DBPC phase III trial including 992 adults with moderate-severe HDM AR despite use of allergy pharmacotherapy. Subjects were randomised 1:1:1 to 1 year of daily treatment with placebo, 6 or 12 SQ-HDM. The primary endpoint was the total combined rhinitis score (TCRS). NNT was based on a responder analysis, where a responder was defined as a subject experiencing at least 80% mild days (a day with no need for antihistamines and no rhinitis symptoms scored higher than 1 (mild)). NNH was estimated based on the number of subjects with: epinephrine use for AEs, treatment-related systemic allergic reactions and treatment-related severe AEs. In this abstract we present the results for the 12 SQ-HDM dose.
Results: For 12 SQ-HDM, the NNT was 7.4 based on the definition of responders (in average 7.4 subjects should be treated to obtain one more responder with 12 SQ-HDM compared to placebo). This corresponds to 31% of subjects in the 12 SQ-HDM group having >80% mild days compared with 17% in the placebo group (OR 2.14, $P < 0.0001$). There were no AEs reported as systemic allergic reactions. 5 of 318 subjects with 12 SQ-HDM experienced treatment-related severe AEs and 1 of 318 subjects had an AE that was treated with epinephrine (mild laryngeal oedema). There were no epinephrine use, treatment-related systemic allergic reactions and treatment-related severe AEs in the placebo group. Accordingly the NNH was 53 (one more subject is harmed with 12 SQ-HDM compared to placebo per 53 subject treated).
Conclusion: Having a large proportion of days with no more than minimal awareness of the disease is considered a benefit of direct relevance for patients with moderate-severe HDM AR, especially as it is a perennial allergy. With NNH being 7 times higher than NNT, the SQ HDM SLIT-tablet is considered to have a favourable benefit-risk profile providing patients with a clinically relevant treatment of HDM AR.

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Results from a double-blind, randomised, placebo-controlled, dose-response evaluation of SQ tree sublingual allergy immunotherapy (SLIT)-tablet

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Background: Allergic rhinitis/conjunctivitis (AR/C) significantly interferes with activities of daily living. The SQ tree SLIT-tablet (ALK, Denmark) is being developed for once-daily, home-administered treatment of patients with moderate to severe AR/C induced by pollens from the birch group. Here, we report the results of a phase II trial.
Method: The TT-02 trial (EudraCT 2012-000031-59) was a randomised, double-blind, placebo-controlled, dose-response evaluation of the SQ tree SLIT-tablet with the primary objective to identify an optimal dose interval in adults and adolescents with moderate to severe AR/C (with or without asthma). 637 subjects were randomised equally to 0.5, 1, 2, 4, 7, or 12 development units (DU) or placebo.Treatment was initiated in autumn 2012 and continued until after the birch pollen season 2013 (at least 6 months). The primary endpoint was the average AR/C daily symptom score (DSS) during the pollen season. Pharmacodynamic endpoints included birch-specific IgE and IgG_4. Safety endpoints primarily included adverse events.
Results: The tree pollen season was short and characterised by low pollen counts.For the low doses there were no significant effects. For 7 DU, the DSS was significantly different from placebo (absolute: 1.14, $P = 0.019$; relative: 33%). For 12 DU, the DSS was numerically different but not statistically significant (absolute: 0.54, $P = 0.299$, relative: 16%)).For the objective pharmacodynamic endpoints, there was a clear dose-response.Treatment was well-tolerated with local reactions in the mouth and throat as the most common treatment-related events; the majority were mild or moderate in severity and occurred on day 1. One serious event of asthma (1 DU) was assessed as treatment-related. No deaths, local allergic reactions compromising the airways or anaphylactic reactions were reported.
Conclusion: The trial did not show a dose-response for the DSS during the pollen season; however, there was a statistically significant treatment effect with 7 DU. There was a clear dose-related effect on the immune system (IgE, IgG_4).

The safety evaluations showed a favourable safety profile for all the tested doses. Common treatment-related events were mild-moderate oral reactions.

The low pollen season impacted the severity of symptoms and thereby the dose-response. Based on the combined evidence, it is suggested that the optimal dose for clinical efficacy is above 4 DU.

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Patient-relevant benefit of sublingual immunotherapy (SLIT) with a 300 IR birch pollen extract in patients with allergic rhinoconjunctivitis

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Background: Allergy immunotherapy is a causal treatment for allergic rhinoconjunctivitis (ARC). To date, there is only few data assessing the benefit of such a treatment from the patient's point of view. Patient-relevant benefit of an ARC treatment can be assessed with the validated instrument 'Patient Benefit Index' (PBI-AR). Using the PBI, this study investigated the treatment benefit of a SLIT in patients with birch pollen-induced ARC.
Methods: The open, prospective, multicenter, non-interventional observational study with birch pollen SLIT (Stallergenes, France) was conducted in 75 German study centers. Patients were observed during the 1st treatment year.

Before treatment start, patients rated the importance of predefined treatment needs; at the end of the treatment they rated the extent to which these needs were achieved (0 = not important at all/did not help at all to 4 = very important/helped a lot). Based on these values the so-called PBI, a global benefit parameter, was computed. In addition, demographic data, medical history and clinical parameters were documented, e.g. the impairment due to ARC symptoms.
Results: Data of 291 patients (170 f, 121 m; mean age 38.8 years) were analyzed. For 212 of these patients, the global PBI could be computed. The needs most important were 'to have less nasal symptoms (runny/stuffed-up nose)' and 'to be able to stay outdoors without symptoms' (rated by more than 85% of patients as 'quite' or 'very important'). 43.6–51.7% of the patients rated the extent to which these needs were achieved as 'quite' or 'very'. The patients achieved a mean PBI of 2.2 ± 1.0 (0 = no benefit, 4 = maximum benefit). The impairment due to ARC symptoms decreased during the observation period.
Conclusion: In its first year, patients already assessed birch pollen SLIT as positive. The PBI revealed a patient-relevant benefit of treatment, and patients were less burdened by their symptoms.

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Efficacy of sublingual immunotherapy with house dust mites in elderly rhinitis patients: a multicenter trial for 6 months

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Background and aims: Allergic rhinitis (AR) in elderly is constantly increasing with the extension of life expectancy, but little is known about elderly AR. Immunotherapy in elderly AR is a controversial issue, since no proven evidence in the safety and efficacy of this treatment has been shown. In this study, we were to find the characteristics of elderly AR sensitized to house dust mites (HDMs) and evaluate the safety and efficacy of sublingual immunotherapy (SLIT) with HDMs.
Methods: Total 25 patients older than 60 years old with AR sensitized to HDMs showing >3+ A/H ratio on skin prick test and/or >0.35 IU/l by ImmunoCAP to *D. farinae* and *D. pteronyssinus* were enrolled from 4 University Hospitals in South Korea. The patients had taken medications according to their symptoms. To evaluate the additional effects of SLIT with HDMs, they were randomized to either SLIT-treated group with LAIS® Mites Sublingual tablets (Lofarma, Milano, Italy, 2 tablets/week) or observation group with pharmacological treatment. Total rhino-conjunctivitis symptom scores (TRSSs)/adverse reactions/ Korean rhinitis-specific quality of life questionnaire (RQLQ)/ asthma control test (ACT) scores in cases of AR with asthma, were monitored every 3 months.
Results: Of 25 patients, 16 (64.0%) patients were in the treated group, and 9 (36.0%) patients were in the observation group. Mean age was 67.5 years ranged from 60 to 81 years, and male/female ratio was 12/13. There were no significant differences in demographics, TRSS, skin reactivity to HDMs, and total/ specific IgE levels to HDMs ($P > 0.05$, respectively).

Compared to baseline level, significant reductions of TRSS were noted at visit 3 ($P = 0.001$) and visit 4 ($P = 0.005$) in the treated group, while no significant changes were noted in the observation group. RQLQ and ACT scores tended to decrease in the treated group, whereas they increased in the observation group. No serious adverse events were noted in both groups.

Conclusions: SLIT with tablet form of HDM is worth trying for elderly AR patients and no safety issue has become a problem, but long term follow up studies are needed.

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House dust mite tablet (S-524101/STG320) at 300 IR is effective in both mono and poly-sensitized patients with allergic rhinitis in a phase 2/3 study conducted in Japan

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Background: S-524101/STG320 is a house dust mite (HDM) sublingual allergen immunotherapy tablet containing a 1:1 mixture of extracts of *D. pteronyssinus* and *D. farinae*. We evaluated its efficacy in a double-blind, randomized, placebo-controlled study in patients with allergic rhinitis (AR) caused by HDM allergen. As the post-hoc analysis, the effect of co-sensitization of other allergens on the efficacy was examined.

Method: Patient inclusion criteria were: males and females between 12 and 64 years, serum HDM-specific IgE score being 2 or higher, a positive nasal provocation test, a total of 4 nasal symptom score being 6 or higher out of 15 before randomization. Exclusion criteria were: suspicion of symptomatic seasonal AR, IgE score being 5 or higher to any other allergens, 2 or higher to cat dander or dog dander allergens if exposed to these allergens in daily life, 3 or higher to autumnal allergens.

Results: In total, 968 patients were randomized to a 300 index of reactivity (IR), 500 IR, or placebo group in a 1:1:1 ratio, and treated for 52 weeks. The Average Adjusted Symptom Scores (AASS) at Week 44–52 in the FAS ($N = 927$), the primary endpoint, were 5.00, 5.32 and 6.11 in the 300 IR, 500 IR and placebo groups, respectively, and the AASSs in both active groups were statistically lower than that in the placebo group. In the subset of mono-sensitized patients (without any sensitization other than HDM, 31% of FAS patients), the AASSs were 4.97, 5.28 and 5.90, respectively, and in the subset of poly-sensitized patients (69% of FAS patients), AASSs were 4.91, 5.22 and 6.10, in the 300 IR, 500 IR and placebo groups, respectively. In the mono-sensitized subset, the AASS in the 300 IR group, and in the poly-sensitized subset, the AASSs in both active groups, were statistically significantly improved compared to the placebo. Further, in the subsets of patients with any specific IgE level of co-sensitized allergens (highest IgE level with co-sensitized allergen being 2, 3 or 4), the AASS at 300 IR was significantly lower than that in the placebo group.

Conclusion: Treatment with the 300 IR dose of HDM tablet S-524101/STG320 is effective in both subsets of mono- and poly-sensitized patients, regardless of specific IgE level of co-sensitized allergens.

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Sublingual allergen immunotherapy patterns of use in RAS 3D study

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Background: To improve sublingual application of allergy immunotherapy (SLIT) standardized in IR/ml, a new dosing pump that doubles the dose administered per actuation has been developed by Stallergenes globally and marketed in Spain since September 2013.

The aim is to describe, in routine practice, the use of initial and maintenance SLIT administered using the new dosing pump.

Methods: A retrospective, cross-sectional, multicenter study in adults and children (> 55 years old) with allergic respiratory diseases who were treated with SLIT prescribed between September 2013 and November 2014 was conducted.

A descriptive analysis of all variables collected was performed. Variables are presented according to their character: Categorical variables were summarised using frequencies and percentages. Continuous variables were summarised using measures of central tendency and dispersion: mean, standard deviation, median, 25% and 75% percentiles (Q1 and Q3).

Results: 801 patients were recruited (52.4% male), mean (SD) age 25.9 (17.2) years, 4.56 (4.68) years from diagnosis. 95.3% had rhinitis or rhinoconjunctivitis (91.4% moderate to severe, 63% persistent), 38% asthma. 69.2% sensitized (prick-test) to pollens (54.7% grasses, 47.8% olea), 52.4% to house dust mites and 30.3% to moulds or animal danders.

Mean time from the start of treatment was 13.35 months (4.94). 50.8% were treated with mixed extracts (D. pteronyssinus + D. farinae 31.5%, grasses + olea 17%). 100% initiated with the 300 IR/ml vial. 96.6% had an initiation phase of 3 days (day 1 one actuation-0.2 ml-, day 2 two actuations-0.4 ml- and day 3 four actuations-0.8 ml-). Median (Q1, Q3) maintenance dose was 4.0 (3.0, 4.0) actuations, 3.0 (3.0, 5.0) days per week. 39.7% have received treatment with the previous dosing pump for a mean of 14.95 (3.80) months before the change to the new one.

Conclusion: RAS 3D study shows that, in Spain, most patients follow a conventional short build up scheme of 3 days, all patients starting with the 300 IR/ml vial. The median maintenance dose in RAS 3D study aligns with the recommended maintenance dose, 4 actuations three days a week.

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Sublingual treatment with a mites mixture is safe and well tolerated in patients with mite induced allergic rhinitis/rhinoconjunctivitis

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Background: Allergic rhinitis/rhinoconjunctivitis may significantly impair social life, school performance, work productivity and sleep in both adults and paediatric populations. The investigated sublingual liquid oral preparation contains the mite allergens *D. pteronyssinus* and *D. farinae* combined and is developed for treatment of allergic disorders such as allergic rhinitis and rhinoconjunctivitis, caused by sensitisation to house dust mites (HDM) allergens. The aim of this study was to investigate the safety and tolerability at different dosages of this formulation.

Method: In this randomized, double-blind, placebo controlled, Phase I dose tolerability study, 81 subjects (mean age: 32.8 ± 10.2 years, 47% female), with allergic rhinitis or rhinoconjunctivitis with a positive skin prick test result for HDM and allergen specific serum IgE >0.7 kU/l,

were randomized in 10 German centers. The investigated dosages were 10 000, 25 000, 50 000 and 100 000 AU/ml, and placebo. Medication was taken once daily, starting with one drop and increasing the dose by one drop each day until the maintenance dose (5 drops/day) was reached and subsequently maintained for approximately 1 month. The main safety and tolerability endpoints were occurrence of local and systemic adverse events (AEs) and occurrence of treatment emergent adverse events (TEAEs).

Results: In total 60.5% of subjects developed at least 1 TEAE, which was only slightly higher for the active treatment groups compared to the placebo group, without a clear dose response relationship. The TEAEs were generally of mild intensity (92.8% of TEAEs) and for 48.1% of subjects at least one TEAE was judged to be drug related. In total, 22.2% of subjects developed a systemic AE with a similar incidence between the active treatment groups and the placebo group. The systemic AEs were generally of mild and none of severe intensity. Furthermore, 38.3% of subjects developed a local AE which was slightly higher for the active treatment groups compared to the placebo group, with local AEs generally of mild and none of severe intensity. None of the subjectss developed a drug related SAE.

Conclusion: Following treatment with ascending doses of a mites mixture, local and systemic reactions were generally of mild intensity without a clear dose response relationship in subjects with mite induced allergic rhinitis/rhinoconjunctivitis and show that treatment with doses up 100 000 AU/ml were generally safe and well tolerated.

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Efficacy and safety of sublingual immunotherapy tablet in patients with Japanese cedar pollinosis. A double-blind, randomised, placebo-controlled study

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Background: Japanese cedar (JC) pollinosis is one of the most common seasonal allergic rhinitis and affects more than 20% of the total population during spring (from February to early April) in Japan. The present study was conducted with three doses of a JC pollen SLIT tablet to examine efficacy and safety and determine the optimal dose for long-term treatment of patients with JC pollinosis after a 3-year treatment with the selected dose and a 2-year follow-up. Here, we report the interim result of efficacy and safety of a JC pollen SLIT tablet in the 1st pollen season.

Method: A total of 1042 patients with JC pollinosis aged from 5 to 64 years were equally randomised into four groups and received daily treatment with a JC pollen SLIT tablet containing 2000 Japanese allergy units (JAU), 5000 JAU, 10 000 JAU, or placebo. The treatment was initiated 5 to 6 months prior to the forecasted JC pollen season. The primary endpoint was the Total Nasal Symptom and Medication Score (TNSMS). The efficacy was evaluated in the 3-week peak symptom period, consisting of the week with the worst symptoms, based on the sum of TNSMS for all subjects and in the week before and after the worst week. Secondary endpoints were individual symptom scores, use of relief medication, and quality of life (QOL), which was evaluated using the Japanese allergic rhinitis QOL standard questionnaire.

Results: TNSMS in the peak symptom period was significantly lower for patients treated with 2000 JAU, 5000 JAU, and 10 000 JAU than placebo by 21%, 32%, and 31%, respectively ($P < 0.0001$ in all groups). Compared with the placebo group, the JC pollen SLIT tablet groups had significantly improved individual nasal and ocular symptom scores, medication scores, and QOL. No anaphylactic reaction was observed. The most treatment-related adverse events were mild local reactions at the administration site.

Conclusion: Daily treatment with a JC pollen SLIT tablet was effective for improving nasal and ocular symptoms of JC pollinosis, reducing use of relief medication, and improving QOL, while being well tolerated. A dose of 5000 JAU of JC pollen SLIT tablet is selected as the optimal clinical dose in the long-term study.

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Group 5 allergen composition and protein composition of 9 sublingual immunotherapy therapies against grass pollen allergies

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Background: People suffering from allergies can use two types of allergen immunotherapies to decrease their hypersensitivity, either subcutaneous injection or sublingual. The traditional vaccination involves a monthly subcutaneous administration of the allergen/allergen mixture (SCIT: sub-cutaneous immunotherapy). In the second type, the allergen extract is administered daily sublingually (either as a liquid or a tablet). In this study, 9 sub-lingual immunotherapy products, all intended for grass pollen allergies were first explored to identify their composition, and then analysed to determine their group 5 allergen content, and the actual protein load per administration determined using SDS-PAGE/Immunoblotting.

Methods: *Compositional analysis:* The companies that manufacture the different products were approached to identify the pollen used to prepare their respective SLIT products, this information, as well as localization information, is presented if available. Sample *Characterization and analysis:* Nine products containing mixed grass extracts used for SLIT were obtained for this study. Grass allergen Group 5 contents was determined by a sandwich ELISA using purified Phl p5 as a reference. SDS-PAGE analysis was used to visualize the protein present in the products at therapeutic doses, and immunoblotting used to identify the different allergens. This allowed also to have a visual representation of the amount of protein present in the daily dose.

Results: As expected, the different grass pollen SLIT products not only vary in the pollen types used in their preparation, but also in their grass group 5 allergen contents. The products can be grouped into three categories: 'High', 'Medium' and 'Low' group 5 contents, with the high group containing, on average, 3-times more group 5 than the medium group, and over 20-times more group 5 protein than the 'Low' group (range of group 5 protein contents ~0.07 μg/dose to ~ 3.4 μg/dose).

Conclusion: The 9 SLIT products are composed of different mixtures of grass pollens, but all include *Phleum pratense*, *Poa pratensis* and *Lolium perenne*. Group 5 allergen contents varies widely, although this could be due that some products are made from pollen obtained from different grass species that may contain less group 5 protein.

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Comparison of efficacy of sublingual and subcutaneous immunotherapy for allergic rhinoconjunctivitis in children

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Background: Allergic rhinoconjunctivitis affects 20% of the population. It causes

morbidity and deteriorates quality of life in children. For children whose symptoms persist despite antihistamines and nasal steroids, immunotherapy, either sublingual (SLIT) or subcutaneous (SCIT), is effective. There is little data on the comparative efficacy of these 2 modes of treatment. Using symptom and medication scores, we compared the efficacy of sublingual and subcutaneous immunotherapy for the treatment of allergic rhinoconjunctivitis in children attending a paediatric allergy clinic.

Method: Children treated with SLIT and SCIT for moderate to severe allergic rhinoconjunctivitis due to grass pollen, tree pollen or house dust mite allergy, between 2003 and 2014 were identified. SLIT was administered perennially for 3 years and SCIT was administered perennially for house dust mite and preseasonally for grass and/or tree pollen for 3 years. Symptom scores (graded out of 33) and medication scores (graded out of 15) were measured at baseline on all patients and then yearly, whilst on treatment. Scores were compared between patients treated with SCIT and SLIT.

Results: In total 38 courses of SLIT and 39 courses of SCIT were administered to 74 children. Forty percent received treatment for combined grass and tree pollen allergy. Eczema was observed in 77% of the children. Symptom and medication scores were available for all 3 years in 36 children. The overall yearly reduction in symptom scores from baseline were -5.2 ± 7.1, -9.0 ± 8.2, and -9.8 ± 6.7; reduction in medication scores were -2.2 ± 2.9, -3.1 ± 3.6, and -3.6 ± 3.2. Paired analysis showed significant reduction in symptom and medication score before and after immunotherapy ($P < 0.001$). Comparative analysis suggested that SCIT led to larger reduction in scores than SLIT, but did not reach statistical significance ($P > 0.05$). Linear regression demonstrated that neither immunotherapy route (SLIT/SCIT) nor presence of atopic comorbidity (eczema, food allergy, asthma) affected the symptom score ($P > 0.05$).

Conclusion: Immunotherapy is effective treatment for allergic rhinoconjunctivitis. This small retrospective study has failed to demonstrate any difference in efficacy between SCIT or SLIT. Prospective studies with larger numbers of patients, using head-to-head comparison will be needed to establish differences in efficacy.

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Allergy immunotherapy medication persistence and adherence with a SLIT-tablet and SCIT preparation in Germany

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Background: Medication persistence and adherence in allergy immunotherapy (AIT) are perceived to be higher for subcutaneous immunotherapy (SCIT) than sublingual immunotherapy (SLIT). Disease-modifying effects and long-term benefits of AIT have been demonstrated after three years of continuous treatment, therefore persistence and adherence are crucial for successful treatment. Our objective was to assess medication persistence and adherence over a 3-years period from AIT initiation in Germany, comparing a SLIT-tablet and a SCIT product prescribed for grass pollen allergy.

Method: A retrospective cohort study was performed using data on prescription renewal rates from the German IMS Health Disease Analyzer (DA) database for the period January 2006 to August 2014. Endpoints included the proportion of persistent patients (≥1 prescription in both the second and third years of follow-up) in the SLIT-tablet and SCIT sub-cohorts over a 3-year follow-up period, and adherence, expressed as the Medication Possession Ratio (MPR), for persistent patients.

Results: Of 6,954 patient profiles obtained from the database, 2,429 patients on SLIT-tablet and 2,109 on SCIT fulfilled the inclusion criteria. The proportion of persistent patients was similar in the SLIT-tablet and SCIT sub-cohorts (30% vs 31%, respectively). The discontinuation rate in the first year of treatment was higher for SLIT-tablet vs SCIT. Among those who remained on treatment at year 2, there were relatively fewer discontinuations for those on SLIT-tablet vs SCIT. The proportion of persistent patients on SLIT and SCIT was comparable across age groups. Adherence over the 3-year follow-up period was comparable for SLIT-tablet and SCIT (MPR 81% and 83%, respectively), and was similar irrespective of patient age group or the speciality of the prescribing physician.

Conclusion: Medication persistence and adherence for grass pollen SLIT-tablet and SCIT were comparable in this German population sample and were consistent across age groups, with some differences in persistence when prescribed by different physician specialties. As persistency is comparable between product modalities focus should be on the level of clinical documentation for each product. Compared with SCIT, patients who discontinued SLIT-tablet tended to do so earlier in the treatment. Those who remained on SLIT-tablet for two years were more likely to continue for a third year vs those on SCIT.

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Analysis of gene expression changes in patients allergic to grass pollen treated with immunotherapy - preliminary results

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Background: Grass pollen allergy is a common cause of asthma, allergic rhinitis and coniunctivitis, which decreases patient's quality of life during pollening season. Specific immunotherapy is the only causative treatment of allergic diseases. Although widely used, it's mechanisms remain unknown and many patients do not benefit significantly during treatment. Understanding of the mechanisms of immunotherapy is vital for improvement of the treatment efficacy. The studies conducted on patients allergic to house dust mites and insect venom revealed changes in certain gene expression, which may help in predicting treatment's efficacy. The aim of the study is to find genes, which expression changes significantly during maintenance phase of immunotherapy.

Methods: The studied group consisted of 7 patients treated in the out-patient clinic in Allergology Department of University Clinical Center in Gdańsk. Analysis was performed on blood samples taken before start of immunotherapy (sample A) and after reaching maintenance dose (sample B). Samples provided mRNA for gene expression analysis of 16 chosen genes. The results were also compared with the group of 7 healthy volunteers with negative allergy history. Statistical analysis of normalised gene expression was performed using computer program Statistica 10. T Student, Menn Whitney, Benjamin-Hochberg's methods.

Results: All treated patients were males. Control group consisted of 3 women and 4 men. Time gap between obtaining sample A and B was on average 96 days. In patient group gene expression analysis revealed statistically significant change of AFAP1L1 expression ($P = 0.04$) For other genes and control group comparisons no significant differences were found.

Conclusion: The expression of AFAP1L1 changes during grass pollen specific

immunotherapy. Further studies are planned on larger patient and control groups to confirm this observation.

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Sublingual allergy immunotherapy patterns of use in RAS 3D study pediatric population

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Background: To improve sublingual application of allergy immunotherapy (SLIT) standardized in IR/ml, a new dosing pump that doubles the dose administered per actuation has been developed by Stallergenes globally and marketed in Spain since September 2013.

The aim of RAS3D study is to describe, in routine practice, the use of initial and maintenance SLIT administered using the new dosing pump in pediatric population.

Methods: A retrospective, cross-sectional, multicenter study in children from 6 years of age with allergic respiratory diseases who were treated with SLIT prescribed between September 2013 and November 2014 was conducted.

A descriptive analysis of all variables collected was performed. Variables are presented according to their character: Categorical variables were summarised using frequencies and percentages. Continuous variables were summarised using measures of central tendency and dispersion: mean, standard deviation, median, 25% and 75% percentiles (Q1 and Q3).

Results: 317 pediatric patients were recruited (57% male), mean (SD) age 9.8 (2.5) years, 2.94 (1.77) years from diagnosis. 89.6% had rhinitis or rhinoconjunctivitis (82.6% moderate to severe, 56% persistent), 48.3% asthma. 57.7% sensitized (prick-test) to pollens (44.8% grasses, 37.2% olea), 56.2% to house dust mites and 25.8% to moulds or animal danders. Mean time from the start of treatment was 13.69 months (4.7). 50.8% of patients were treated with mixed extracts (D. pteronyssinus + D. farinae 44.3%, grasses + olea 15.3%). 100% initiated with the 300 IR/ml vial. 95.9% had an initiation phase of 3 days (day 1 one actuation-0.2 ml-, day 2 two actuations-0.4 ml- and day 3 three actuations-0.6 ml-). Median (Q1, Q3) maintenance dose was 4.0 (2.0, 4.0) actuations, 3.0 (3.0, 5.0) days per week. 33.8% have received treatment with the previous dosing pump for a mean of 14.61 (3.31) months before the change to the new one.

Conclusion: RAS 3D study shows that, in Spain, in pediatric population most patients follow a conventional short build up scheme of 3 days, all patients starting with the 300 IR/ml vial. The median maintenance dose in RAS 3D study aligns with the recommended maintenance dose, 4 actuations 3 days a week.

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The combination of oral immunotherapy and a non-digestible oligosaccharide supplemented diet reduced allergic symptoms in a murine cow's milk allergy model

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Background: Non-digestible oligosaccharides have been shown to reduce allergic symptoms in murine models of allergy.

Objective: To assess the capacity of non-digestible oligosaccharides in supporting the efficacy of oral immunotherapy (OIT) in a murine model for cow's milk allergy.

Method: 5-week old female C3H/HeOuJ mice were sensitized intragastrically (i.g.) to the cow's milk protein whey (20 mg whey + 15 μg cholera toxin in PBS) once a week for 5 weeks (d0-d28). Subsequently, mice were fed a 1% short chain fructo-oligosaccharide/long chain fructo-oligosaccharide (9:1) diet (FF) or a control diet (d35-d78). In addition, mice were treated i.g. with 10 mg whey in PBS or PBS alone for 5 days/week for three weeks (d41-d59). The acute allergic skin response, anaphylaxis score and body temperature were measured upon intradermal (i.d.) challenge (d64), mast cell degranulation was measured upon i.g. challenge (d70) and allergen-specific immunoglobulins were monitored during and after OIT and upon intraperitoneal (i.p.) challenge (d77). Cellular parameters were measured in spleen and mesenteric lymph nodes (MLN) at d0, d35, d50, d63, d71 and d78 and in lamina propria (LP) on d63 and d71.

Results: Whey sensitized mice receiving OIT+FF showed a decreased acute allergic skin response compared to sensitized mice receiving OIT or FF alone. Body temperature, anaphylactic shock symptoms and mMCP-1 levels in serum were improved in the OIT+FF group compared to sensitized control mice on control diet. During OIT, a rise in whey-specific IgG1, IgG2a and IgA was observed independent of the diet. The increase in whey-specific IgE observed in sensitized mice after i.d. challenge (d64), was prevented in OIT+FF mice. Halfway through immunotherapy (d50), a reduction of IL-5, IL-10 and IFNγ in splenocyte culture supernatant and an increase in the percentage of CD4+ CD25+ FoxP3+ Tregs in the MLN was observed in OIT+FF mice. Directly after OIT (d63), the percentage of activated Th1 cells was increased in MLN and a tendency towards an increased percentage of Tregs was observed in the LP of the OIT+FF group. Total short chain fatty acid content was increased in the caecum of mice on FF diet.

Conclusion: OIT in combination with a diet supplemented with non-digestible oligosaccharides effectively reduced allergic symptoms upon i.d. and i.g. challenges. Cellular and cytokine parameters suggest Th2 suppression and the induction of Tregs during immunotherapy in combination with the diet.

Poster Discussion Session PDS 28

Pediatric asthma: Risk factors and management

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Risk factors of asthma

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Background: Asthma is a heterogeneous condition with clinical expressions that depend on age, gender, genetic background and environmental exposures. Wheezing, its major clinical expression, is a nonspecific sign associated with airflow restriction through narrowed airways. Because of the heterogeneity of the disorder, there is limited ability to identify infants and young children with recurrent wheezing who are at increased risk of developing persistent asthma.
Method: Two hundred girls(31%) and 451 boys(69%) 651 children admitted to our clinic with wheezing periods between 1997–2015 years were evaluated retrospectively for asthma risk factors.
Results: In this group of wheezy children asthma frequency was 51% (*n*:376). When it is compaired with non asthmatic group, maturity, consanguinity, family income, passive smoking, father's, sister's and brother's asthma were not found as risk factors whereas family's, parents', sisters' and brothers' atopia, mother's and family's asthma, personal allergic rhinitis and atopic dermatitis, having respiratory symptoms between wheezing periods, immunodeficiencies were identified as risk factors ($P < 0.05$). Selective IgA deficiency was common in atopic asthma and transient hypogammaglobulinemia of infancy was common in nonatopic asthma. Sixty-two percent of the asthmatic patients (*n*:235) were atopic and 38% were non-atopic. Aeroallergen-spIgE was 55% positive in asthmatic group and 89% of patients with aeroallergen-spIgE positivity were asthma wheras asthma frequency was 43% in the negative group and the difference was significant ($P < 0.05$). Negative predictive index(NPI) of absence of asthma bronchiale history in family was high (% 83) and positive predictive index(PPI) was low (27.9%). PPI of modified asthma predictive index (mAPI) for asthma was 65.1% and NPI was 82.3% whereas PPI for atopic asthma was 78% and NPI was 86%.
Conclusion: Modified APİ is a good index as a predictor for asthma in wheezy children in addition family's, parents', sisters' and brothers' atopia, mother's and family's asthma, personal allergic rhinitis and atopic dermatitis, having respiratory symptoms between wheezing attacks, immunodeficiencies were identified as risk factors. Negative mAPI can be a criteria for predicting transient wheezing.

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Early life risk factors for asthma in school age children with grass pollen induced allergic rhinitis

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Background: Results of population-based studies have determined several genetic and environmental risk factors for asthma in school age children. Aeroallergen sensitization is one of the well-established asthma risk factors. However, every child with aeroallergen sensitization does not present with asthma symptoms. The aim of our study was to examine whether parental and perinatal risk factors were associated with asthma in children with seasonal allergic rhinitis.
Method: We analyzed the data of our cohort consisted of children with allergic rhinitis and followed in our outpatient department. Monosensitized children with grass pollen allergy were enrolled whereas children with other sensitizations were excluded. A detailed questionnaire regarding demographic features, parental history and perinatal events was applied to the parents. Laboratory investigations including complete blood counts with differential, total IgE levels, skin prick tests and pulmonary function tests were performed.
Results: A total of 293 children (200 male (68.3%); with a median age [interquartile range] of 10.2 [7.4–13.0] years were included. 109 children (37.2%) had accompanying asthma. The age of onset of rhinitis symptoms were earlier (5.3 years [4.0–8.0] vs 7.0 [5.0–10.0]), $P = 0.001$), history of prematurity (16.7% vs 6.5%) ($P = 0.006$), history of preeclampsia (5.5% vs 0%) ($P = 0.001$), history of NICU admission (15.1% vs 6.0%) ($P = 0.01$), history of phototherapy (17.9% vs 7.1%) ($P = 0.004$), history of formula feeding (58.7% vs 41.2%), ($P = 0.006$) and parental asthma (asthma at any parent) (25.0% vs 11.4%) ($P = 0.002$) were more frequent in children with asthma. Multivariate logistic regression analysis revealed prematurity (Odds Ratio [Confidence Interval]) (2.78 [1.24–6.24]; $P = 0.13$), history of formula feeding (1.81 [1.09–3.01]; $P = 0.01$) and parental asthma (2.37 [1.22–4.63]; $P = 0.011$) were associated with asthma in school-age children with grass pollen induced allergic rhinitis.
Conclusion: Risk factors for asthma may be different in risky populations such as children with grass pollen sensitization and allergic rhinitis. Our results revealed that prematurity, formula feeding and parental asthma as independent risk factors for asthma in school age children with allergic rhinitis.

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Longitudinal trajectory of multiplexed immunoglobulin E sensitization from prenatal stage to the first year of life

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Background: The longitudinal trajectory of allergen-specific IgE levels from the prenatal stage to early life has remained largely unexplored.
Method: One hundred and three mother-infant pairs that were part of an ongoing population-based prospective birth cohort study of early childhood allergic diseases were included in this study. We examined the relationship of 20 allergen-specific IgE levels (including: *D. pteronyssinus*,

D. farinae, Blomia tropicalis, cat dander, dog dander, German cockroach, Bermuda grass, Timothy grass, ragweed, Aspergillus fumigatus, Candida albicans, egg white, milk, codfish, wheat, peanut, soybean, almond, crab and shrimp) with blood samples of mothers, cord blood and infants at 12 months of age using the McNemar test.

Results: A significant level of agreement was observed for most examined allergen-specific IgE levels in blood samples of mothers, cord blood and infants at 12 months of age. When we further examined the association between allergic related risk factors and atopic disease in infants at the first year of life, we found that there was positive association between colic pains at night and atopy in infants at 12 months of age (adjusted odds ratios (AOR) = 3.51; 95% confidence interval: 1.13–10.96; $P = 0.03$).

Conclusion: Together, the findings from this study suggest that the influence of maternal allergen-specific IgE levels on infant immune response might occur at birth and then wane in infants at 12 months of age, and provide supportive evidence for the 'Allergy March' theory of allergy development in an Asian study sample.

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IL-13 gene polymorphisms and their association with asthma in Iranian children patients

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Background: Asthma is a hereditary disorder which environmental and genetic factors like high level of IgE play key roles in its pathogenesis. Interleukin 13 (IL13) is directly involved in the secretion of total serum immunoglobulin E (I g E) .IL13 strongly causes Cbronchial hyperreactivity in response to allergen, airway inflammation airway remodeling, and airway smooth muscle proliferation. The aim of this study was to evaluate the associations between two single nucleotide polymorphism (SNP) located in interlokin 13 gene and the development of asthma in Iranian children population.

Method: In this study, First we prepared questioner for 80 patient that clinical trial of asthma disease.IL13 gene polymorphisms rs20541 (A2044G) and rs1295685 (A2525G) detected by PCR amplification followed by direct sequencing using DNA extraction and RFLP(restriction fragment length polymorphism) and SSCP(single-strand conformation polymorphism) in 80 asthmatic patients.

Results: We investigated 80 patients (26 females and 45 males). In the polymorphism A2525G on agarose gel after digestion by NheI enzyme were observed between 20 patients 7 the heterozygous genotype AG whith three bands and 10 patients show a single band with GG genotype and 1 patiant show AA genotype wih a single band. In The exon 4 nonsynonymous polymorphism A2044G by NlaIV enzyme were observe among 40 patiants, 10 the heterozygous genotype AG until now. A similar finding was also observed among the control group with differences by case group patients.

Conclusion: our study indicate in A2044G and A2525G polymorphisms are important risk factors for asthma susceptibility and severity, with implications for asthma healthcare management. Hence, IL-13 2044 and 2525 may be candidate forfuture gene therapy targeted at reducing the ill-effects of these polymorphisms.

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The relationship between secondhand smoke and wheezing in infants, children and adolescents in the city of Cordoba, Argentina, from an epidemiological perspective

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Background: Breathing in other people's tobacco smoke is known as passive, involuntary or secondhand smoking (SHS). It may also be called environmental tobacco smoke exposure. Globally, an estimated 40% of children are reported to be exposed to SHS. In the UK around 2 million children are estimated to be regularly exposed to SHS at home, being the main source of exposure. Children are particularly vulnerable to the effects of SHS exposure, which has been linked to an increased risk of a range of illnesses, including lower respiratory tract infections, asthma, and wheezing.

Method: Both the epidemiological study EISL (on 12 months of age) (*n*: 1062), and ISAAC (on 6-7 years, (*n*: 998) and 13–14 years, (*n*: 3045)), have been performed by our group in the city of Córdoba, including on these surveys the passive smoking. We searched for the association of the mentioned variables with the presence of wheezing, using parametric tests (ANOVA one-tailed), setting $P < 0.05$ as statistically significant.

Results: In minors under 12 months, we found that 8.4% of mothers smoked during pregnancy, 21.8% of mothers currently smoke, and 33.4% of other residents smoke at home. In the group of 6–7 years, 17% of mothers smoked during pregnancy, 32% of mothers currently smoke, and other smokers are present in 35% of homes. And in the group of 13 and 14 years, 36% of them had their mothers as regular smokers, and 38% have other people smoking at home. We found in the group of <12 months of age a significant association of current smoking mother with severe recurrent *wheezing* (≥ 6 exacerbations in 12 months) OR = 2.7; 95% CI I 1.4–5.18; $P = 0.0009$). We also found a significant association with mothers who smoked during pregnancy OR = 4; 95% CI 1.8–8.5 $P = 0.0001$) and with other people smoking at home. OR = 1.8; 95% CI: 1.01–3.4; $P = 0.002$). Taking all variables together for multiple regressions evidenced that smoking during pregnancy variable had the strongest association with severe recurrent wheezing.

Conclusion: Epidemiological studies like EISL and ISAAC allow to reinforcing the potential preventable actions that are needed (like education on stop smoking in pregnancy), in order to reduce the significant impact of SHS in children and particularly in the most vulnerable group of age, children under 12 months. It is desirable that health authorities promote a strong education campaign to reduce or abolish smoking indoors.

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Association between sensitization to mold and impaired pulmonary function in children with asthma

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Background: Recent data indicate that sensitization to mold may contribute to the severity and persistence of asthma symptoms. The aim of this study was to examine the relationships between sensitization to mold and pulmonary function parameters in children with asthma.

Method: A total of 551 children with asthma were recruited by Allergy Clinic of Korea University Anam Hospital. They underwent spirometry, methacholine

challenge tests, and measurement of blood eosinophils, serum IgE and concentrations of eosinophil cationic protein (ECP) and exhaled nitric oxide (NO). Skin prick test with common aeroallergens in South Korea including house dust mites (*Dermatophagoides pteronyssinus, Dermatophagoides farinae*), animal dander (cat, dog), pollen, cockroach and mold allergens (*Alternaria alternata, Aspergillus fumigatus)* was performed. Children were divided into 3 groups according to their sensitization profiles. Children who did not show positive result to any aeroallergen were designated as Group 1. Group 2 represented children who sensitized to aeroallergens other than mold and Group 3 included children who sensitized to mold allergens.

Results: Among the 551 children, 441 (78.6%) showed a positive response to at least 1 aeroallergen. Three hundreds and seventy-five (86.6%) children were sensitized to house dust mites and followed by animal dander 121 (27.9%), pollen 117 (27.0%), cockroach 37 (8.5%), and mold 67 (15.5%). The mean (± SD) FEV1 in children with Group 3 (86.9 ± 12.1%pred) was significantly lower than those of the Group 1 (93.4 ± 15.4%pred) and Group 2 (92.0 ± 14.8%pred). The log mean (± SD) methacholine PC20 in children with Group 3 (0.08 ± 1.91 mg/ml) was significantly lower than those of the:

Group 1 (2.29 ± 1.66 mg/ml).
Group 2 (1.31 ± 1.69 mg/ml).

Blood IgE levels and eosinophil profiles were significantly higher in the Groups 2 and 3 than those of the Group 1 but no significant difference was found between the former 2 groups.

Conclusion: Sensitization to mold is associated with severe asthma, particularly lower lung function and severe bronchial hyperresponsiveness in children with asthma. However, it may not be necessarily related to eosinophilic inflammation.

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Does asthma influence school performance among adolescents in a Swedish population based birth cohort?

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Background: Previous studies have shown that children and adolescents with asthma tend to be more absent from school. However, it remains unclear if having asthma influences school performance.

Objective: In this study we have examined the association between asthma and school performance among adolescents.

Method: In the 16-year follow up in the population based birth cohort BAMSE, the adolescents answered questions about asthma symptoms. In earlier follow ups at age 1, 2, 4, 8 and 12 years, the parents answered similar questions about their child's asthma symptoms as well as questions about socioeconomic status. Current asthma was defined as more than 3 episodes of wheeze in the last 12 months and/or at least 1 episode of wheeze in combination with inhaled steroids occasionally or regularly in the last 12 months. Early onset/transient asthma was defined as having asthma only early in life (i.e. at age 1, 2 and/or 4 but not at age 8, 12 or 16). Late onset asthma was defined as having asthma only later in life (i.e. at age 8, 12 and/or 16 but not at age 1, 2 or 4) and persistent asthma as having asthma both early and later in life. Information on school grades was obtained from Statistics Sweden. The grade used in this study was the final grade from the compulsory school called merit value which is the sum of grades in 16 subjects. Linear regression analysis was used to estimate the likelihood of performing less well (i.e. getting a lower merit value) if having asthma with adjustments for gender and socioeconomic status.

Results: In the total study population (n = 1736), 7.8% of the adolescents had current asthma, 5.1% early onset/transient asthma, 9.8% late onset and 5.2% persistent asthma. The median grade or merit value in the total population was 265 (ranged from 0 to 320). The adolescents mean age was 16.6 years when answering the questionnaire and 15.9 when receiving the grade. Dividing the grade in 2 categories, with the highest grades in the reference group (i.e. merit value > 240) showed that adolescents with current asthma had a tendency to be more likely to get a lower grade (OR_{adj} = 1.38, CI = 0.93–2.03). Adolescents with late onset and persistent asthma was significantly more likely to get a lower grade (OR_{adj} = 1.53, CI = 1.06–2.20 and OR_{adj} = 1.88, CI = 1.15–3.05 respectively).

Conclusion: Adolescents with asthma tend to perform less well in school than adolescents without asthma, in particular adolescents with late onset or persistent asthma.

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Identifying relationship between the presence of sleep-related breathing disorders and disease control in asthma patients under 5 years old

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Background: It was reported that sleep-related breathing disorders (SRBD) are an independent risk factor for asthma control and they are observed more common in asthmatic patients than normal population. Identifying and controlling SRBD in asthmatic children may affect asthma control in a positive way. In our study, it was aimed to investigate relationship between asthma control and the presence of SRBD in patients under 5 years old who were followed due to asthma in our clinic for at least 1 year.

Method: In this study, patients under 5 years old who were followed due to asthma in our clinic between the dates of June 2013 – December 2014 and came to examination at least 3 times were invited for examination and they were questioned for asthma risk factors, clinical and demographic characteristics. The patients were evaluated according to asthma control and sleep-related breathing disorder. 'TRACK' (Test for Respiratory and Asthma Control in Kids) and 'SRBD' standart questionnaires were used for evaluation of patients. The relationship between presence of SRDB and disease control was investigated.

Results: A total of 130 patients with asthma were examined. Eigty nine (68.5%) patients were male and the average age was 4.04 ± 1.2 (2–5.9) years. In all patients, 92 (70.8%) patients were uncontrolled in terms of GINA, 59 (45.4%) patients were uncontrolled in terms of TRACK. The age of onset of the complaints of the patients was 10.7 ± 10.6 (3–48) months and age of diagnosis was 21.5 ± 14.0 (6–60) months. In all patients, 30 (23.1%) patients had allergic rhinitis, 18 (13.7%) patients had humidity-moisture in their home, and crowd (≥5 people) conditions in their home were present for 43 (33.1%) patients. Passive smoking exposure was available for 71(54.6%) patients. Passive smoking exposure significantly affected diseases control (P = 0.032) but no correlation was found between asthma control and other risk factors affecting the asthma control (P > 0.05). SRBD was identified in 33 (25.4%) patients. In terms of GINA and TRACK scores, no

relationship was detected between disease control and SRBD ($P = 0.241$ and 0.731 respectively).
Conclusion: SRBD frequency was found to be higher in child patients with asthma than normal population and no relationship between presence of SRBD and asthma control was detected. However, there is a need for large-scale studies.

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Disease specific tools are more effective in determining the relationship between asthma control and quality of life in children with asthma

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Background: Asthma has major effects on the life of children and there are various life quality measurement tools available. The aim of the study is to investigate and compare the efficiency of general life quality scales and disease specific scales for asthma and to determine the relationship between quality of life and asthma control status in children with asthma.
Method: Children with asthma who were followed in our pediatric allergy unit were consecutively recruited. Children and parents completed the Childhood Asthma Control Test (C-ACT), Pediatric Asthma Quality of Life Questionnaire (PAQLQ), Pediatric Quality of Life Inventory (PedsQL) and underwent spirometry. An asthma specialist determined the asthma control status of each patient according to GINA guidelines.
Results: A total of 82 children (55 male, 67.1%) with a median age of 10.1 (8.9–10.5) years were included. Asthma was controlled in 52 children (63.4%), partly controlled in 17 children (20.7%) and uncontrolled in 13 children (15.9%). 91 patients (71.6) were in the mild asthma group. C-ACT, PAQLQ and PedsQL child scores were significantly higher in children with controlled asthma. When the correlation between parameters obtained from scales and asthma were analyzed, the most significant correlations were found between C-ACT and asthma control ($r = 0.572$; $P < 0.001$). There were also significant correlations between PAQLQ scores and asthma control. Significant but weak correlations were also determined between PedsQL scores and asthma control.
Conclusion: Assessing quality of life in children using disease specific quality of life measures may provide an important contribution to controlling the disease.

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Smoking among adolescents from a countryside city of Brazil and the future impact in respiratory diseases

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Objective: Despite the anti-smoking prevention programs, many teens start smoking at school age. The objectives were to determine the prevalence and risk factors associated with smoking in adolescents living in Uruguaiana, RS, Brazil.
Methods: A prospective study conducted in adolescents (12–19 years), enrolled in municipal schools, who answered a self-administered questionnaire on smoking.
Results: 798 adolescents were enrolled in the study, with equal distribution between genders. The tobacco experimentation frequency (ever tried a cigarette, even one or two puffs) was 29.3%, and 14.5% started smoking before 12 years of age and 13.0% of them said they smoked at least one cigarette/day last month. Teens reported having ease in getting cigarettes (OR: 1.77; 95% CI: 1.33–2.35) as well as having been affected by a smoker friend (OR: 1.78; 95% CI: 1.44–2 20). Using electronic cigarettes (OR: 2.10; 95% CI: 1.50–2.94), try hookah (OR: 2.37; 95% CI: 1.91–2.94) and live with smokers (OR: 1.93; 95% CI: 1.57–2.38) were significantly associated with active smoking among teenagers. The non smoking father's or non smoking mother's advices were identified as a protective factor for adolescents not initiate smoking (OR: 0.61; 95% CI: 0.49–0.76).
Conclusion: The prevalence of smoking among adolescents in Uruguaiana is high. The implementation of measures to reduce/stop tobacco use and its new forms of consumption, such as electronic cigarettes and hookah in schools are urgent and imperative.
Keywords: tobacco, adolescent, risk factors, cigarette.

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The reality of asthma control in Japanese children

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Background: The goal of asthma management is to achieve and maintain control of the disease. However, recent surveys have shown that many patients with asthma have uncontrolled symptoms and decreased quality of life due to their disease, indicating that this goal defined by current guidelines is not fully achieved. The Japanese pediatric guideline for the treatment and management of asthma (JPGL) recommends higher levels of control compared to other international guidelines such as GINA, and the rate of hospitalization for childhood asthma seems to decrease in Japan. However, there are few population-based studies that have evaluated the asthma control in Japanese children receiving asthma treatment.
Methods: In June 2012, a web-based survey was conducted to identify Japanese children aged 6 to 11 years. who had been using asthma medication regularly for the previous 1 month. We further collected information regarding the prescription and evaluated the control of asthma using the Childhood Asthma Control Test (C-ACT) for the 1450 patients under asthma treatment. The C-ACT score ranged from 0 (poorest asthma control) to 27 (optimal asthma control) and a score of 19 or less was classified as uncontrolled asthma. The study protocol was approved by the independent review board of the Tokyo Metropolitan Children's Medical Center. All parents and their children were provided with an online explanation of the purpose and the procedure of the study and gave informed consent by proceeding to the questionnaire.
Results: Among the 1450 children under asthma treatment, 903 (62.3%), 641 (44.2%) and 566 (39.0%) children received leukotriene receptor antagonists (LTRA), inhaled corticosteroids (ICS) and tulobuterol patch, respectively. Uncontrolled asthma was found in 382 children (26.3%). The proportion of uncontrolled disease was 32.1% in children with ICS, 23.0% in those with LTRA only, and 20.6% in those with tulobuterol patch only, respectively. Meanwhile, optimal asthma control was found in only 75 children (5.1%).

Conclusion: This study provided the reality of asthma control in Japanese children who had received asthma treatment. A quarter of them had uncontrolled diseases and a few children got optimal control, showing that the goal of JPGL was not achieved in a large proportion of Japanese children with asthma.

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Childhood asthma prevalence and therapy in Chorzow (Poland) - an epidemiological study

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Background: Asthma is the most frequent chronic disease in children. Proper therapy should ensure full symptom control. For this reason, the appropriate choice of prescribed medicine is crucial. The aim of this study was to assess asthma prevalence and choice of pharmaceutical therapy as well as its correctness according to current recommendations.
Method: Population based survey was performed in town of Chorzów (Poland) using standard questionnaire in a randomly selected group of 2032 children in the age from 5 to 15 years. Questionnaire was completed by parents.
Results: Previously diagnosed asthma was reported in 12.6% of children ($n = 255$) children. In 44.3% of children with asthma, during last 12 months at least one of the following respiratory symptoms occurred: wheezing – 38.0%, dry cough attacks - 16.9% and respiratory symptoms after exertion – 38.1%. Asthma exacerbation during last year was noticed in 28.4% of children with asthma. Any allergic disease was present in 93.1% of children with asthma. Any pharmacological therapy was received by 65.8% of children with asthma. In a group of children with asthma where respiratory symptoms were present in the last 12 months, pharmaceutical therapy was applied in 89.2% while in group with no current presence of symptoms only 46.7% of children were treated ($P = 0.0001$). Inhaled glucocorticosteroids were prescribed in 68.8% of asthmatic children with current respiratory symptoms and in 30.4% of asthmatic children with no current symptoms ($P < 0.001$), anti-leukotrienes in 46.0 vs 20.0% ($P < 0.001$), short-acting beta2-agonists (SABA) in 28.8 vs 8.9% ($P < 0.001$), antihistamines in 23.4 vs 13.3% ($P < 0.001$), long-acting beta2-agonists (LABA) in 13.5 vs 6.7% ($P = 0.04$), systemic glucocorticosteroids in 0.4% vs 0.0% ($P = 0.2$), theophylline in 0.4 vs 0.0% ($P = 0.02$) respectively.

Among children treated by LABA only, 2% did not use inhaled steroids at the same time. Detailed analysis revealed that asthma treatment consistent with GINA recommendation was completed in 65.9% of children with asthma.
Conclusion: Almost half of children with diagnosed asthma suffer from respiratory symptoms and over 10% of them do not receive any treatment. More than one third of children with asthma are not treated in line with current GINA guidelines.

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Wider neck circumference is associated with asthma in obese children

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Background: Obesity is a well-established risk factor for asthma. Previous studies reported that central obesity is more associated with asthma. The aim of the study is to investigate the association between fat distribution, which is determined by anthropometric measures including neck circumference (NC) and asthma in school-age children.
Method: Children diagnosed with asthma were enrolled along with control subjects who admitted to our outpatient department with allergic symptoms such as rhinitis, urticaria and atopic dermatitis. Anthropometric measures including height, weight, NC, waist circumference and hip circumference were obtained. Skin prick tests, blood eosinophil counts and serum total IgE level measurements were performed.
Results: A total of 196 children (92 male, 46.9%) with a median age of 9.3 (7.3–11.6) years were included. Asthma was present in 102 (52.1%) patients. 91 of the patients (46.4) were overweight and 45 patients (22.9) were obese. NC of children with asthma (median, interquartile range) [29.0 cm (23.5–38.5)] was significantly higher than children in control group [28.5 cm (26.9–30.0)] ($P = 0.04$). Grades defined according to neck circumference percentiles were also significantly different between groups ($P = 0.04$). In children with asthma, the prevelance of children with NC higher than 90th percentile (grade 6) was more frequent when compared to control subjects ($P = 0.02$). The median NC of obese-overweight children with asthma [32 cm (29.3–33.0)] was significantly higher compared to obese-overweight subjects without asthma [30 cm (28.0–32.0)] ($P = 0.01$). Result of multivariable logistic regression analysis revealed that presence of NC > 90th percentile were associated with asthma in obese-overweight children (odds ratio; [95% confidence interval] (3.67 [1.25–8.21]; $P = 0.001$).
Conclusion: Neck circumference, which is a simple anthropometric measure, is associated with asthma in obese children.

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Is neck circumference associated with asthma severity in children?

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Background: Obesity is a risk factor for asthma in children and neck circumference (NC) is associated with the disease's risks. The aim of the study was to investigate if wider neck circumference is related to severe asthma in children.
Method: Cross-sectional study including 1466 elementary school students aged between 6–12 years old at public schools in Santos, Brazil. Asthma characteristics were evaluated using the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. Anthropometric measures including height, weight, waist circumference and neck circumference were obtained. The association between asthma and the risk factors studied was assessed by logistic regression analysis at a 5% statistical significance.
Results: The result of multivariable logistic regression analysis revealed that after allowing for sex, age and family history of asthma neck circumference (NC>95th percentile) was associated with severe asthma (odds ratio; [95% confidence interval] (1.57 [1.20–2.06]; $P = 0.001$).
Conclusion: Neck circumference is associated with severe asthma in children.

Poster Discussion Session PDS 29

Asthma management

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Omalizumab treatment decreased IL-1β and irisin but increased chemerin without any impact on NK cells and APC cells in cases of severe asthma

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Background: Irisin is a thermogenic protein that sources energy outgoing by converting white adipose tissue to brown adipose tissue. Chemerin is originally identified as a chemoattractant protein mainly mediating the chemotaxis of dendritic cells (DC) and natural killer (NK) cells. The aim of this study is to assess potential impact of immune modulation related chemerin and irisin concentrations together with cell surface markers (CSM) in allergic asthmatic patients in treatment of omalizumab.
Methods: The study participants were age and sex matched 30 healthy controls (Group I) and consecutive patients who had severe persistent asthma disease (Group II). Asthma patients took Omalizumab treatment for 12 months within every 2 weeks. Flow cytometry analysis was used to evaluate CSM, ELISA for IL-1β expression. In addition, NK activity (NKA) and induced cytokine expression (by bioassay and ELISA, respectively) before and after omalizumab therapy were evaluated.
Results: Chemerin, irisin and IL-1β concentrations were significantly higher in severe persistent asthma patients compared to controls in serum ($P = 0.01$; $P = 0.03$; $P = 0.008$, respectively). IL-1β level decreased with treatment and it was statistically significant. Although leves decreased, no statistically significant difference were observed for Irisin, CD80, CD56/16 levels. Chemerin level kept rising after treatment and this was significant statistically.
Conclusions: This is the first study to assess NKA and adipokins in asthma patients and their relationship with CSM. We observed that the level of these molecules are higher in asthma and are influenced by Omalizumab treatment. Since no obvious change was observed for NKC, Omalizumab may be considered safe against cancer development.

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Seven years of clinical experience with omalizumab for moderate-severe allergic asthma treatment

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Background: Omalizumab is a monoclonal antibody approved for treatment of moderate to severe persistent allergic asthma whose symptoms are inadequately controlled with inhaled corticosteroids. Its use has shown to be safe and effective, demonstrating decrease in the number of asthma exacerbations, emergency room visits and hospitalizations with less influence on lung function and FEV1 levels.
Objective: To evaluate the efficacy and tolerability of omalizumab in moderate-severe allergic asthma and analyze its impact on cost savings.
Method: An observational and retrospective study was performed in a tertiary referral hospital in Madrid. All patients with moderate-severe allergic asthma who received omalizumab were evaluated. Demographic data, baseline levels of serum IgE, skin prick tests and/or specific IgE, treatment used and dose and frequency of administration of omalizumab were collected from January 2007 to July 2014. Number of asthma exacerbations and hospital admissions for each patient were also compared before and after receiving treatment with omalizumab.
Results: A total of 30 patients were included. Average of age was 40 ± 20.72 years and 83.3% were women. Average of serum total was IgE 478 KU/L. Average time of omalizumab treatment was 3.4 ± 1.9 years. Seven patients abandoned treatment with omalizumab during this period. A symptomatic improvement was obtained in 97% of patients. Reduction of oral corticosteroids intake was achieved in 61.5%, for inhaled corticosteroids in 56.7% and 83.3% of the patients could reduce the rescue medication of short action beta-agonists. Oral corticosteroids were withdrawn in 38.5% of the patients. After starting the treatment with omalizumab an average reduction of 82.4% ($P < 0.01$) in the number of exacerbations and 69.9% ($P = 0.012$) in the number of hospital admissions was observed. A cost analysis study revealed a mean cost savings of 7060 € (ranging from 1528 € to 12 362 € per patient and year. No relevant adverse effects were observed.
Conclusions: Omalizumab has shown to be a safe and a cost-effective treatment in moderate-severe allergic asthma. A remarkable reduction of asthma exacerbations, hospital admissions, use of rescue medication and sanitary cost savings were stated.

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Effect of mepolizumab in severe eosinophilic asthma patients in relation to their baseline ACQ-5 and SGRQ scores

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Background: Clinical studies have demonstrated improvements in symptoms and health-related quality of life (HRQoL) with mepolizumab, as indicated by asthma control questionnaire (ACQ-5) and St George's Respiratory Questionnaire (SGRQ) scores, respectively, in patients with severe eosinophilic asthma (SEA). This analysis determined whether responses to mepolizumab was influenced by baseline disease severity, as assessed by ACQ-5 and SGRQ scores.
Method: The MENSA (NCT01691521) trial included patients with SEA, aged

≥12 years, receiving high-dose inhaled corticosteroids plus additional controller(s), with a history of ≥2 exacerbations in the past 12 months. Patients ($N = 576$) received mepolizumab 100 mg subcutaneous (SC) or 75 mg intravenous (IV), or placebo every 4-weeks for 32-weeks. ACQ-5 and SGRQ scores were assessed at baseline and Week 32. No specific SGRQ or ACQ-5 thresholds were required for inclusion in the study. In this post hoc analysis, ACQ-5 and SGRQ scores at Week 32 were stratified by baseline

ACQ-5 score (<1.5, ≥1.5 units) and SGRQ score quartiles (≤31.9, >31.9–≤46.4, >46.4–≤60, >60 units). Results are provided as differences in change from baseline between groups.

Results: Numerically greater improvements in ACQ-5 scores were observed with mepolizumab SC vs placebo in patients with baseline ACQ-5 scores ≥1.5 (difference: −0.53 [95% CI: −0.78, −0.27]) than with scores <1.5 (difference: −0.31 [95% CI: −0.54, −0.08]). Similarly, improvements in SGRQ scores were greater with mepolizumab SC vs placebo in patients with baseline ACQ-5 scores ≥1.5 (difference: −7.9 [95% CI: −12.0, −3.8]) than with <1.5 (difference: −5.5 [95% CI: −10.5, −0.6]). When stratified by baseline SGRQ scores, improvements in SGRQ score with mepolizumab SC vs placebo were similar regardless of baseline scores (baseline score ≤31.9, difference: −7.8 [95% CI: −12.5, −3.1]; baseline score >60, difference: −8.3 [95% CI: −16.5, 0.0]). A similar pattern of results was seen with mepolizumab IV vs placebo.

Conclusion: Mepolizumab improved asthma symptoms and HRQoL in patients with SEA, regardless of baseline disease severity, measured by ACQ-5 or SGRQ. Patients with worse asthma control at baseline (ACQ-5 scores ≥1.5) demonstrated the largest numerical improvement in symptoms and HRQoL with mepolizumab in this post hoc analysis, suggesting a greater clinical benefit of mepolizumab in these patients, although prospective studies are required to confirm this.

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Evaluation of methotrexate in the treatment of severe persistent asthma

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Background: Methotrexate (MTX) was proposed in 2014 by international ERS/ATS guidelines in patients with severe steroid-dependent asthma who require daily oral corticosteroids (OCS) and limited to specialised centres. Our objective was to evaluate the efficacy and tolerance of methotrexate in the treatment of severe persistent steroid-dependent asthma.

Method: A 5-year retrospective study was conducted and included patients followed by pneumologists with uncontrolled severe persistent steroid-dependent asthma and who received at least one dose of methotrexate during their follow-up.

Results: 9 patients were treated by methotrexate for severe persistent asthma. The duration of treatment averaged 27.4 months with a median of 11.5 months. In 5 patients, methotrexate was administered by oral route. The average dose was 13.8 ± 2.3 mg with a median of 15 mg. A decrease of the doses of OCS was observed in 7 patients. A reduction in the number of exacerbations and clinical improvement was observed in 6 patients. In 3 patients, mean FEV_1 showed an improvement after the addition of treatment with MTX. The tolerance was acceptable for 7 patients. In one case, methotrexate was stopped due to poor tolerability with onset of bronchospasm and skin rash. In another case, the treatment was stopped because of repeated infections. Folic acid supplementation was reported in 6 patients.

Conclusion: Treatment with MTX is still continued in 3 patients. Given its relatively good tolerance (particularly due to the low doses used), MTX may be considered as an additional alternative treatment for severe persistent asthma allow corticosteroid dose reduction.

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Real-world experience with a new dry powder inhaler for asthma/chronic obstructive pulmonary disease (COPD): patient satisfaction, preference and ease of use

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Background: Budesonide/formoterol fumarate dihydrate (BF) Spiromax® is a multi-dose dry-powder inhaler licensed in Europe since 2014 for the management of asthma and COPD in patients (pts) aged ≥18 years. This prospective, open-label, multi-centre, phase 4, non-interventional, observational study was conducted in Germany, as recommended by the local regulatory authorities, to collect post-approval data on pt satisfaction and preference, and ease of use with BF Spiromax within the labelled indication.

Method: Pts were adults with asthma or COPD, for whom an inhaled corticosteroid (ICS)/long-acting β2 agonist (LABA) combination was indicated, and who were scheduled to commence treatment/switch treatment to BF Spiromax. Physician rationale for prescribing/switching to BF Spiromax was determined at Visit 1 (Week 1). Pts were assessed at Visit 1 and at Weeks 4 and 12 for their Satisfaction With Inhalers and Preference (SWIP) questionnaire and inhalation technique/handling errors. Here, we report ad interim on the patients' device preference and satisfaction findings of the study.

Results: 2500 pts were enrolled, 1891 pts completed Visit 1 (44% male, mean age 56.8 years, 67%/33% with asthma/COPD diagnosis). 1305 pts (with completed electronic case-report forms [e-CRFs]) completed the study. SWIP data (not part of the e-CRF) were available for 578 pts at Week 1 and for 449 pts at Week 12. Prior to starting BF Spiromax, 50% of pts were receiving an ICS/LABA combination (56% on BF Turbuhaler®). The main reasons cited by physicians for prescribing/switching to BF Spiromax were: low requirement for training (46%), one-step inhalation (41%) and anticipated improvement in adherence (36%). Results from the SWIP questionnaire indicated that 65% were 'satisfied' or 'very satisfied' with Spiromax at Week 1; this increased to 91% by Week 12. 58% of pts preferred BF Spiromax to their old inhaler at Week 12. The most common observed errors were 'not exhaling fully before inhalation' (20% [116/580] Week 1; 10% [47/486] Week 12) followed by 'Inhalation is not as strong as possible from the beginning' (18% [102/580] Week 1, 9% [46/486] Week 12). At the end of the observation period (Week 12), 88% of pts continued treatment with BF Spiromax.

Conclusion: These real-world data suggest that pt satisfaction with BF Spiromax was high and increased from Week 1 through Week 12, with more adult pts indicating a preference for BF Spiromax over their previous inhaler.

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Switch from mid-dose inhaled corticosteroid/long-acting beta$_2$ agonist (ICS/LABA) treatment to fluticasone furoate/vilanterol (FF/VI) 100/25 μg

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Background: The results of the first period of a study that aimed to evaluate the proportion of Japanese patients (pts) whose asthma remained 'well-controlled' after switching from mid-dose twice-daily (bd) ICS/LABA, to once-daily (qd) FF/VI 100/25 μg are presented.

Method: The study (GSK 201135) was divided into two periods: Period 1 (P1; open label); and Period 2 (P2: pts who remained well-controlled at the end of P1 were randomised to receive FF 100 μg qd or fluticasone propionate [FP] 100 μg bd or FP 250 μg bd [double blind]). Well-controlled asthma was defined as having no exacerbation/asthma worsening, no night-time symptoms, a best pre-bronchodilator forced expiratory volume in 1 second ≥80% at clinic, and ≥2 per week of: daytime symptoms on ≤1 day, rescue use on ≤1 day, or morning peak expiratory flow ≥80% of the best effort value. The results of P1 are presented here, where, after completing a run-in period of 4 weeks, pts with well-controlled asthma taking an ICS/LABA equivalent to salmeterol/FP 250/50 μg bd were transferred to FF/VI 100/25 μg qd for an 8-week open-label treatment period. Asthma control status was assessed in the last week of P1. Other assessments included time to withdrawal due to poorly controlled asthma (requiring step-up treatment), percentage of symptom- and rescue-free 24 h periods, and Asthma Control Test (ACT). Safety assessments included adverse events (AEs). The study was descriptive with no sample size calculation.

Results: A total of 551 pts were enrolled: 430 were included in the open-label population (i.e. received ≥1 study medication dose in P1). At the end of P1, 373 (90.5%; 95% CI 87.29, 93.18) pts' asthma remained well controlled with FF/VI 100/25 μg qd. 39 (9.5%) pts were not assessed to be well controlled ($n = 29$ not controlled; $n = 10$ not evaluable). 17 pts were withdrawn due to poorly controlled asthma during P1; the median time to withdrawal was 53 days (range 15–58). Lung function was maintained over the 8 weeks following the switch from mid-dose ICS/LABA bd to FF/VI 100/25 μg qd, as was the ACT score. Rescue- and symptom-free 24 h period percentages were also maintained at over 95% each week. During P1, 37% of pts reported AEs and nasopharyngitis (16%) was the only AE reported by ≥3% of pts. No serious AEs/deaths were reported.

Conclusion: Well-controlled asthma was maintained by 90.5% of pts who were transferred from mid-dose ICS/LABA to FF/VI 100/25 μg qd.

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LJ/CG: GSK employee/GSK stocks.

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Stepping down from fluticasone furoate/vilanterol (FF/VI) to inhaled corticosteroids (ICS) alone

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Background: We report results of Period 2 (P2) of a study that aimed to clarify the positioning of FF 100 μg, by comparison of asthma control with fluticasone propionate (FP) when 'well-controlled' pts stepped down from FF/VI 100/25 μg to ICS alone.

Method: The study (GSK 201135) was split into 2 periods: Period 1 (P1) assessed pts who remained well-controlled when transferred from mid-dose ICS/long-acting beta$_2$ agonist to open-label FF/VI 100/25 μg. In P2, pts who remained well controlled were randomised (1:1:1/double blind) to FP 250 μg twice-daily (bd), FP 100 μg bd or FF 100 μg once daily (qd) for 12 weeks. Co-primary endpoints: % of pts with well-controlled asthma in the last week of P2; time to withdrawal due to poorly controlled asthma requiring step-up therapy.

Results: The intent-to-treat population in P2 included 371 pts (mean age 47.6 years; 58% female; all Japanese). When well-controlled pts stepped down from FF/VI to ICS alone, control was maintained in ~80–90% pts per group; ~5–7% of pts were withdrawn due to poorly controlled asthma (Table 1), with similar time to withdrawal curves across groups (data not shown). Comparable reductions occurred across the 3 arms in secondary endpoints (forced expiratory volume in 1s, peak expiratory flow, % rescue-/symptom-free 24 h

Table 1 Asthma control in pts stepped down from FF/VI to ICS alone

	FF 100 μg qd ($N = 123$; $N = 114$ for analysis of well-controlled asthma)	FP 100 μg bd ($N = 124$; $N = 117$ for analysis of well-controlled asthma)	FP 250 μg bd ($N = 124$; $N = 117$ for analysis of well-controlled asthma)
Pts withdrawn due to poorly controlled asthma[+], *n* (% [95% CI])	6 (4.9 [1.81,10.32])	7 (5.6 [2.30, 11.29])	9 (7.3 [3.37,13.33])
FF 100 μg qd vs FP 250 μg bd HR* (95% CI)	–	–	0.92 (0.32,2.62)
FF 100 μg qd vs FP 100 μg bd HR* (95% CI)	-	1.16 (0.39, 3.47)	-
Pts with well-controlled asthma[‡] at the end of the study, *n* (% [95% CI])	102 (89.5 [82.33,94.44])	93 (79.5 [71.03,86.39])	98 (83.8 [75.81,89.93])
FF 100 μg qd vs FP 250 μg bd OR[†] (95% CI)	–	–	1.37 (0.62,3.04)
FF 100 μg qd vs FP 100 μg bd OR[†] (95% CI)	–	1.75 (0.81,3.78)	–

[+]Defined as asthma worsening/exacerbation or ≥3 per week of: day symptoms on ≥2 days, rescue use on ≥2 days, morning PEF <80% of best effort on ≥1 day, night symptoms on ≥1 or more day, a best pre-bronchodilator forced expiratory volume in 1s (FEV1) <80% at clinic.

[‡]Defined as having no exacerbation/asthma worsening, no night-time symptoms, a best pre-bronchodilator FEV1 ≥ 80% at clinic, and ≥2 per week of: daytime symptoms on ≤1 day, rescue use on ≤1 day, or morning peak expiratory flow ≥80% of the best effort value (patients not assessed as well controlled may have been unevaluable for assessment).

*Cox proportional hazards.

[†]Logistic regression; CI, confidence interval; HR, hazard ratio; OR, odds ratio.

periods, Asthma Control Test). Adverse events (AEs) were reported by 36% (FF 100 μg qd), 48% (FP 100 μg bd) and 49% (FP 250 μg bd) of pts. 2 serious AEs were reported ($n = 1$ FF 100 μg qd; $n = 1$ FP 250 μg bd); neither were drug related. Drug-related AEs were reported by 4% (FF 100 μg qd), 4% (FP 100 μg bd) and 2% (FP 250 μg bd) of pts; the most frequent was oral candidiasis.

Conclusion: After stepping down well-controlled pts from FF/VI to ICS alone, maintenance of asthma control was comparable between FF 100 μg qd, FP 100 μg bd and FP 250 μg bd. The safety profile of the 3 groups was generally similar but ~12–13% fewer pts reported an AE with FF, compared with either FP dose.

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LJ/CG: GSK employee/GSK stocks.

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Combination therapy of inhaled steroids and long-acting beta2-agonists in asthma-COPD overlap syndrome

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Background: +Although asthma and COPD are known to have different entities and independent etiologies, they shares some features in specific phenotypes, and may overlap each other (Asthma-COPD Overlap Syndrome, ACOS). There is limited evidence of the effect of ICS (inhaled corticosteroid) in patients with ACOS while its beneficial effect in those with asthma. Limited literatures on the use of ICS on ACOS patients depended on the cellular criteria for definingACOS. We aim to evaluate the benefit of ICS/LABA (long acting beta2-agonist) in patients with ACOS when compared to those with COPD only.

Method: A total of 152 stable COPD patients were classified into ACOS and COPD-only groups. ACOS was defined when a patient.

1 Had bronchodilator response (increase in FEV1 of 200 mL and 12%).
2 Was diagnosed as having asthma previously.
3 Had a history of wheezing in the past year.

Patients were recruited prospectively and treated with ICS/LABA during 3 months.

Results: After 3-month treatment of ICS/LABA, the increase of FEV1 was significantly greater in ACOS patients (ACOS group, 240.1 ± 33.5 ml; COPD-only group, 124.6 ± 19.8 ml; $P = 0.004$). Thee multivariate logistic regression model revealed that ACOS was a significant predictor of FEV1 increase even after adjustments of age, body mass index, smoking status, baseline FEV1, and blood eosinophils. In patients with mild to moderate airway obstruction, the degree of FEV1 increase was significantly higher in ACOS group than in COPD-only groups (ACOS group, 223.2 ± 42.9 ml; COPD-only group, 84.6 ± 25.3 ml; $P = 0.005$). However, the difference was not shown in those with severe to very severe obstruction (ACOS group, 269.4 ± 54.6 ml; COPD-only group, 197.1 ± 28.4; $P = 0.202$).

Conclusion: ICS/LABA was beneficial in ACOS patients regardless of the level of peripheral blood eosinophils and the severity of airway obstruction.

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High levels of group 2 innate lymphoid cells were inhibited with glucocorticoid treatment in allergic airway inflammation patients in China

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Background: Group 2 innate lymphoid cells (ILC2s) are closely associated with the human allergic disease, such as asthma (AS) and allergic rhinitis (AR). However, the effects of ILC2s to the severity of the diseases and the relationship between ILC2s and glucocorticoid treatment, is not well understood. The purpose of this study was to investigate whether glucocorticoid treatment was involved to ILC2s in AS and AR patients.

Methods: Peripheral blood mononuclear cells were collected from subjects with AS, AS with AR before or 1 and 3 months with the glucocorticoid treatments and ILC2s were evaluated using flow cytometry. PBMCs were cultured with IL-2, IL-25 and IL-33, and the levels of ILC2, IL-5, IL-13 were examined. In addtion, ILC2s were also sorted and evaluated its functions. Elisa was used to measure the cytokine in plasma or cell-free supernatant.

Results: The levels of ILC2s and IL-13 in blood were higher in the asthma group and asthma with allergic rhinitis patients compared to the healthy controls. IL-13 levels in asthma but not AS with AR had positive collection with ILC2 levels. ILC2 levels in PBMCs were significantly decreased 1 and 3 months after glucocorticoid treatment and there was no differences for the ILC2 levels between the patients with 3 month-treatment and the healthy controls. We further identified that IL-5 and IL-13 dramaticlly increased in AS, AS with AR patients. Furthermore, glucocorticoid significantly inhibited the production of IL-13 in cultured ILC2s. We successfully sorted ILC2s from AS PBMCs and found that ILC2s were the main cells to produce IL-13 under the stimulation.

Conclusion: There were high ILC2s inasthma patients with or within allergic rhinitis in China and were decreased after the treatment ofglucocorticoid.

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Once-daily tiotropium add-on to at least ICS demonstrates improved asthma control and reduced exacerbation risk in patients with symptomatic asthma, independent of serum IgE or blood eosinophil levels

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Background: Once-daily tiotropium (via the Respimat soft-mist device), as add-on to ICS ± other maintenance therapies, has been shown to improve asthma symptom control and reduce the risk of severe exacerbation and asthma worsening in adults with symptomatic asthma, independent of serum IgE ≤ or >430 μg/l and blood eosinophils ≤ or $>0.6 \times 10^9$/l, in conventional subgroup analyses. We assessed whether improvements in asthma control and exacerbation risk were observed in modelling estimates across a continuous range of IgE and eosinophil values following tiotropium add-on therapy.

Method: Four Phase III double-blind, placebo-controlled, parallel-group trials: PrimoTinA (two 48-week trials; NCT00 776984/NCT00772538; $n = 912$), once-daily tiotropium 5 μg or placebo (via Respimat) as add-on to ICS (≥800 μg budesonide or equivalent) + LABA; MezzoTinA (two 24-week trials; NCT01172808/NCT01172821; $n = 2100$), once-daily tiotropium 5 μg or 2.5 μg (via Respimat), twice-daily salmeterol 50 μg (via HFA-MDI) or placebo (double-dummy protocol) as add-on to ICS (400-800 μg budesonide or equivalent). Patients had symptomatic asthma requiring at least ICS therapy for ≥4 weeks before screening; COPD was excluded. *Post hoc* logistic regression modelling analyses of ACQ-7 responder rate and *post hoc* Cox

regression modelling of severe exacerbation and asthma worsening were performed across continuous ranges of IgE (2-2000 μg/L) and eosinophils (0.05-2.00 × 10^9/L) to investigate tiotropium or salmeterol (MezzoTinA only) vs placebo.

Results: Tiotropium consistently improved ACQ-7 responder rate vs placebo across all IgE and eosinophil ranges (odds ratio [OR] >1). Salmeterol also improved ACQ-7 responder rate vs placebo across IgE levels (OR >1). In the modelling with salmeterol, as eosinophil levels increase, the ACQ-7 responder rate OR decreases, becoming <1 at levels above ~1.4 × 10^9/l. In modelling of exacerbation risk, in general, tiotropium consistently reduced the risk of severe exacerbation and asthma worsening at all levels of IgE and eosinophils vs placebo (hazard ratio <1); salmeterol also reduced the risk of severe exacerbation and asthma worsening vs placebo.

Conclusion: Once-daily tiotropium, as add-on to ICS ± other maintenance therapies, improved asthma symptom control and reduced exacerbation risk in patients with moderate or severe symptomatic asthma, across the range of IgE and eosinophil values, supporting findings from conventional subgroup analyses.

Poster Discussion Session PDS 30

Epidemiology of drug allergy

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Hypersensitivity to fluorescein - experience of a tertiary hospital

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Background: Fluorescein is a photoluminescent molecule frequently used in ophthalmology as a contrast agent in retinal angiographies. Adverse reactions to fluorescein, namely after intravenous administration, are rare (3%) and can be divided into mild, moderate and severe (including anaphylaxis). Fluorescein allergy prevalence is unknown. We attempted to summarize the experience of our center in the management of these patients.
Method: A specialized appointment period dedicated to contrast media and dye allergy was created in our department, to which were referred patients with suspected allergy to these drugs, including fluorescein. Data were compiled from patient files followed for suspected fluorescein allergy.
Results: Currently, nine patients are being followed in our outpatient clinic for suspected allergy to fluorescein, three males and six females, with ages between 18 and 78 years-old. Fifty-five percent of the patients had previous history of allergic disease (rhinitis or asthma). All of the patients had presented with immediate-type reactions after the intravenous administration of fluorescein. In most patients (five), the symptoms were systemic manifestations (syncope), while in the remaining patients these were solely cutaneous manifestations (pruritus, urticaria). During workup, all skin prick tests with fluorescein were negative. Intradermal tests with fluorescein were positive in two patients, one with the 1:10 dilution and another with the 1:100 dilution.
Conclusions: The literature estimates that half of the patients with previous reactions to fluorescein will experience another reaction after a new administration. However, it is believed that most reactions to fluorescein are due to non-allergic, non-specific mechanisms (e.g. non-IgE mediated histamine release). This could explain the low number of patients with positive skin tests in the studied group. Our data seems to support current literature in showing anaphylaxis as a rare, yet real, complication of fluorescein administration. Therefore, physicians overseeing these procedures must be prepared to identify and promptly treat these potentially life-threatening events.

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Perioperative anaphylaxis due to selective hypersensitivity to cefazolin

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Background: In the last decades hypersensitivity to cephalosporins has been increasingly reported related with their growing prescription. Perioperative use of cefazolin, a first-generation cephalosporin commonly included in prophylaxis protocols, has been associated to severe allergic reactions, and the patients are labelled as penicillin allergic afterwards. The aim of our study was to describe a group of patients with immediate reactions to cefazolin, whose allergological workup confirmed as being selective hypersensitivity reactions.
Methods: Systematic review of all patients followed in our center with cefazolin-related reactions, between January 2012 and December 2015. All patients were investigated according to the European Network for Drug Allergy (ENDA) recommendations through skin testing (major and minor penicillin determinants, penicillin, amoxicillin, cefazolin, cefuroxime and ceftriaxone) and oral challenges tests.
Results: A total of six patients with confirmed IgE-mediated cefazolin hypersensitivity were identified. All patients developed anaphylactic reactions, three with hypotension, immediately (less than 10 minutes) after cefazolin injection. All reactions occurred in perioperative setting. Median age was 39.5 [32; 42] years, four female and two patients had history of other allergic diseases (drug-related, skin and respiratory allergy). Cefazolin skin tests were positive in all patients by intradermal tests (IDT), with cefazolin 0.25 mg/ml (2), cefazolin 2.5 mg/ml (2) and cefazolin 10 mg/ml (2). Two patients experienced systemic reactions during IDT, treated with oral medication. All patients were successfully challenged with amoxicillin, and three patients also with cefuroxime (the other three tolerated cefuroxime).
Conclusions: Cefazolin can be responsible for immediate severe allergic reactions in perioperative setting. Allergological workup is essential to perform accurate diagnosis and explore cross-reactivity between cefazolin and other beta-lactams. Our experience confirmed that patients with IgE-mediated hypersensitivity reactions to cefazolin can tolerate other beta-lactams, but a selective avoidance of first-generation cephalosporins is reasonable in these patients. This selective pattern of clinical reactivity may be explained by its particular chemical structure, whose R1 side-chain is different from other beta-lactams.

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Descriptive analysis of patients evaluated in our allergy unit with allergic reactions to fluoroquinolones

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Background: Fluoroquinolones (FQ) are the second most frequent cause of hypersensitivity to antibiotics after betalactams. In recent years, an increase in the number of patients allergic to these drugs has been detected. The aim of our study was to describe the clinical characteristics and the methods used for diagnosing patients with allergy to FQ.
Method: All patients who were studied in our Allergy Unit in the last 10 years (2005–2014) with a suspicion of hypersensitivity to FQ were evaluated. Diagnosis was achieved by clinical history, basophil activation test (BAT) and/or drug provocation test (DPT).
Results: A total of 381 patients were evaluated and 146 (38.32%) of them were confirmed as allergic. Over two-thirds (73%) were female, and the mean age was 52.50 ± 12.5 years. Most reactions were immediate with 73% of cases having a reaction less than 1 h after FQ

administration. The FQ involved were Moxifloxacino in 51.2% of cases, Ciprofloxacin in 33.6%, Levofloxacin in 11.6% Norfloxacin in 2.1% and Ofloxacin in 1.44%. The most frequent clinical entity was anaphylaxis (56%) followed by generalized urticaria (25%), exanthema (5%) and angioedema (7%). In patients with immediate reactions, the diagnosis was achieved using clinical history in 51. 9%, DPT in 8.5% and BAT in 34.9%. In patients with non-immediate reactions diagnosis was performed by clinical history in 39.5% and DPT in 59.5%.

Conclusion: These data indicate that most reactions induced by FQ are immediate (anaphylaxis) and induced by Moxifloxacin. The BAT seems to be a useful method for diagnosing these patients.

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Study of patients sensitized to quinolone drugs

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Background: Quinolone antibiotics represent the second most used group of antibiotics, following betalactams in industrialized countries. Ciprofloxacin is the second most prescribed antibiotic in USA. We present the follow-up through 5 years of 24patients diagnosed of hypersensitivity to quinolones.

Method: We performed skin prick tests (SPT) and intradermal tests(IDTs) with different concentrations of quinolones. Ciprofloxacin: SPT 2 mg/ml and IDT 0.02 mg/ml; levofloxacin: SPT 5 mg/ml and IDT 0.05 mg/ml; moxifloxacin: SPT 4 mg/ml. The patients underwent oral provocation tests when needed.

The allergy work-up was fulfilled after informed consent.

Results: The 24patients (14 female/10 male), with a median age of 53.8(24-80), referred an adverse reaction with the following quinolones: ciprofloxacin (12patients), levofloxacin (5) and moxifloxacin (7). The symptoms were pruritus along with urticaria (5), angioedema (1), urticaria/angioedema (3), erythema/angioedema (1), exanthema (4), erythema (5), dysphagia (1) and 4 had anaphylaxis. The time between the reaction and the skin tests varies from 1 week to36 months.

We diagnosed four cases of anaphylaxis by anamnesis. Of them, 2 were due to moxifloxacin (only one with positive SPT to moxifloxacin), 1 due to ciprofloxacin (with negative skin tests to ciprofloxacin); these 3patients had negative skin and provocation tests for levofloxacin. The 4th anaphylaxis, with levofloxacin, had positive IDT to the culprit drug and tolerated ciprofloxacin.

We obtained positive skin tests with ciprofloxacin IDT (7); levofloxacin SPT (3) and IDT(1); moxifloxacin SPT (5). 7 patients had a positive provocation test: ciprofloxacin (4), levofloxacin (1), moxifloxacin (1).

Three patients with positive tests for levofloxacin tolerated ciprofloxacin and another 3patients with positive tests with ciprofloxacin tolerated levofloxacin. Besides, the 2patients with an anaphylaxis with moxifloxacin tolerated levofloxacin.

Conclusion: We diagnosed most of the patients through skin tests (22 out of 24).

In our patients, we observed tolerance to levofloxacin in patients sensitized to moxifloxacin or to ciprofloxacin, and the opposite, there is a good tolerance to ciprofloxacin in patients sensitized to levofloxacin.

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Study of reactions due to quinolones

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Background: The increasing use of quinolone antibiotics is associated with an increase in hypersensitivity reactions. The objective of this study is to analyse the cases of suspected allergy to quinolones referred to an allergy department in a tertiary level centre during 2015.

Method: We performed a descriptive, retrospective study. Cases were identified from the medical record administrator programme by searching the terms 'quinolone, norfloxacin, moxifloxacin, levofloxacin or ciprofloxacin'. We collected demographic and medical data, information of the adverse events, results of the allergy evaluation and the recommendations for the future use of quinolones.

Results: We analysed 46 cases (18 male/28 female) with an average age of 56 y.o. (6-88). The most frequent clinical manifestations were cutaneous (65%), 12% digestive, 8% respiratory, 8% neurological, 5% cardiovascular and 2% renal. The most common symptom were pruritus (30%) and erythema (24%). The reaction was immediate in 35% and delayed in 48%. The type of reaction was not described in 17% of cases. The involved quinolones were ciprofloxacin (40%), levofloxacin (31%), norfloxacin (11%) and moxifloxacin (8%). The most frequent route of administration was oral (70%), followed by intravenous (24%), topical (2%) and otic (2%). In two cases quinolones were directly banned after the adverse event due to the patient clinical condition or it was not considered necessary due to the lack of evidence on the reliability of the tests. Skin tests were performed in 22% of cases, with positive results for ciprofloxacin (82%), moxifloxacin 83%, levofloxacin 67% and none for norfloxacin. In a case of a generalized delayed reaction, patch tests were negative. In one case a fixed drug erythema due to norfloxacin was diagnosed without further check. In 10 cases referring severe immediate reactions, basophil activation test (BAT) was performed being positive in 4 times for ciprofloxacin and 3 for levofloxacin. In these cases quinolones were banned. All patients tolerated alternative quinolones to the one eliciting the adverse event, even when skin tests were positive.

Conclusion: Ciprofloxacin and levofloxacin were the most frequently involved quinolones. Skin symptoms were the most common ones. The allergy evaluation included skin tests, BAT and controlled exposure to identify the responsible quinolones and search for safe alternatives.

The sensitivity and specificity of these tests are still undetermined.

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Anti-neoplastic chemotherapy agents-related adverse drug reactions reported to the Korea Adverse Event Reporting System (KAERS)

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Background: With the increased use of antineoplastic chemotherapy agents, adverse drug reactions from these agents also increased. Since the introduction of regional pharmacovigilance centers in Korea, the number of adverse drug reactions reported is also rapidly increased. To find the adverse reaction profiles of these chemotherapy agents, we analyzed data from Korea Adverse Event Reporting System of Korea Institute of Drug Safety (KIDS-KAERS database).

Method: Data were extracted from the KIDS-KAERS database from January 2012 to December 2013 then analyzed data related to chemotherapy-related adverse events.

Results: In the total of 322 512 cases, 45 715 cases of antineoplastic chemotherapy agent-related adverse drug reactions reports were identified after excluding cases

for concomitant drugs and cases assessed as 'unlikely', 'unclassified' and 'unassessable'. Of the patients with adverse drug reactions, about 51% were males and the median age was 56 years. In the total of them, 6378 cases (14%) from 2,907 patients (11.7%) were reported as 'serious' ADR. The most common antineoplastic agent class was platinum compounds (19.8%), followed by pyrimidine analogs (18.8%), tyrosine & mTOR kinase inhibitors (11.9%) and monoclonal antibodies (8.8%). The most common clinical manifestation of organ involvement was gastrointestinal disorder (27.4%), followed by hematologic disorder (25.1%), skin involvement (17.0%) and nervous system (7.8%).

Conclusion: Most common causative class of antineoplastic agents was those of platinum compounds and gastrointestinal disorder was the most common clinical manifestation of adverse drug reactions. Further evaluations to define the unlabeled ADR in these cases were needed.

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Hypersensitivity reactions to iodinated contrast media in a Mediterranean population

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Background: The incidence of hypersensitivity reactions to iodinated contrast media (ICM) has increased along with their augmented use, despite the standard preference of low osmolar ICM. Several investigators have reported positive skin tests in patients with previous adverse reactions, supporting an underlying allergic mechanism. However, the prognostic value of skin tests for the selection of safe alternative ICM in patients with previous adverse reactions remains poorly characterized.

Method: Patients referred to our Unit for ICM hypersensitivity reactions within the last 4 years underwent the following allergological work-up:

- A thorough medical history.
- Skin prick tests (SPTs) to undiluted and intradermal tests (IDs) with 100-fold and 10-fold diluted solutions of the culprit ICM and of different class ICM.
- Patch tests (PTs) with undiluted ICM in cases of non-immediate reactions.
- Subsequent re-exposure to ICM was evaluated by phone interview.

Results: Ninety one patients (mean age 54 years, SD $\pm$ 15) were included in the study. 39/91 (42.9%) experienced an immediate reaction (IR), whereas 50/91 (54.9%) a non-immediate reaction (NIR). An atopic background was documented in 11%. Skin tests were positive in 3/19 with a previous IR and in 5/23 with a previous NIR. Time interval between the reaction and skin tests was 33 months (SD $\pm$ 62 months).

Thirteen patients (6/13 with an IR and 7/13 with NIR) underwent a subsequent contrast enhanced radiological procedure with an alternative ICM, according to clinical history and skin tests results. All patients were pre-medicated and tolerated the alternative ICM.

Conclusion: The combined use of clinical history, skin test results and premedication offers a safe and practical approach for protecting patients from a subsequent hypersensitivity reaction to ICM, in both immediate and non-immediate reactions. As in most cases of drug hypersensitivity patients were reluctant on re-exposure to ICMs even after allergological evaluation.

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The evaluation of patients developed severe cutaneous drug reactions

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Background: The severe cutaneous drug reactions are rare but life-threatening reactions. Acute generalized exanthematous pustulosis (AGEP), Drug reaction with eosinophilia and systemic symptoms (DRESS), Steven-Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN) are reactions in this group. The purpose of this study is the evaluation of patients in our clinic diagnosed with severe cutaneous drug reactions.

Method: The diagnosis of patients who were diagnosed with severe drug reaction, the time between suspected drug / drugs intake and development of clinical findings, clinic and laboratory findings, treatments they have received and their recovery time were recorded.

Results: 19 patients whose ages are 10.29 $\pm$ 4.18 [(median IQR: 10.3 (7.2-12.2)] were in the scope of this study. 56.6% of these patients were male. 52.6% (*n*:10) of these patients were diagnosed with SJS, 21% (*n*:4) were diagnosed with DRESS, 16% (*n*:3) were diagnosed with AGEP, 5.2% (*n*:1) were diagnosed with TEN and 5.2% (*n*:1) were diagnosed with SJS/TEN overlap syndrome. The suspected drugs were antibiotics in 12 (63.1%) patients, antiepileptic drugs in 5 (26.3%) patients, salazopyrin in 1 (5.3%) patient and non-steroid antiinflammatory drug (metamizole) in 1 (5.3%) patient. 57.9% (*n*:11) of patients were provided intravenous immunoglobulin (IVIG), 42.1% (*n*:8) were provided systemic steroids, 16% (*n*:3) were provided IVIG+systemic steroids treatments. A patient who has SJS/TEN overlap syndrome, lagophthalmus remained sequelae. No mortality was observed.

Conclusion: The early diagnosis and appropriate treatment of severe cutaneous medication reactions will reduce the incidence of mortality and morbidity.

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Delayed hypersensitivity skin reactions: a case/non-case study from a Tunisian pharmacovigilance database

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Background: Drug hypersensitivity reactions represent a heterogeneous clinical entity with diverse pathogenesis and result in a considerable burden of morbidity and mortality. Diagnostic procedures rely on clinical history, skin testing and in some cases, provocation tests. Drug imputability is still difficult to establish due to the weakness of sensitivity of some skin tests and the impossibility to perform provocation test in case of severe reactions. The aim of our study is to evaluate delayed-type cutaneous allergic reactions associated with drug use.

Methods: The data were obtained from a Tunisian pharmacovigilance database of adverse drug reactions (ADRs). Analyzed reports were retrieved from the pharmacovigilance unit of Monastir (Tunisia) database collected from 2004 to 2015. The association between drugs and skin reactions was assessed using the case/non-case method, calculating the adverse reaction reporting odds ratio (ROR) and their 95% confidence intervals as a measure of disproportionality. The 'cases' were defined as reports of type III and IV skin allergic reactions (according to gelle and Coombs classifications). The 'non-cases' were all other reports.

Results: Overall 1800 reports of adverse reactions related to drug use were analyzed; of which 1523 (84%) were judged as type III and IV skin allergic reactions (cases) and the remaining were considered non-cases. Drug classes associated with a significant increase of ROR were anticonvulsive agents (ROR = 2.2, 95% CI [1.4–3.3], $P < 10^{-3}$) and antibacterial drugs

(ROR = 1.6, 95% CI [1.3–2], $P < 10^{-3}$). Among antibacterial agents, betalactams were associated with a significant ROR (1.5; 95% CI [1.2–1.8], $P < 10^{-3}$). Regarding betalactams, only oxacilline and the third generation cephalosporins were associated with a significant risk (ROR = 1.9; 95% CI [1.1–3.2], $P = 0.01$) and (ROR = 1.81; 95% CI [1.3–2.4], $P < 10^{-3}$), respectively, while only carbamazepine (ROR = 3; 95% CI [1.6-5.7], $P < 10^{-3}$) and phenobarbital (ROR = 2.3; 95% CI [1.1–5.2], $P = 0.03$) have shown a significant ROR values among anticonvulsive agents.

Conclusion: Results highlight the frequency of association of delayed hypersensitivity skin reactions with betalactams, carbamazepine and phenobarbital. Given the widespread use of these drugs, awareness should be raised among patients and prescribers about these risks.

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Role of skin tests in the diagnosis of hypersensitivity reactions to taxanes. Results of a multicenter study

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Background: Taxanes (paclitaxel and docetaxel) are chemotherapeutic drugs largely utilized for the treatment of a number of neoplasms and represent an important cause of hypersensitivity reactions (HSRs). HSRs usually develop during the first or second exposure to Taxanes, but the exact pathomechanims are not yet clarified. In the recent years, despite premedication with steroids, an increased incidence of HSRs to taxanes was described. Skin tests were very recently introduced in the allergological work-up of these patients. The main aim of our multicenter study was the evaluation of the role of skin tests (ST) in the diagnosis of HSRs to taxanes.

Method: ST with paclitaxel and docetaxel were performed in patients that developed HSRs to one of these drugs. The concentration of skin tests were the follows. For paclitaxel 6 mg/ml on prick test and, if negative, 0.06 and 0.6 mg/ml on intradermal tests, for docetaxel 10 mg/ml on prick test and, if negative, 1 mg/ml on intradermal test. ST were also performed in patients treated with taxanes without HSRs as control. Patients with positive skin tests and/or severe reactions underwent a 12-step desensitization procedure; in the other cases a challenge was proposed.

Results: 56 patients (males 6 females 50, median age 54 years) with HSRs, 55 immediate, 1 delayed and 30 controls were enrolled in the study. HSRs occurred at the first exposure in 33 patients at the second in 17, 1 at the third and at the fourth in 5 and were severe in 18 cases. The culprit drug was paclitaxel in 42 cases, docetaxel in 14. Prick tests resulted negative in all patients, whereas intradermal tests were positive for paclitaxel in 8 subjects and for docetaxel in 6. One patient with immediate HSR to paclitaxel had an intradermal test positive to both taxanes. In the aggregate, tests were positive in 13 patients (23%). About the controls, skin tests were negative in all the cases. 35 patients with positive tests and/or severe reactions underwent a 12-steps desensitization procedure and concluded the planned schedule. 14 patients with non-severe reactions or negative skin tests performed a challenge procedure without problem, whereas the remaining 7 subjects underwent an alternative chemotherapy.

Conclusion: These, non definitive, results show that intradermal tests could be useful in the diagnosis of HSRs to taxanes and in the selection of patients who need a desensitization procedure to safely complete the chemotherapy with taxanes.

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Pristinamycin-induced acute generalized exanthematous pustulosis (AGEP): a case series

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Background: Acute generalized exanthematous pustulosis (AGEP) is a rare but severe drug induced skin eruption, characterized by acute occurrence of an erythematous rash, covered by sterile nonfollicular pustules accompanied by fever and leukocytosis. Pristinamycin is considered among the most frequently implicated drugs.

Aim: To study the clinical, biological and chronological characteristics of pristinamycin-induced AGEP.

Method: We carried a descriptive study including all AGEP cases notified to the pharmacovigilance unit of Monastir (Tunisia) between 2004 and 2015.

Results: Ten patients (8 male and 2 female) with a mean age of 57 years (35–80 years) were included in the study. Clinical features showed in all cases an acute eruption with erythema, rapidly covered by nonfollicular pustules. The rash onset was mainly noted on the face or in the folds moving to the trunk and/or to the limbs. Fever (> 38°C) was objectified among six patients. No mucosal involvement was noted in our cohort. Laboratory findings showed marked neutrophilia among seven patients (8500–25 500/mm^3). Polynuclear neutrophils count wasn't precized in one case. Histologic findings showed a spongiform subcorneal and/or intraepidermal pustules and a perivascular infiltrate in two cases. Clinical symptoms onset varied between 1 and 19 days. After culprit-drug withdrawal, outcomes were favorable within 15 days for all patients. Skin tests were positive with pristinamycin in 4/7 cases. An accidental rechallenge was positive in one case. Thus, the AGEP Euro-SCAR score ranged between 6 to 11 corresponding to a final score 'probable' or 'definite'. The Naranjo score was 'possible' or 'probable'.

Conclusion: Physicians should be aware of such clinical entity to avoid confusion with other diagnosis and inappropriate treatment. Indeed, symptoms spontaneous resolution is possible once the culprit drug is withdrawn. In addition to pharmacovigilance algorithms, skin tests are a useful diagnosis tool allowing us to refine the imputability of pristinamycin in inducing AGEP.

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Value of re-exposure in patients with previous hypersensitivity reactions to intravenous iron

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Background: The occurrence of hypersensitivity reactions (HSRs) to intravenous iron is well known. Formerly used high molecular weight iron dextran products elicited immune complex-mediated anaphylaxis due to preexisting anti-dextran IgG antibodies. With newer products, these reactions have become less frequent. However, many clinicians still consider intravenous iron dangerous. Our objective was to review patients evaluated at our center because of HSRs to intravenous iron, to determine the safety of controlled re-exposure. We also assessed the usefulness of diagnostic tests performed to better select candidates for re-administration.

Method: We retrospectively reviewed the charts of 31 patients referred to our institution because of HSRs to intravenous iron. Two products, ferric carboxymaltose (FCM) and iron sucrose (IS) were

implicated. Allergological workup included a detailed history, skin prick tests with respiratory allergens, as well as skin tests with the iron products and basophil activation tests in some patients. Controlled re-exposure with the product in question or an alternative one was done if indicated.

Results: 31 Patients (30 females and 1 male) aged 17 to 77 years (mean age 36.7 years) were referred to our hospital because of a HSR to intravenous iron between 2007 and 2015. The causing agent was FCM in 19 and IS in 11 patients (1 unknown). 9 patients had a previous HSR to intravenous iron, 14 had other risk factors. Severity grades were: local (n = 1), grade I (n = 15), grade II (n = 7), grade III (n = 3), grade IV (n = 3), and death (n = 1). Skin prick tests with the causative agent were done in 11 patients. 7 patients were tested with a different product. Intradermal tests were done in 1 patient. All skin tests were negative. The basophil activation test was done in 10 patients, all were negative. 17 re-exposures to intravenous iron were carried out. 13 patients tolerated the procedure (including 2 with a previous grade IV HSR). One patient developed urticaria after a 'test dose' from FCM and subsequently tolerated IS. A second patient developed urticaria and dyspnea during an FCM infusion.

Conclusion: The mechanism of HSRs from intravenous iron products is unclear. Skin tests and basophil activation tests in our patients provided little additional information and were not useful in predicting subsequent reactions. Controlled re-exposure in an intensive care setting can be safely performed in most patients.

Poster Discussion Session PDS 31

Management of food allergy

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The safety profile of oral immunotherapy with cow's milk and hen's egg: a 10 year experience

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Background: Oral immunotherapy (OIT) can modulate the immune response and induce a clinical desensitization in IgE-mediated food allergy(FA). OIT safety remains to be better quantified and risk factors identified before it can be formally added to recommendations for the FA management.
Method: We reviewed the clinical records of children (4–18 years) who underwent OIT in CTs in our Allergic Pediatric Unit for cow's milk (CM) and hen's egg (HE) FA confirmed by DBPCFC since 2006. The specific AEs occurred during OIT up-dosing regimens were empirically classified into mild, moderate and severe.
Results: OIT was performed in 68children: in 40with CM & in 28with HE (17with dehydrated egg white, DEW; 11with whole egg, WE). In the CM-OIT group, 4 (10%) pts (3 with history of asthma) had severe systemic AEs (asthma, rhinitis, generalized urticaria&hypotension) and received rescue therapy. They discontinued OIT. The doses of undiluted CM causing severe AEs were: 2 ml in 2 pts, 4 ml in 1 pt and 8 ml in 1pt, respectively. Those pts had a median sIgE level of 98 kU/l for α-lactalbumin, 115 kU/l for β-lactoglobulin, 138 kU/l for casein.

35 pts completed CM-OIT successfully. 1, during the maintenance phase, interrupted OIT due the onset of eosinophilic esophagitis. 20(30%) pts had mild to moderate AEs during the up-dosing phase. 7pts with moderate AEs needed symptomatic drugs. 20% of pts who continued the maintenance regimen had mild to moderate AEs during exercise, infections or menses but none discontinued the CM consumption.

Concerning DEW-OIT, 1 of 17 pts had severe AEs (wheezing, generalized urticaria, rhinitis) and stopped OIT (dose 3 mg). 2 pts had mild AEs and continued OIT.During the maintenance HE-containing diet, after DEW desensitization, 2 pts had AEs during exercise or infection.

No pt desensitized with WE ($n = 11$) stopped OIT for severe AEs. 3 pts had self-limited mild AEs.During the maintenance phase a girl had local urticaria during menses and continued the egg-containing diet.
Conclusion: Overall, severe AEs were not rare (9%, 6/68 pts) and led to the OIT discontinuation since the induction phase. No fatal AEs occurred. No child had AEs among placebo groups of our CTs ($n = 32$). Mild to moderate AEs could be easily managed by symptomatic drugs.

We attempted to grade AEs caused by OIT, with the aim of achieving an appropriate and reproducible grading system.

OIT produces beneficial effects but it should be practiced only in clinical setting by well-trained physicians and nurses.

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Outcome of open peanut challenges and guided reintroduction after negative double blind placebo controlled challenges

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Background: Failure of reintroduction after a negative double blind placebo controlled food challenge (DBPCFC) is not rare and can indicate false-negative food challenge outcome. In this prospective study we investigated the outcome of subsequent open challenges and course and success rate of guided reintroduction of peanut after negative or inconclusive DBPCFC.
Method: Children with suspected peanut allergy ($n = 83$) underwent a DBPCFC. Those with negative ($n = 29$) or inconclusive results ($n = 18$) subsequently underwent open peanut challenge. Children who were still considered negative ($n = 37$) introduced peanut at home using a standardized protocol and diary. During this introduction period they were guided by telephone calls of the physician.
Results: Peanut allergy was diagnosed after open challenge in another 10 patients: 2/29 (7%) with a negative and 8/18 (44%) with an inconclusive DBPCFC. Regular ingestion of peanut at home failed in 10/37 (27%) children. Reasons for not introducing were: aversion ($n = 4$), oral symptoms ($n = 3$), fear of the child ($n = 1$), an exacerbation of eczema that was considered peanut related by parents ($n = 1$) and uncertainty of parents about the challenge outcome ($n = 1$). Three children 3/37 (6%) were diagnosed with a mild peanut allergy after reintroduction at home.
Conclusion: Open challenges and subsequent reintroduction are indispensable to exclude or diagnose peanut allergy accurately in children with negative or inconclusive DBPCFC outcomes. Reintroduction after negative or inconclusive peanut challenges should be monitored to detect (peanut related) problems early.

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Yogurt challenge test in cow's milk allergy with children

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Background: Prevalence of food allergy is increasing worldwide. Cow's milk allergy is the most important food allergy in infants. In our country, yogurt is consumed more than milk on daily basis. Structural changes occur in the protein content of yogurt after being fermented from milk. The aim of this study was to evaluate whether the children diagnosed with cow's milk allergy could tolerate yogurt.
Method: Food challenge with yogurt was applied to 34 children, who were previously diagnosed with cow's milk allergy in our Pediatric Allergy clinic. Twenty-four of the children were male and 10 were females. The mean age of the children was 23.53 ± 4.2 months. The mean age of diagnosis was 10.44 ± 7.9 months.
Results: Medical history revealed that 10 (29.4%) patients had wheezing. In 16

patients (47.2%) atopic dermatitis and in 4 patients (11.8%) gastrointestinal system symptoms were prominent. After food challenge with yogurt to 34 patients with cow's milk allergy, 17 patients (50%) had allergic reaction, while no reaction developed in the other 17 patients (50%). There was statistically significant difference in cow's milk specific IgE levels between the patient with or without reaction to yogurt ($P = 0.016$). When eosinophil count was taken into consideration, there was no difference between the two groups ($P = 0.57$). There was no statistically significant difference in the age of the patients diagnosed with cow′s milk allergy, who can tolerate and can not tolerate yogurt ($P = 0.6$). There was no difference in the occurrence of endurances after skin prick test with commercial milk, natural milk and natural yogurt between the two groups.

Conclusion: It is important to apply yogurt food challenge to children, who were diagnosed with cow's milk allergy, as yogurt is a widely consumed food in our country. By applying this test, some of the children with cow's milk allergy can continue consuming yogurt, which is a valuable nutrient for children.

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Long-term follow-up of baked milk containing diet in patients with IgE-mediated milk allergy

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Background: Previous studies have reported that oral immunotherapy (OIT) with baked milk is a good alternative for IgE-mediated milk allergy.

Objective: The aim of this study was to evaluate the safety and efficacy of baked milk OIT and to observe side effects in long term follow up among our patients.

Patients and methods: 84 children with milk allergy were recruited. Participants underwent an oral challenge with baked milk and continued to consume similar baked milk products for 6 month. In the second phase the participants underwent an oral challenge with a baked cheese-pizza. Six months later, a challenge with unheated milk was offered to patients who tolerated baked cheese challenge.

Results: Between 2012 to 2015, a total of 84 children were recruited. The average age was 5.1 years. Patients were followed for a median of 20 months (range 6–41 months). 68/84 (81%) passed the initial baked milk challenge. 50% of those who reacted to baked milk (16/84) had a history of asthma. Among 68 children initially passed one of the milk challenges, 25% now tolerate unheated milk, 49% tolerate some form of baked milk/cheese and 26% avoid all forms of milk. However, on a long term follow up, 29/68 (42%) stopped consumption of baked milk products in their diet at home, half of them due to mild allergic reactions and the rest with no apparent reason.

Conclusions: OIT with baked milk was well tolerated in most of our patients. However, on a long term follow up, there was a high rate of drop out due to mild allergic reaction and avoidance from unapparent reasons.

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Could a slow-progression schedule with baked milk be a safer way for milk oral immunotherapy?

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Background: Avoidance is currently the only accepted management for children with cow's milk allergy (CMA). Oral immunotherapy (OIT) has emerged as a promising alternative but has a potential risk of anaphylactic adverse reactions. Baked milk seems to be safer than raw milk in most cases. The aim of this study is to compare the safety of a raw-milk cluster vs a baked-milk slow-progression schedule

Methods: Children aged over 3 years with a persistent severe CMA were randomly assigned to one or the other arm. Allergic reactions (AR) and tolerated dose were collected as primary outcomes.

Results: 41 patients underwent OIT, with baseline tolerated dose 150 ± 200 mg [2–680] of milk proteins (m.p.). 56.1% of children experienced at least one AR during OIT, mainly mild (60%). After a mean period of 16.4 ± 2.8 months, 36.6% tolerated more than 2720 mg of m.p./ day, 26.8% between 340 and 2720 g of m.p./ day, and 36.6% less than 340 mg of m.p./ day. No difference was found between the arms in respect with the final tolerated dose.

Five children (13.5%) have needed an epinephrine injection at home. No difference was found between the two arms in respect with the number and the severity of AR, especially the need for an epinephrine injection.

Conclusion: Risk of severe AR remains possible even if using a baked-milk slow-progression schedule. Choice between OIT schedules could also be guided by children and caregivers preferences.

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Home baked milk introduction for children less than 3 years old with IgE mediated cow's milk allergy

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Background: Cow's milk in baked form has reduced allergenicity due to heating and the food matrix. As milk allergy resolves it is accepted earliest so is widely used in oral challenges to determine evolving tolerance. Most centres recommend in IgE-mediated cow's milk allergy (CMA) where the skin prick test (SPT) remains positive that supervised oral challenges are performed. These inpatient challenges potentially prolong food exclusion where facilities are limited. In Leicester, our recent practice has been to recommend that all children where the SPT <8 mm (except with previous severe symptoms or multiple food allergies) be assessed from 12mo of age for home introduction of baked milk using the milk ladder with regular dietetic support. We audited our practice to assess its safety and efficacy.

Method: Children with IgE-mediated CMA at clinic review were advised to introduce baked milk at home. Parents were provided with a written milk ladder, allergy action plan and antihistamines. Data collected was: age of first clinic appointment, presenting symptoms at diagnosis, SPT size, age of baked milk reintroduction and any symptoms during reintroduction. Tolerance was scored from 0 (no baked milk) to 6 (fresh milk tolerance).

Results: Of 53 children studied, age at first clinic appointment was <7 months in 26, 8–14 months in 20 and 14–60 months in 7 children. Symptoms at diagnosis were mild ($n = 31$) or moderate ($n = 22$) and SPT size <4 mm ($n = 27$), 5–7 mm ($n = 24$) and 8 mm+ ($n = 2$). Age at baked milk reintroduction was <12 months in 12, 12–18 months in 28, 18–24 months in 9 and >24 months in 4 children. 45 children successfully introduced some baked milk into their diet by 18mo of age, 17 of whom had

achieved level 4 (all baked milk and some whole milk items). At subsequent review 32 had achieved level 4 tolerance. Mild symptoms were reported on 27 occasions, all but 2 when too much non-baked milk or cheese flavouring was ingested. Two moderate reactions occurred (1 to cheese flavouring, 1 with misunderstanding the ladder). All children with no tolerance had delayed baked milk until after 33 months.

Conclusion: Children with IgE mediated CMA should be assessed early for milk reintroduction even when SPT is positive. We showed guided home reintroduction in all but high-risk patients is safe with good outcome for resolution of milk allergy, but may not be as favourable if started at later age. Milk ladders should promote caution/delay with cheese flavouring, and patients should be equipped to treat allergic reactions.

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Oral immunotherapy and omalizumab for food allergy

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Background: The treatment of food allergy is based on food immunotherapy. The implementation of these treatments may be difficult if the food threshold's patients are very low.

Omalizumab (Novartis, Switzerland), a humanized anti-IgE antibody has been proposed as adjunctive therapy in severe food allergies. The anti-IgE pre-treatment can increase the reactivity thresholds and enable fast food desensitization.

Method: We report 8 observations of immunotherapy in children (age 11.6 ± 3.3 years) highly allergic (5 to milk, 2 to peanut, 1 to fish), for which a sublingual or oral immunotherapy were initially unsuccessful.

The clinical reactions presented by these patients are asthma for 7 patients and mild anaphylaxis reaction for 1 patient.

Results: The pre-treatment with omalizumab for 3 to 5 months allowed all patients to increase them threshold's reactivity: 100 fold for peanuts, 875 for fish and 4.5 for cow's milk. It allowed an immunotherapy leading to a current consumption of the culprit food for 5 patients and consumption of more than 6 ml of milk for 3 children.

The characteristics of patients are summarized in table 1.

The size of skin prick tests decreases in 3–5 month after the beginning of treatment.

The acquisition, even partial, of oral tolerance puts these patients away to the risk of anaphylaxis by accidental ingestion.

Conclusion: This study shows the possibility of inducing oral tolerance with omalizumab for highly allergic patients with a very low threshold reactivity and at risk of severe anaphylactic reactions. The questions to the terms and duration of treatment are not resolved.

Allergens	Initial cumulative dose reactogenic (ml)	Dosage of omalizumab	Tolerated dose	duration of treatment (month)	Acquired tolerance
Peanut	65	225 mg/15 days	10 g to 7 month	17	4 peanuts, 2 or 3 by week
Peanut	44	375 mg/15 days	10 g to 5 month	19	8 peanuts, 2 or 3 by week
Fish	8	300 mg/15 days	7 g to 3 month	6	Daily consumption
Cow's milk	5	300 mg/month	10 ml to 2 month	21	Daily consumption
Cow's milk	<0.05	225 mg/15 days	6,8 ml to 4 month	29	20 ml/d, 2 yogurt, grated cheese
Cow's milk	3	150 mg/15 days	23 ml to 16 month	3	200 ml/d
Cow's milk	4	225 mg/month	3 ml to 3 month	>24	In progress, 13 ml/d
Cow's milk	<0.05	300 mg/month	0.3 ml to 3 month	>30	In progress, 6 ml/d

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Efficacy of LTP immunotherapy in patients with food anaphylaxis

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Background: Lipid transfer proteins (LTP) are panallergens responsible for IgE cross-reactions between unrelated vegetal food allergen sources. They survive food processing and can frequently induce severe food allergic reactions, especially in Mediterranean countries. Sublingual immunotherapy (IT) with natural LTP obtained from peach is a recent option to treat LTP allergic patients.

Method: Patients with suspected food allergy due to LTP were studied. Diagnostic procedure included skin prick test (LTP, pollens and vegetal foods) and resolved component diagnosis to detect specific IgE to LTP (rPru p 3, rAra h 9 and r Cor a 8). Those patients fulfilling the following criteria were treated with peach sublingual immunotherapy (ALK Abelló, Spain): 1) Repeated episodes of urticaria/angioedema or anaphylaxis due to LTP (vegetal foods), 2) sIgE to rPru p 3 with significantly higher value than the other LTP.

Patients received peach sublingual IT during a whole year. Then, an oral open challenge test with peach unpeeled (up to 135 g) was performed in all patients to asses IT efficacy, and with other implied foods: apple unpeeled (up to135 g), hazelnut and peanut (up to 15.5 nuts) in some who referred symptoms with their intake.

Results: Sixteen patients started LTP IT. Eleven of them completed 1 year of treatment: 5 men (45.4%) and 6 women (54.5%) with a mean age of 28.3 years. There were 4 withdrawals due to poor compliance and one IT suspended by a systemic reaction. The challenge test was performed with unpeeled peach in the 11 patients: 10 tolerated the 135 g and one tolerated 65 g. Challenge tests with peanut were performed in 4 patients, being negative in 3; with hazelnut in 1 patient with positive result and with apple in 2 patients with a good tolerance. Five out of the 11 patients are currently asymptomatic, with no dietetic restrictions.

Conclusion: Peach immunotherapy is a safe treatment with excellent results in LTP allergy patients who develop symptoms with different vegetal foods. It avoids restricted diets and dismish the risk of severe allergic reactions.

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The role of cofactors in allergic reactions to food

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Background: Cofactors, like exercise, alcohol consumption and use of several types of medication are associated with more severe allergic symptoms to food. However there is limited evidence on how often cofactors play a role in allergic reactions to food. The aim of this study was to get more insight in the frequency of exposure to cofactors and their influence on allergic reactions in adult food allergic patients.
Method: A baseline questionnaire was completed by every patient ≥16 years of age visiting the outpatients department Allergology for the first time. Patients with food allergy documented by typical allergic symptoms to food and a positive skinprick-test, ImmunoCAP or food challenge were included. Outcome measures were the frequency of medication use from medication groups that are known as cofactors (antacids/acid neutralizing medication, NSAIDs, beta blockers, ACE inhibitors and angiotensin-receptor) and the frequency of patients reporting an association between physical exercise, alcohol consumption or use of analgesics with more allergic symptoms to food.
Results: 496 patients were included between 2003–2011. Most patients had multiple food allergies (mean: 2.9 different foods). The most common food allergies were fruit (68%) hazelnut (43%) and peanut (38%). Prevalence of other atopic diseases were asthma (62%), allergic rhino conjunctivitis (74%) and atopic dermatitis (67%). The frequency of medication use from medication groups that are known as cofactors was: antacids/acid neutralizing medication (5%), NSAIDs (2%), beta blockers (0.6%), ACE inhibitors (0.6%) and angiotensin-receptor inhibitors (0.2%). Of all patients, 13% reported more symptoms to food after involvement of one of the cofactors; exercise was reported by 9%, alcohol consumption by 5% and use of analgesics by 0.6%. Of all patients 65% did not know if these cofactors caused more symptoms; 22% reported that these cofactors had no effect. Patients with mild or moderate food allergy reported significantly less frequent the involvement of these cofactors (physical exercise, alcohol consumption and use of analgesics) than patients with severe food allergy.
Conclusion: Only a small percentage of patients used medication that might aggravate allergic reactions to food. Exercise and alcohol were the most frequently reported cofactors, but occurring in less than 10% of the patients. A lot of patients seem to be unaware of the possible influence of cofactors.

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Threshold dose distribution for cashew nut allergy in children

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Background: The prevalence of cashew nut allergy seems to increase and this allergy especially affects young children. Cashew nut consumption by allergic patients can cause severe reactions, maybe even more severe than peanut consumption does. Threshold dose information is necessary for clinical management but also for informing policy on precautionary labeling. However, there is only one study on threshold dose distribution for cashew nut allergy. The aim of our study was to analyse atopic characteristics and threshold dose distribution of 52 children allergic to cashew nut.
Methods: Open food challenges for cashew nut with positive outcome for allergic reactions were selected from the clinical database of children tested to diagnose food allergy at the University Hospital of Nancy in France between November 2013 and August 2015. Individual thresholds were analyzed using Interval-Censoring Survival Analysis and fitted to parametric model with Weibull distribution. The eliciting dose predicted to provoke reactions in a proportion of the allergic population was estimated.
Results: Individual positive oral food challenges were available for 52 children (58% of boys) aged from 2 to 14 years old. Atopic characteristics were as follow: atopic dermatitis (73%), asthma (44.2%), rhinoconjunctivitis (32.7%), polysensitization (86.5%) and other food allergies (65.4%). All children had a positive skin prick test to cashew nut; 40.4% had prior history of allergic reaction to cashew nut. Threshold dose ranged from 0.784 mg to 2940 mg of cashew nut proteins. The severity of the allergic reaction was grade 3 or 4 according to the Astier's classification for 48.1% of the children. The severity of the reaction was not associated with the threshold dose. The protein doses at which 5%, 10% and 50% of the allergic population is likely to respond were respectively 0.81 mg, 2.48 mg and 46.12 mg.
Conclusion: Almost half of the children have moderate to severe reactions, regardless of the threshold dose. Threshold doses range from 0.784 mg to 2940 mg of cashew nut proteins. The eliciting dose for 5% of the allergic pediatric population is slightly higher than those described by Blom et al for any type of symptoms (0.32 mg) but lower than those described for peanut by Taylor et al (1.82 mg).

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Prepackaged foods are the most frequent cause of unexpected allergic reactions which are usually moderate to severe

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Background: Food allergy is a serious health problem, affecting daily life. Despite precautionary measures, accidental ingestion of allergens resulting in unexpected reactions still occurs. Aim of the study was to evaluate the frequency, severity, causes and consequences of unexpected allergic reactions to food in adults.
Method: A prospective study was carried out, with 1 year follow up, in adults with a doctor diagnosed food allergy. Outcome parameters were the frequency, severity, causal products, labeling, patient's behavior and consequences (medical treatment and sick-leave) of unexpected allergic reactions to food.
Results: A total of 157 patients were included, with a mean of 3.5 (SD 2.1) confirmed food allergies, most frequently for fruit (61%), hazelnut (55%), other nuts (48%), peanut (47%) and vegetables (32%) A total of 153 reactions were reported. Remarkably 54% of the patients did not report any unexpected reaction, whereas 26% of the patients reported 1 reaction and even 20% ≥2 reactions (range 2–11).Reported unexpected allergic reactions were mild reactions (local symptoms) in 22%; moderate (skin and mucosa symptoms and gastro-intestinal symptoms) in 50%, and severe (respiratory and cardiovascular symptoms) in 28%.Of the 153 unexpected reactions, 41% were on prepackaged products, 24% on composite meals outdoors, 20% on fresh products, 9% on products or meals in a foreign country and 7% on composite meals at home. In 37% of reactions on prepackaged

products, composite meals at home or composite meals outdoors with a label, the allergen was not mentioned as ingredient or warning on the labels. Reactions took also place in a restaurant (16%); even though the patient informed the restaurant, cook or waiter before in 68% of cases. In only 67% of the reactions, patients used medication. In not more than eight reactions, medical care was sought; 2 times by a general practitioner and 6 times by a hospital, of which 2 patient were hospitalized. Sick leave of some hours until 1 day was reported in case of 13 unexpected reactions.

Conclusion: Unexpected allergic reactions to food are frequent and generally moderate to severe, occurring in almost half of the food allergic patients. Prepackaged foods are the main causal products followed by composite meals outdoors. There is a strong and undesirable underuse of emergency medication and medical care.

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Weaknesses of treatment guidelines for the management of anaphylaxis and healthcare utilization following an anaphylaxis event

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Background: Intramuscular adrenaline (epinephrine) is classified as first-line treatment of choice for anaphylaxis in international and national guidelines. Patients at risk are recommended to be prescribed two epinephrine auto-injectors (EAIs) which they should carry with them at all times. A prospective, web-based survey was conducted to obtain insight into adherence to treatment recommendations according guidelines, and post-injection behavior in patients at risk for anaphylaxis and their caregivers.

Method: 159 patients aged 18–65 years (mean = 29.7; 32.1% male) and 215 caregivers of individuals under aged 18 years (mean = 23.3; 39.5% male) were recruited in the United States. All participants had been prescribed an EAI for self-administration or for administration as a caregiver. The survey took place between November 15 and November 30, 2015.

Results: At home, 18.2% of patients and 15.4% of caregivers had no EAI and 61.0% of patients and 62.8% of caregivers indicated 1 EAI was available. At their workplace, 83.0% of patients did not have access to at least one EAI and 35.8% of caregivers reported that their child did not have access to at least one EAI at school. Only 8.0% of caregivers identified that two EAIs were available for their children at school. Of those who received emergency care, 5.9% received their first dose of epinephrine in the Emergency Department. 3.0% of patients reported having to call emergency services due to the lack of a secondary EAI with 2.0% being admitted to inpatient care. Following utilization of an EAI, 13.0% of patients and 15.0% of caregivers did not seek follow-up medical attention.

Conclusion: Given the lifesaving ability of EAIs, the effect of treatment according to guidelines is imperative to successful manage anaphylactic events. The survey results revealed suboptimal realization of treatment guidelines and call into question the efficacy of current educational programs and safety information delivered to patients and caregivers regarding the use of EAIs to treat anaphylaxis.

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Incorrect and incomplete demonstration of epinephrine auto-injectors by pharmacists in the Netherlands

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Background: Anaphylaxis is a severe, progressive, allergic reaction that is rapid in onset and can cause death. Successful treatment of anaphylaxis in the community relies on early and correct use of epinephrine auto-injectors (EAI). In the Netherlands, pharmacists supply EAIs to patients, and have a crucial role instructing patients in how and when to use them. However, there are currently no data on the quality of such instruction provided by pharmacists. Therefore, the aims of this study were to investigate the quality of EAI instructions and demonstrations to patients by pharmacists and pharmacy assistants, and to investigate the knowledge, attitudes and beliefs regarding food allergy among pharmacists and pharmacy assistants in the Netherlands.

Methods: Quality of instructions and demonstration accuracy were assessed in simulated patient visits to randomly selected pharmacies. Pharmacists and pharmacy assistants were asked to fill a prescription of an EAI (Epipen® or Jext®) by the simulated patient. The simulated patient assessed whether or not the pharmacists and pharmacy assistants gave correct instructions and adequately demonstrated the use of an EAI.

Pharmacists and pharmacy assistants were asked to complete an online questionnaire. For the statistical analysis descriptive methods were used.

Results: In total, ten pharmacies were included in simulated patient visits. Five of them (50%) demonstrated the EAI. None of them (0%) demonstrated the EAI use correctly.

In total, 25 out of 115 questionnaires were completed (response rate 22%). Eight (32%) respondents stated an incorrect administration site. Two (8%) respondents filled in the correct answers concerning a correct EAI demonstration. Twenty-one (84%) respondents found that the provision of instructions was the responsibility of pharmacists.

Conclusion: Food-allergic patients at high risk for anaphylaxis who receive their EAI at a community pharmacy in the Netherlands are often not instructed on how and when to use an EAI or receive incorrect instructions. Pharmacists and pharmacy assistants show considerable gaps in knowledge about food allergy and its management. These data suggest that opportunities exist to improve the quality of care provided by pharmacies for high risk food-allergic patients.

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Emergency epinephrine use for food allergy reactions in Hortaleza District schools in Madrid

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Background: We have previously described the prevalence of food allergen-free diets in school canteens in Hortaleza District in Madrid. Given the increase in childhood food allergy, national and local policies have been developed to encourage schools staff, families and students to aware epinephrine auto-injectors in case of an anaphylactic emergency.

Objective: To describe the use of epinephrine auto-injectors in Hortaleza District schools during the 2014–2015 school year, specifically for food-induced allergic reactions.

Methods: A food allergy structured survey was performed in 86 schools in Hortaleza District in Madrid prior to the start of the 2014–2015 school year. Data on previously use of epinephrine and type of food allergy were collected, and frequencies were computed in the winter of 2015.

Results: 5.07% food allergen-free diets were reported in the studied year (1,132 of

22,326 total served meals). From these 1,132 students, 255 had an action plan in the case of an allergic reaction (22,5%) with the following age distribution: <3 years: $n = 34$, ≥3 to <6: $n = 100$, ≥6 to <15: $n = 113$ and ≥15: $n = 8$. 188 of the later, had an indication of epinephrine use (73,72%), accounting 0.8% of total food allergen-free diets served this year to allergic students. The most frequent food allergy indication for its use were: egg (60.5%), nuts (50.5%) and milk (34.5%).

Conclusions: This is the first large, urban school District in Spain to develop and implement a food allergy survey which can help 188 students and school staff to avoid potential morbidity and mortality due to food allergy. The impact of this initiative during its first year underscores the need for stocking epinephrine in schools across our District.

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The knowledge and attitudes of patients about epinephrine autoinjector use in Turkey

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Background: Vast majority of anaphylaxis episodes occur outside the hospital and intramuscular epinephrine is the cornerstone of the treatment. Determination of knowledge gaps and concerns of patients about epinephrine autoenjector usage (EAI), will help to provide an decrease in morbidity and mortality. We aimed to evaluate epinephrine autoenjector prescribed patients' and parents' knowledge and attitudes about the use of EAI and also experience of patients who had to use it.

Method: Individuals who had been prescribed an epinephrine auto-injector were evaluated by a phone survey. Demographic data, the reason(s) for EAI prescription, triggering agent, clinical findings, knowledge about symptoms of anaphylaxis and use of EAI and experience of anaphylaxis after prescription were evaluated.

Results: A total of 1200 patients were surveyed. Mean age of the patients was 25.57 ± 19.0 years (1–79 years) and 564 (53.6%) of them were male. A written anaphylaxis action plan was given to 10.9% of patients (Table 1) and only 55.4% of patients were able to describe anaphylaxis symptoms. All steps of EAI use was known by 37.9% and only 62.6% were taking their EAI together with them. Knowledge of EAI use was better in patients or parents with high educational levels ($P = 0.025$) and in who were informed by demo ($P < 0.001$). Among the 129 patients who were experienced recurrent reaction, 60 (46.5%) of them made adrenalin injection.

Conclusion: Knowledge and practice of EAI use among patients was unsatisfactory. Educational level and giving a practical demonstration are the key factors in the ability to effectively use the device. Allergists should instruct and spend time with the patients and parents to explain the importance of carrying the EAI.

Variable	*N* (%)
Prescribed at first attack	445 (42.2)
Prescribed after second or more attack	398 (37.8)
Instructed how to use (yes)	966 (91.7)
Video(visual)Information	30 (2.8)
Instructed by training device	759 (72.1)
Verbal Information	177 (16.8)
Instructed when to use (yes)	1013 (96.2)
Written anaphylaxis action plan (yes)	115 (10.9)
Prescribed by allergist	1020 (96.9)

[Autoinjectors prescription patterns]