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ABSTRACTS

SUNDAY, 18 JUNE 2017
OAS 01
FOOD ALLERGY MECHANISMS AND BIOMARKERS

0001 | Innate immune hyperactivation in paediatric food allergy

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Introduction: The prevalence of paediatric food allergy is increasing globally. Food allergy results from the failure to induce tolerance towards innocuous dietary antigens. A proportion of food allergic children will outgrow their allergy without intervention, indicating an immunological shift towards a tolerogenic immune phenotype in these individuals. The immunological mechanisms responsible for the development of food allergy in infancy and the induction of natural tolerance in childhood are not well understood.

Objectives: Using longitudinally collected samples from a population-based cohort of challenge-confirmed egg allergic infants with either persistent or transient egg allergy, we aimed to define the immune profiles associated with egg allergy and assess the immunological changes that occur with the development of natural tolerance in childhood.

Results: We show that egg allergy is characterised by a hyperactive innate immune phenotype in the first year of life. This is particularly evident in infants with egg allergy that persists into childhood, with significant increases observed in circulating monocytes and dendritic cells that naturally produce more inflammatory cytokines (IL-1β, IL-6, IL-8) and less regulatory (IL-10) cytokines. Follow up analysis revealed that infants with persistent egg allergy continue to show a hyperactive innate phenotype in childhood and that this is not observed in children who develop natural tolerance. Reductions in the number of circulating memory B cells were also associated with persistent egg allergy, and children who develop natural tolerance in childhood appear to recover this deficit over time.

Conclusions: We report the complete innate and adaptive cellular immune profiles of infants and children with carefully defined clinical phenotypes of egg allergy. We used longitudinally collected samples and clinical outcomes from the HealthNuts study to reveal that hyperactivation of the innate immune system in the first year of life is a biomarker of persistent egg allergy in childhood.

0002 | Genetic variation at the Th2 immune gene IL13 is associated with IgE - mediated paediatric food allergy

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Introduction: Food allergies pose a considerable worldwide public health burden with incidence as high as one in ten in 12-month old infants. Few food allergy genetic risk variants have yet been identified. The Th2 immune gene *IL13* is a highly plausible genetic candidate as it is central to the initiation of IgE-class switching in B cells.

Objectives: Here we sought to investigate whether genetic polymorphisms at *IL13* are associated with the development of challenge-proven IgE-mediated food allergy.

Method: We genotyped nine *IL13* 'tag' single nucleotide polymorphisms (tag-SNPs) in 367 challenge-proven food allergic cases, 199 food sensitised-tolerant cases and 156 non-food allergic controls from the HealthNuts study. 12-month old infants were phenotyped using the gold standard oral food challenge. SNPs were tested using Cochran-Mantel-Haenszel test adjusted for ancestry strata. A replication study was conducted in an independent, co-located sample of four paediatric cohorts consisting of 203 food allergic cases and 330 non-food allergic controls. Replication sample phenotypes were defined by clinical history of reactivity, 95% PPV or challenge and *IL13* genotyping was performed.

Results: *IL13* rs1295686 was associated with challenge-proven food allergy in the discovery sample (P=.003; OR=1.75; CI=1.20-2.53). This association was also detected in the replication sample (P=.03, OR=1.37, CI=1.03-1.82) and further supported by a meta-analysis (P=.0006, OR=1.50). Carriage of the rs1295686 variant A allele was also associated with elevated total plasma IgE.

Conclusions: We show for the first time, in two independent cohorts, that *IL13* polymorphism rs1295686 (in complete linkage disequilibrium with functional variant rs20541) is associated with challenge-proven food allergy.

0003 | A proposal for the adverse outcome pathway (AOP) for food sensitization

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Introduction: The introduction of whole new foods in a population may lead to sensitization and food allergy. This constitutes a potential public health problem and a challenge to risk assessors and managers as the existing understanding of the physiopathological processes and the currently available biological tools for prediction of the risk for food allergy development and the severity of the reaction are not sufficient.

There is a substantial body of *in vivo* and *in vitro* data describing molecular and cellular events potentially involved in food sensitization. However, these events have not been organized in a sequence of related events that is plausible to result in sensitization, and useful to challenge current hypotheses.

Objectives: The aim of the COST Action network FA1402, called 'ImpARAS', was to collect and structure the current mechanistic understanding of sensitization induction to food proteins by applying the concept of adverse outcome pathway (AOP).

Results: The proposed AOP for food sensitization is based on information on molecular and cellular mechanisms and pathways evidenced to be involved in sensitization by food and food proteins and uses the AOPs for chemical skin sensitization and respiratory sensitization induction as templates. Available mechanistic data on protein respiratory sensitization were included to fill out gaps in the understanding of how proteins may affect cells, cell-cell interactions and tissue homeostasis. Analysis revealed several key events (KE) and biomarkers that may have potential use in testing and assessment of proteins for their sensitizing potential.

Conclusions: The application of the AOP concept to structure mechanistic *in vivo* and *in vitro* knowledge has made it possible to identify a number of methods, each addressing a specific KE, that provide information about the food allergenic potential of new proteins. When applied in the context of an integrated strategy these methods may reduce, if not replace, current animal testing approaches.

The proposed AOP will be shared at the www.aopwiki.org platform to expand the mechanistic data, improve the confidence in each of the proposed KE and key event relations (KERs), and allow for the identification of new, or refinement of established KE and KERs.

0004 | Metabolomic phenotyping based biomarkers in food allergy linked to respiratory allergy

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Introduction: Allergies are increasing steadily worldwide, only current prevalence in western world is estimated for about 25% of total population. In recent decades there have been both; a progression and an increment in the severity of this kind of diseases including food and respiratory allergies. In the case of food allergy, the model using allergen profilin has shown that allergens may have an effective access to the body through the oral mucosa. This fact has created from profilin a unique model to study the evolution of allergic inflammation. Along with it Spain which is characterized by an extreme climatic variation, is an ideal place to study the correlation between allergen respiratory exposure and development of severe food allergy. To date, there is a huge lack of deep knowledge concerning the molecular mechanism involved in this pathology. To tackle down, metabolomics emerged as a science capable of managing complex multifactorial diseases thought the analysis of all possible metabolites in a biological sample obtaining a global interpretation of biological systems.

Objectives: The aim of this study was to obtain the metabolic pattern of allergic patients and controls, in order to highlight potential biomarkers which might predict the diagnosis and prognosis of the disease while understanding the metabolic changes underneath. Plasma samples from 3 groups; controls, mild and severe allergic patients to profilin, were analysed by high throughput liquid chromatography-mass spectrometry (LC-MS) technique. The study was multicentre comprising 4 different hospitals from Spain.

Results: Preliminary results from the statistical models showed differences between the groups. Significant masses of each comparison were tentatively identified using available online databases. The tentative list of compounds encompassed metabolites from the energy metabolism, amino acids, fatty acids, sphingolipids, phospholipids and bile acids, which were found significantly correlated to the severity of the disease.

Conclusions: These findings indicated that plasma metabolomics screening may be a guide to the prediction of severity in food allergy linked to respiratory allergy, and a potential approach to the understanding of this condition. This was the first study of its kind that exhibited the potential of using metabolomics in this field.

0005 | Nutritional wheat alpha-amylase/trypsin inhibitors but not a control storage protein from corn exacerbate allergen-induced gut and lung inflammation in humanized mice

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Introduction: The non-gluten proteins wheat alpha-amylase/trypsin inhibitors (ATIs), which have been identified as potent nutritional activators of various innate immune cells via TLR4, are implicated as central triggers of wheat-induced asthma and gastrointestinal hypersensitivity to wheat.

Objectives: The aim of this study was to analyse whether ATIs are also involved in gut and lung inflammation induced by other allergens in a recently developed humanized mouse model of allergy.

Results: Therefore, NOD-scid gamma chain knock-out mice first received a gluten- and thus ATI free diet over at least three weeks before starting the experiment. Then, mice were engrafted with human PBMC from highly sensitized grass or birch pollen allergic donors together with the respective allergen or saline as control by intraperitoneal injection, and were fed with different ATI-containing diets or injected with purified ATIs. Three weeks later, mice were challenged with the allergen rectally, and gut inflammation was monitored by a high-resolution video mini-endoscopic system evaluating translucency, granularity, fibrin production, vascularity, and stool consistency. Airway hyperreactivity (AHR) was measured by invasive body plethysmography after an additional intranasal allergen challenge. Allergen-specific human IgE in mouse sera, which was detectable only in PBMC plus allergen-treated mice, was strongly enhanced in mice receiving an ATI-containing diet compared to mice that continued on the gluten-free diet. Consequently, allergen-induced IgE-dependent colitis and AHR were also enhanced in ATI-fed mice. Even modestly enhanced gut inflammation was detectable in ATI-treated mice, which had been engrafted with PBMC alone in the absence of the respective aeroallergen, while this effect was not observed with the control storage protein zein from corn.

Conclusions: These results underline that ATIs are important activators and adjuvants of allergy independent of their intrinsic function as allergens in wheat allergy which might be exploited for nutritional therapeutic strategies to address allergen- and wheat-induced intestinal as well as extraintestinal inflammation.

0006 | Tri-pentamers in galacto-oligosaccharide mixture induce galectin-9 release in CpG dna-primed activated human intestinal epithelial cells

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Introduction: A diet containing short chain galacto- and long chain fructo-oligosaccharides (scGOS/lcFOS, 9:1), and *Bifidobacterium breve* (containing CpG-DNA, TLR-9 ligand) suppresses allergic symptoms in mice and humans in association with enhanced local intestinal and/or systemic galectin-9 levels.[1] In mice as well as a co-culture model of human intestinal epithelial cells (IEC) and activated peripheral blood mononuclear cells (PBMC), IEC were identified as a source of galectin-9[1,2]. Galectin-9 inhibits IgE mediated mast cell degranulation. In addition, galectin-9 was shown to enhance regulatory IL-10 secretion and Th1 polarization in the co-culture model upon IEC exposure to synthetic CpG-DNA, which was further enhanced by scGOS/lcFOS.

Objectives: These findings prompted us to identify the most bioactive components in the scGOS mixture.

Methods: scGOS consists of galactose chains, starting with glucose, with a degree of polymerization (DP) between 2 to 10 (GOS-DP). Different oligomers were separated according to these DP from the scGOS mixture by size exclusion chromatography. To determine the most bioactive oligomer, various GOS-DP were tested in combination with synthetic CpG-DNA through apical exposure of HT-29 cells (IEC) co-cultured with anti-CD3/CD28-activated PBMC, derived from buffy coats. The basolateral medium was collected after 24 hours and IEC were used for qPCR to measure galectin-9 mRNA expression. In the supernatant, galectin-9, IL-10 and Th1 and Th2 associated cytokines were measured.

Results: Only in the presence of CpG DNA all GOS-DP enhanced galectin-9, IFN-γ and IL-10 secretion after 24 hours in the co-culture model. Especially the trimer-pentamer mixture of scGOS (GOS-DP3-5) further enhanced galectin-9 and regulatory IL-10 secretion when compared to exposure with CpG-DNA alone ($P<.01$). The trimer mixture (GOS-DP3) closely resembled the effects of GOS-DP3-5 and further increased IFN-γ concentrations on top of the effect of CpG-DNA ($P<.05$). CpG DNA induced galectin-9 mRNA and protein levels in IEC, which remained unaltered by the presence of the GOS-DP3-

5 mixture. However, IEC released galectin-9 only in the presence of GOS-DP3-5 ($P<.01$).

Conclusions: GOS-DP3-5 facilitate immunomodulatory galectin-9 secretion by CpG-DNA exposed IEC under inflammatory conditions.

References:

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SUNDAY, 18 JUNE 2017
OAS 02
BIOMARKERS IN ASTHMA

0067 | Control of asthma by omalizumab: the role of CD4+ Foxp3+ regulatory T cells

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Introduction: Allergic asthma is an inflammatory disease of the airways, resulting from an inappropriate immune response to inhaled allergens, which involves multiple inflammatory cells and mediators that contribute to airway hyperresponsiveness. Omalizumab is a monoclonal antibody, specifically directed to the C-ε 3 domain of immunoglobulin E (IgE), indicated as an additional therapy in severe allergic asthma. Data on circulating $CD4^+Foxp3^+CD25^+CD127^{lo}$ Treg related to asthma control are scarce, as are biomarkers linked to asthma control in general.

Objectives: To investigate whether the proportion of circulating $CD4^+Foxp3^+CD25^+CD127^{lo}$ Treg could be used as a biomarker for asthma control in patients treated with omalizumab.

Results: We performed a prospective cross-sectional study in a tertiary care hospital. All the children were older than 6 years and had severe allergic asthma remaining uncontrolled for at least 6 months despite high-dose inhaled corticosteroids (ICS). Asthma control was assessed at baseline and after 16 weeks of omalizumab. We monitored in parallel blood eosinophils and the proportion of circulating $CD4^+Foxp3^+CD25^+CD127^{lo}$ T cells. Twenty-one children aged 11.2±3.5 years were recruited consecutively. The percentage of children with well or partly controlled asthma increased after 16 weeks of omalizumab from 0% to 42.9% and 52.4%, respectively. Dose of ICS was on average 1300 μg of equivalent budesonide (IQR 1000-2000) and remained unchanged during the follow-up. The mean proportion of circulating $CD4^+Foxp3^+CD25^+CD127^{lo}$ Treg increased from 4.6±1.6% of $CD4^+$ T cells at baseline to 5.7±1.9% after omalizumab treatment (P=.02), whereas mean blood eosinophilia decreased from 460±316 to 320±200/mm^3 (P=.02). Proportions of $CD4^+Foxp3^+CD25^+CD127^{lo}$ Treg significantly changed according to asthma control after treatment (P<.01). The proportion of $CD4^+Foxp3^+CD25^+CD127^{lo}$ Treg was confirmed to be independently associated with disease control [OR 2.3; 95% CI 1.04-5.2; P=.04].

Conclusions: Thus, circulating Tregs appear more as a marker of omalizumab-induced control. A more extensive analysis is warranted to validate the proportion of $CD4^+Foxp3^+CD25^+CD127^{lo}$ Treg as a biomarker to assess omalizumab efficacy. If confirmed, these results also support the role of $CD4^+Foxp3^+CD25^+CD127^{lo}$ Treg in controlling the inflammation process in asthma.

0068 | Quantification of histamine-secreting microbes from the gut differentiates obese vs non-obese asthma patients

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Introduction: Microbiome-host interactions are believed to significantly influence host immunologic homeostatic mechanisms, whereas microbial dysbiosis has been associated with many inflammatory diseases. The importance of microbiota in asthma progression or in the protection against this disease is being extensively examined. The potential influence of gut bacteria on asthma development is not only related to their relative abundance or their taxonomic affiliation, but also to the metabolites that they produce. Histamine is one such bacterial metabolite with important immunomodulatory effects.

Objectives: The aims of this study were to quantify histamine secreting bacteria within the gut of asthma patients and healthy volunteers and to identify the bacterial species responsible for histamine secretion.

Results: Following PCR screening of faecal samples from 148 patients and healthy volunteers we observed that the bacterial histidine decarboxylase (HDC) gene copy number was significantly higher in asthma patients compared to healthy volunteers. Within the asthma group, non-obese asthma patients had the highest burden of bacteria capable of producing histamine, compared to obese asthma patients. Faecal samples were cultured on multiple growth media, under multiple different growth conditions, in the presence of histidine, to isolate histamine secreting microbes. *Escherichia coli*, *Lactobacillus vaginalis* and *Morganella morganii* (*M. morganii*) were identified as the species capable of high levels of histamine secretion within the gut of asthma patients. Histamine from these strains activated the histamine 1 receptor, as demonstrated using a reporter cell line. Increased levels of *M. morganii* correlated with increased disease severity in non-obese asthma patients, but not obese asthma

patients. Finally, *M. morganii* was administrated to mice following induction of airway inflammation but the study had to be prematurely terminated due to the negative effects of *M. morganii* administration on animal health.

Conclusions: In conclusion, the level of histamine-secreting bacteria increases in the gut of non-obese asthma patients, but not obese asthma patients. More accurate endotyping of asthma patients may be assisted by further analysis of the composition and metabolic activity of an individual's microbiome, while novel therapeutics directly targeting microbiome activities may be considered as complementary to existing approaches.

0069 | Decreased lung function relates to increased type-2 inflammation in asthma subjects from the Swedish ga2len study

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Introduction: Decreased lung function and fixed airway obstruction (fixed-AO) are long-term complications in asthma due to airway remodelling. Chronic airway inflammation in asthma is believed to play a crucial role in these changes. Therefore, we analysed four proposed markers of type-2-inflammation in relation to impaired lung function in subjects with asthma.

Objectives: The study was done in 403 individuals from the Swedish GA2LEN study, aged 17-75 years, with pre- and post-bronchodilation spirometry measurements. Fixed-AO was defined as post-bronchodilation FEV1/FVC<lower limit of normal. A low FEV1 was defined as <80% of predicted. The following markers of type-2-inflammation were measured: serum periostin (S-periostin) (elevated defined as ≥74 ng/ml), urinary eosinophil-derived neurotoxin (U-EDN) (elevated≥65.95 mg/mol creatinine), fraction of exhaled NO (FeNO) (elevated ≥25 ppb) and serum eosinophil cationic protein (S-ECP) (elevated ≥20 μg/ml).

Results: In univariate analyses, an elevated level of U-EDN related to fixed-AO as well as a FEV1<80% predicted both pre- and post-bronchodilation. In multiple logistic regression models, fixed-AO was independently related to elevated S-periostin (OR: 2.9 (95% CI: 1.3-6.1), U-EDN (OR: 2.8 (1.3-5.9)) and S-ECP (OR: 3.6 (1.6-7.9)), but not to elevated FeNO (OR: 0.85 (0.39-1.8)). A FEV1<80% of predicted was related to elevated S-ECP, both pre- (OR: 2.0 (1.1-3.9) and post-bronchodilation (OR: 2.8 (1.4-5.8), but not to the other markers. Adjustments were made for sex, age, atopy, smoking status, study centre, use of inhaled corticosteroids (ICS) and leucotriene receptor antagonists, BMI, age of asthma onset, asthma duration, sampling time, pack-year smoking and COPD.

Conclusions: Fixed-AO related to increased levels of three type-2 markers: U-EDN, S-Periostin and S-ECP while having FEV1<80% predicted related to elevated S-ECP after adjustments. This indicates that increased type-2 inflammation relates to fixed airway obstruction, especially systemic eosinophil activation. Further studies are warranted to examine whether controlling systemic eosinophilic inflammation may prevent fixed airflow obstruction.

0070 | Asthma symptoms, medication use, and lung function before and after an asthma exacerbation

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Introduction: For patients with allergic asthma, exacerbations constitute the greatest medical need and a significant source of anxiety.

Objectives: Here, we present descriptive clinical data on asthma symptoms, medication use, and lung function in the period before and after an asthma exacerbation in order to improve our understanding of how and for how long patients remain affected by an asthma exacerbation.

Results: The randomised DBPC MT-04 trial (EudraCT 2010-018621-19) included 834 adult subjects with house dust mite (HDM) allergic asthma not well-controlled by inhaled corticosteroids (ICS). Subjects were randomised to treatment with the SQ HDM SLIT-tablet (ACARIZAX) in doses 6 or 12 SQ-HDM, or placebo for up to 18 months. During a 6-month efficacy evaluation period where ICS was gradually reduced, subjects recorded lung function (PEF), asthma symptoms, nocturnal awakening, and SABA use in an electronic diary twice daily. The primary endpoint was the time to first moderate or severe asthma exacerbation (as defined in Virchow et al. JAMA 2016).

204 subjects experienced a moderate or severe asthma exacerbation during the trial. Plotting the raw means of subjects' lung function data for the 4 weeks before and 4 weeks after their first asthma exacerbation revealed a characteristic pattern. From a baseline level of approximately 400 l/min, average morning PEF decreased gradually starting 7 days prior to the exacerbation to approximately 360 l/min on the day of exacerbation followed by a gradual return to baseline over 7 days following the exacerbation. Evening PEF showed a very similar pattern. Subjects' asthma symptoms as well as the frequency of waking at night due to asthma increased starting 10 days before an exacerbation followed by a return to baseline 10 days after the exacerbation. This pronounced peak in asthma symptoms was accompanied by an increase in subjects' SABA intake

starting 14 days prior to an exacerbation. Specifically, subjects' SABA use increased from an average 1 puff/day at baseline to 2 puffs/day on the day of exacerbation followed by a return to baseline over 21 days.

Conclusions: The presented data on asthma symptoms, medication use, and lung function reveal characteristic patterns during the time preceding an asthma exacerbation and highlight the fact that subjects remain affected for several weeks after experiencing an asthma exacerbation. Further, these data substantiate the relevance of the asthma exacerbation definitions used in the trial.

0071 | Nocturnal variabilities of tidal airflow and heart rate show mutual association in young children with asthma symptoms

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Introduction: Wearable impedance pneumography (IP) -based technique was recently introduced to describe tidal airflow variability (TAFV) during night sleep at home. These indices were associated with risk of persistent asthma in a cohort of young children (Eur Respir J 2016; 47:1687-96). From the same overnight recordings heart rate variability (HRV) indices were derived and found as well to associate with risk of persistent asthma (under review).

In the present study, recordings were successful in 34 young children (age 3-7 y) with recurrent or persistent lower airway symptoms. Most of the children were without regular medication and were considered to have either high (n=14) or lower (n=13) risk of persistent asthma according to modified asthma predictive index (mAPI); additionally, 7 children were on inhaled corticosteroids due to asthma.

Signals for both TAFV and HRV analysis were collected with custom-made wearable recorders (design of Tampere University of Technology) through four skin electrodes at home during night sleep. The airflow parameters of minimal curve shape correlation (CSR_{min}) and minimal noise limit (NL_{min}) reflect overnight change in expired flow-volume curve shape, and smallest detected respiratory chaoticity, respectively. HRV parameters represent high (HF) and normalised low (LFn) frequency power. HRV organisation index (OI) describes regularity of the HF components of HRV spectrum.

Objectives: The objective of this study was to assess the association between novel IP-derived indices of TAFV and HRV indices measured during sleep.

Results: HRV indices LFn and OI correlated significantly with NL_{min}, but none of the HRV indices correlated with CSR_{min} (Table 1). Exclusion of the ICS group (n=7) did not change significances of the correlation analysis.

Conclusions: The results show that indices of TAFV and HRV are related in young children with asthmatic symptoms. Specifically, reduced NL_{min} indicating lowered chaoticity in tidal breathing flows relates to reduced LFn indicating parasympathetic dominance and increased organization of the HF band of the HRV spectrum, which may suggest lack of adaptability of the parasympathetic nervous system. It is possible that the same neural mechanisms contribute to both heart rate and airflow variability, or changes in HRV spectrum reflect altered respiratory modulation in asthma.

		HRV indices			
		HF	LFn	OI	(NL_{min})
TAFV indices	CSR_{min}	0.02	−0.01	−0.13	(0.48 §)
	NL_{min}	−0.05	0.36*	−0.50 §	

0072 | Phenotypes related with the clinical improvement of patients treated with omalizumab in routine clinical practice. Fenoma study

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Introduction: A better knowledge of asthma phenotypes allows an optimal treatment choice and improves disease control.

Objectives: The main objective was to describe the phenotypes of patients (pts) with severe asthma (SA) who achieved disease control during the first year of treatment with omalizumab (OMA). Secondary objectives included: symptoms and non-severe asthma exacerbations (NSAE) reduction, work absenteeism, unscheduled visits, lung function and medication intake.

Results: Observational, retrospective and multicentric study in pts≥18 years with uncontrolled persistent SA which achieved disease control after 1 year of treatment with OMA (according to Spanish Guidelines asthma management). Phenotypes were classified as: early onset allergic asthma, SA with frequent exacerbations, asthma with persistent airway obstruction, steroid-dependent SA, hypereosinophilic SA and SA in obese women. Measured variables were: lung function, NSAE and symptoms reduction, oral (OC) and inhaled corticosteroid (IC) intake, work absenteeism, and unscheduled visits. 345 pts (66.7% women, mean age 48.6 years) were included. Main phenotypes were: SA with frequent exacerbations (29.9%), early onset allergic asthma (23.7%), steroid-dependent SA (18.0%), hypereosinophilic SA (13.6%), asthma with persistent airway obstruction (9.3%) and SA in obese women (5.5%). Before starting OMA, 50.1% and 18% had daily or several times per day, respectively. After one

year with OMA, 55.4% were asymptomatic and 44.6% had symptoms 1-2 days per week. 36.4% and 49.9% of pts reduced OC and IC doses, respectively. Mean change (95% CI) in lung function (FEV_1%) was 14.7% (13.2-16.3; $P<.0001$). After 1 year of OMA, NSAE and work absenteeism days decreased (9.8 [29.3] vs 1.3 [2.2] episodes and 12.4 [39.2] vs 0.6 [2.3] days respectively; both $P<.0001$). Unscheduled visits to the primary care physician and to the specialist due to asthma worsening also decreased significantly (4.7 [95% CI: 4.2-5.2] vs 0.7 [95% CI: 0.5-0.9] and 1.7 [95% CI 1.5-1.9] vs 0.3 [95% CI: 0.2-0.4], respectively).

Conclusions: Most common phenotypes in SA pts treated with OMA who achieved full control were early onset allergic asthma and SA with frequent exacerbations. In the first year with OMA there was a decrease in non- severe asthma exacerbation, maintenance and rescue medication use, unscheduled visits and work absenteeism, and an improvement in lung function.

SUNDAY, 18 JUNE 2017
OAS 03
IGE AND ALLERGENS

0133 | Human IgE monoclonal antibodies with natural heavy and light chain pairing and specificity for asthma-associated allergens

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Introduction: IgE antibodies are polyclonal, present at low concentrations in blood, and not feasible for structural studies of allergenic epitopes.

Objectives: The aim was to isolate human IgE monoclonal antibodies from patients with allergic diseases with the natural pairing of heavy and light chain for antigenic studies.

Results: Cultured human B cells from peripheral blood of allergic patients were fused with a human myeloma cell line using electrical cytofusion. Resulting hybridomas were screened with allergens purified from the natural source (Can f 1, Fel d 1 and Der p 2). Four high affinity allergen-specific IgE mAbs were isolated from 4 different patients which bound Can f 1 (1J11), Fel d 1 (11A12) and Der p 2 (2G1 and 5D10) with EC50 of 14.6, 2.6, 7.1 and 15.6 ng/ml, respectively. Allergen-specificity of IgE antibodies and the relative position and isoform recognition of the Der p 2-specific antibodies were assessed by ELISA. Both anti-Der p 2 IgE mAbs bound two isoforms (Der p 2.0101 and Der p 2.0103, expressed as recombinant proteins in *Escherichia coli* or *Pichia pastoris*, respectively) and Der f 2. The murine anti-Der p 2 IgG mAbs 1D8 and 7A1 that bind to opposite sides of the allergen did not interfere with the binding of either human IgE mAb using two-site ELISAs. Human IgE mAbs 2G1 and 5D10 bound to epitopes overlapping with the binding site for the murine mAb αDpX, which binds both Der p 2 isoforms and Der f 2. Since the two IgE mAbs came from two different patients, these results suggest that this could be an immunodominant area for IgE epitope/s recognized by human IgE.

Conclusions: The first human IgE monoclonal antibodies with natural pairing of heavy and light chains and with specificity for three asthma-associated allergens were isolated by human hybridoma technology. These antibodies will facilitate the identification of allergenic epitopes for the future design of hypoallergens for immunotherapy.

0134 | Bet v 1 microarray: recognition pattern of serum allergen-specific IgEs

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Introduction: As the number of IgE-mediated allergic reactions is increasing, there is growing demand for strategies targeting early diagnosis and treatment of allergic sensitisation. By component-resolved diagnostics it is possible to identify the specific disease-eliciting allergen and, thus, to introduce more personalised and effective immunotherapy treatments for sensitised individuals.

Objectives: A rapid, simple and cost-effective allergen microarray was established where allergens are printed as an array into the wells of a 96-well microtitre plate. Simultaneous measurement of IgE binding to 36 different allergens printed into one well can be performed with subsequent detection by an HRP-labelled human IgE-specific secondary antibody using only microlitres of serum. Signal amplification is carried out using fluorescently labelled tyramide. Fluorescence imaging of the microarrays is used to quantify the intensity in each spot, thus resulting in a semi-quantitative profile of IgEs present in the sample.

Results: The performance of the established allergen microarray was evaluated by analysing the binding of more than fifty serum samples specific to the major birch pollen allergen Bet v 1. Birch pollen serum samples used in the analysis were collected in spring 2014 which was reported to be a birch pollen-rich season in Finland. The allergen microarray results were compared with the results obtained with the well-established Immuno-Solid phase Allergen Chip ISAC 112 (Thermo Fisher). Our semi-quantitative result shows that there is a good correlation between these two methods. In addition, a competition immunoassay in microarray format was established to analyse different binding properties of serum IgE towards wild-type Bet v 1 and its natural hypoallergen.

Conclusions: The developed allergen microarray provides a promising, simple and cost-effective tool for simultaneous analysis of allergy-associated IgE antibodies. The assay has apparent potential for the analysis of the allergenic activity of allergens as well as high-throughput IgE profiling in large patient cohorts targeting wide variety of allergens. Additionally it enables the biological activity measurement of the allergens by the competitive immunoassay using only tiny amounts of serum.

0135 | Identification of novel oyster allergens using a combined transcriptomic and proteomic approach for improved diagnosis of oyster allergy

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Introduction: Increasing production and consumption of mollusc is associated with the rise in prevalence of mollusc allergy worldwide, currently ranging from 0.2% to 1.3% of the general population. However, the elucidation of mollusc allergens for better diagnostics still lags behind other seafood groups such as fish and crustacean. Genomic data have been utilized previously for improved identification of non-food allergens by performing similarity searching using the BLAST program.

Objectives: Based on the published genome of the Pacific oyster (*Crassostrea gigas*) we aimed to identify the complete potential oyster allergen repertoire using bioinformatics analysis and to investigate identified protein allergenicity using a combination of immuno-chemical methods and proteomic analysis.

Results: Ninety-five potential allergenic proteins of the Pacific oyster were discovered using *in silico* analyses. These proteins shared over 50% amino acid identity with their homologous allergens. The allergenicity of these proteins was characterized using a combination of immunoassay and genome-derived proteomics analysis. The 2D-immunoblotting using a serum pool from five shellfish-allergic patients showed twenty-two IgE-reactive spots in the raw extract of the Pacific oyster and five spots in the heated extract. The identity of these IgE-reactive proteins was investigated by mass spectrometry. Eighteen allergens were identified, some with two or more isoforms.

Conclusions: The combination of genomics coupled to proteomics and IgE-reactivity profiling is a powerful method for the identification of novel allergens from food sources. Using this combination approach we were able to expand the current knowledge on IgE-reactivity to various proteins of the Pacific oyster. These newly identified allergens and knowledge of their gene sequences will facilitate the development of improved component resolved diagnosis and future immunotherapy approach for oyster allergy.

0136 | Identification and immunological characterization of the ligand of pru p 3

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Introduction: Nowadays, an increasing evidence suggests that molecules carried by allergens could be implicated in the development of allergy. The lipid transfer proteins (LTPs) family includes a large number of allergens from different sources. LTPs are basic proteins of about 9 kDa with a conserved structure made of four a-helices that presents a characteristic tunnel-like cavity capable of binding lipophilic molecules. This work focuses on Pru p 3, a plant LTP and major allergen from peach.

Objectives: Identification and preliminary immunological characterization of the ligand of Pru p 3.

Results: The ligand carried by Pru p 3 was purified by organic extraction (methanol: formic acid) and chromatographic methods (LH20 exclusion chromatography). Mass spectrometry was employed to identify the chemical nature of the ligand which was then confirmed by commercial standards. The immunological properties of the purified ligand of Pru p 3 were investigated by using monocyte cell line THP1 and monocyte-derived dendritic cells.

The ligand of Pru p 3 was identified as a mono-hydroxylated derivative of the alkaloid camptothecin linked to phytosphingosine. While the hydrophobic tail of the sphingoid moiety is inserted into the hydrophobic cavity of the LTP, the camptothecin derivative that bears a number of polar groups is exposed to the aqueous solvent. The results obtained from several immunological assays showed that the ligand of Pru p 3 should be an important factor in the activation of the immune cells investigated.

Conclusions: The ligand of the major peach allergen Pru p 3 has been characterized and clear evidence of its immunological role has been found.

0137 | Clonality and mast cell activation

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Introduction: In allergy, that affects more than 20% of western populations, IgE antibodies presented by FcεRI on mast cells and basophils cause degranulation when crosslinked by allergen.

Increasing IgE clonality from 2 to 3 significantly increased reactivity (maximal activity) but not sensitivity (EC_{50}) of mast cells challenged with house dust mite or grass extract.

Objectives: We explored activity of the mast cells with further increase in clonality.

We used 3 different rIgE antibodies specific to the major cat allergen Fel d 1 and a recombinant Fel d 1 dimer. The three antibodies bound to Fel d 1 dimer in the low nanomolar range and reacted with non-overlapping epitopes as determined by ELISA and NMR spectroscopy (Vogel and Bachmann, unpublished results). Clonalities of 2, 4 and 6 could be created when sensitizing with 1, 2 and 3 antibodies, respectively.

Thus, if natural Fel d 1 is dimeric, this would be a unique situation where the first sensitizing IgE-clone would already lead to clinical symptoms.

Results: Mast cells were cultured during 8 weeks from CD133 + stem cells from anonymous peripheral blood. From week 6 they were sensitized with Fel d 1-specific rIgE-clones. The cells were challenged with Fel d 1-allergen, marked with an anti-CD63-FITC-antibody, and measured on a flow cytometer. Reactivity and EC_{50} were estimated by nonlinear curve fitting.

With a dimeric Fel d 1, single antibody response, the reactivity was 60.75% and EC_{50} $1.61{\cdot}10^{-10}$ mole/l. When using 2 antibodies, a reactivity of 80.95% and EC_{50} of $6.75 \cdot 10^{-14}$ mole/litre was observed. Further increasing to 3 antibodies, the reactivity was 80.98% and EC_{50} $1.33 \cdot 10^{-13}$ mole/l.

Thus, there is a 20.20% increase in reactivity (P=.0045) and 2385-fold increase in EC_{50} (P=.0046) when increasing the clonality from 2 to 4. There is no significant increase in mast cell activity when increasing clonality to 6.

Conclusions: The reactivity and EC50 of the mast cells increase when increasing clonality from 2 to 4, but reach a plateau beyond 4, which could indicate that dimeric Fel d 1 is saturated with 4 IgE-clones. The change in EC_{50} has not been seen with previous experiments done on Der p 2 and Phl p 5, suggesting that dose-response-curves differ between allergens.

0138 | An anti-IgE single domain antibody disrupts the interaction of IgE with fceri by acting as functional high affinity mimic of cd23

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Introduction: Anti-IgE therapeutics interfere with the ability of IgE to bind to its receptors on mast cells and basophiles, and block the IgE-mediated release of inflammatory mediators from these cells. The so far only IgE-neutralizing antibody approved for treatment is the humanized IgG1 antibody Omalizumab. Understanding of the mechanistic basis of novel anti-IgE molecules can provide insights needed for optimization of anti-IgE therapeutics and understanding IgE biology.

Objectives: An anti-IgE single domain antibody was expressed in *E. coli*. The human IgE-Fc was produced in HEK293 cells. Functionality and affinity of resulting antibodies were shown by ELISA and SPR analyses. The complex was successfully crystallized and diffracted in x-rays to 3.4 A. The structure was solved by molecular replacement. Cellular assays were used to assess competition of the anti-IgE with the IgE-receptors. Furthermore, conformational changes in solution were assessed using small angle x-ray scattering (SAXS).

Results: The epitope of the anti-IgE single domain antibody shows minor overlap with the FcεRI binding site but a significant overlap with the binding site of CD23. The structural data revealed the mechanism of inhibiting the interactions with the high affinity IgE receptor and CD23. The single domain antibody acts as functional homologue of CD23 and induces conformational changes into the closed conformation incompatible with FcεRI binding. For the first time SAXS data documented substantial conformational rearrangements of the IgE-Fc upon anti-IgE binding. Mutational analysis and inhibition studies corroborated the obtained data. Notably the single domain antibody is able to compete with and displace IgE from CD23 providing evidence for novel mechanisms of disruptive IgE inhibitors.

Conclusions: In summary, our data provide structural and functional insights into the mode of action of a single domain antibody in complex with the IgE-Fc and contribute to understanding the structural basis for anti-IgE approaches.

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OAS 04
ALLERGY EPIDEMIOLOGY: EARLY LIFE AND ENVIRONMENT

0169 | Does leucocyte telomere length play a role as a candidate biomarker for prenatal stress exposure and the risk of atopic dermatitis development?

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Introduction: Prenatal maternal stress affects the offspring's development of allergic diseases. No specific biomarker, however, has succeeded in evaluating the risk of allergic diseases development or the degree of exposure to the potential risks factors. Recently the shortening of leucocyte telomere lengths (LTL) begins to be highlighted as potential marker for the exposure to oxidative stress.

Objectives: We aimed to explore whether LTL shortening varies according to the high exposure to maternal stress and the later development of atopic dermatitis (AD).

Results: We chose four groups of samples from the COCOA birth cohort according to the exposure to maternal prenatal distress and the later development of atopic dermatitis at 1 year. We measured the LTL from the cord-blood and 1-year peripheral blood of the sample populations. The LTL was shorter in 1-year samples than in the cord blood ones ($P<.001$), which was evident in the prenatally distressed and later AD-developed group ($P=.004$), the prenatally non-distressed and later AD-developed one ($P=.026$), and the prenatally distressed but later AD-undeveloped one ($P=.003$). However, the LTL was not different in the prenatally non-distressed and AD-undeveloped group. ($P=.434$). The LTL was shorter in prenatally-distressed groups than non-distressed the others ($P=.002$ in the cord blood and $P=.015$ in the 1-year peripheral blood), but the difference is not significant between the group of later AD-developed and non-developed ones ($P=.310$ in the cord blood and $P=.154$ in the 1-year peripheral blood).

Conclusions: We observed a significant difference in LTL according to the exposure to prenatal maternal distress but less significant trend according to the later AD development. The result implies that oxidative stress may partly be involved in the pathogenesis of prenatal stress exposure and the later AD development, but also implies that there might be other moderating factors.

0170 | Late preterm birth protects against atopies in adulthood

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Introduction: Development of immunological pathways occurs during intrauterine life.

Objectives: To examine whether preterm birth predicts the risk of atopy in adulthood.

Methods: Young adults born during 1985-9 in Northern Finland participated in Ester preterm birth-cohort study at mean age of 23.5±1.7 years. Participants were tested for atopy for common allergens (birch, timothy, mugworth, dpteron, cat, dog), as part of a clinical health check and provided health details and history of atopic diseases (incl. physician-diagnosed asthma) via a questionnaire. Mean wheal diameter (largest + perpent/2) ≥3 mm and no reaction in the negative control, was considered a positive result. Participants were grouped into early preterm: GA≤34 weeks, n=134, late preterm: 34<37 weeks, n=235) and full-term-born (≥37 weeks, n=331).

Results: 43% of all participants were tested positive for any atopy and 22% to birch. Likelihood of birth pollen sensitisation was considered as uniform across the population and a good representative of a random sensitisation. Logistic regression analysis showed that late-preterm individuals had a significantly lower risk of any atopy (OR: 0.7, 95% CI: 0.5, 0.9, birch: 0.8, 95% CI: 0.7, 1.0) than full-term-born. The association was not observed within the early-preterm-born (any atopy: 0.9, 95% CI: 0.6, 1.4, birch: 0.7, 95% CI: 0.4, 1.0). Parental educational attainment or maternal smoking during pregnancy, were not association with atopy in this population.

Conclusions: Preterm birth has a significant effect on the early development of immunity. Late preterm birth may expose individuals to environmental antigens earlier, possibly priming immunological maturation and protecting against atopy. Severity of early preterm birth may counterbalance this benefit.

0171 | Influence of early feeding patterns on eczema development in high-risk infants

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Introduction: This study examined the relationship between timing of introduction of complementary feeding, type of early milk feeding (breast milk, standard cow's milk formula [CMF], prebiotic-supplemented hydrolysed cow's milk formula [pHF-OS]) and development of eczema in infants at increased risk of allergy.

Objectives: This is a sub-study of the PATCH randomized-controlled trial which evaluated the effect of pHF-OS on prevention of eczema [ISRCTN65195597]. PATCH participants were randomized to receive pHF-OS or CMF for 6 months. A breastfed reference group was included. Cumulative incidence of eczema was assessed at age 18 months and 5 years. Age at introduction of complementary feeding was categorized as early-introduction (<18 weeks) or standard-introduction (≥18 weeks). The relationship between timing of introduction of complementary feeding, type of milk feed and eczema was examined using multivariate Cox proportional hazard models.

Results: Data regarding age at introduction of complementary feeding were available for 821 infants; 397 (48%) introduced complementary feeding <18 weeks, 424 (52%) ≥18 weeks of age. The risk of developing eczema by 18 months was 35% lower (HR: 0.65; 95% CI: 0.49-0.85) in the early-introduction group compared to standard-introduction group, and 34% lower (HR: 0.66; 95% CI: 0.47-0.92) at 5 years of age, after adjustment for family history of eczema and ethnicity. Regarding the type of milk feed, pHF-OS was associated with reduced eczema risk compared with CMF, only in the standard-introduction group (HR 0.54, 95% CI: 0.34-0.85).

Conclusions: Introduction of complementary feeding before 18 weeks was associated with a lower risk of eczema development in high-risk infants, compared to introduction after 18 weeks. Further studies are required to understand the optimal timing for introduction of complementary feeding to support tolerance and underlying mechanisms. Where breastfeeding is not possible, pHF-OS may offer an approach to reduce eczema risk in high-risk infants with delayed introduction of complementary feeding.

0172 | Farmhouse-like indoor microbiota protects children from asthma also away from farms

Kirjavainen PV[1]; Karvonen AM[1]; Täubel M[1]; Adams R[2]; Jayaprakash B[1]; Roponen M[3]; Renz H[4]; Pfefferle P[4]; Schaub B[5]; Remes S[6]; Hyvärinen A[1]; Lauener R[7]; Von Mutius E[8]; Pekkanen J[9]

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Introduction: Asthma prevalence has increased in epidemic proportions along urbanization, while growing up on traditional farms offers protection. The protection in farms has been associated with rich indoor dust microbiota. It remains elusive whether compositionally similar microbiota predicts lower asthma risk in non-farm homes, independently of the farm environment and lifestyle.

Objectives: To determine compositional predictors of asthma protective microbial exposures in farm homes and whether similar microbiota is associated with lower asthma-risk also in homes away from farms.

Results: We modelled the primary distinguishing features between indoor dust microbiota composition of farm homes and other rural homes in a prospective birth cohort of 204 children with half living on farms. The samples were collected when the children were 2 months old and microbiota analysed by DNA amplicon sequencing. Living in a house with microbiota more similar to farm than non-farm homes was a stronger predictor of the farm-associated asthma protection by 6 years of age than living in an actual farm home. The microbiota feature distinct to farm homes was high proportion of livestock-associated taxa over those commonly found in human airways. Applying the approach to a second Finnish, mainly suburban, birth cohort of 182 children, showed that also in non-farm homes the risk of asthma was inversely associated with the similarity of the predominant microbiota in farm homes. The similarity was characterized by high abundance of bacteria associated with outdoor environment and associated with reduced proinflammatory cytokine responses against bacterial cell wall components ex vivo.

Conclusions: These results identify the phylogenetic composition of indoor dust microbiome as characterizable predictor of asthma-risk and a novel modifiable target for asthma preventive interventions.

0173 | Indoor microbiome and asthma: a case-control study within ECRHS

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Introduction: Both protective and adverse effects of indoor microbial exposure on risk of asthma have been reported, mainly among children. There are very few studies on adults.

Objectives: Xxxxxxxxx.

Methods: A cross-sectional case-control study of 198 asthmatics and 199 controls aged 29-55 in 14 centres was done within the European Community Respiratory Health Survey (ECRHS) II. DNA was extracted from mattress dust samples for bacterial microbiota analysis using denaturing gradient gel electrophoresis (DGGE). Based on sequencing of selected DGGE bands, four qPCR assays (targeting Clostridium cluster XI, Corynebacterineae/Pseudonocardineae group, Corynebacteria amycolatum cluster and Staphylococcus group) were developed or optimized for mattress dust samples. Clostridium cluster XI and Corynebacterineae/Pseudonocardineae qPCRs were also measured from different types of repeated indoor dust samples (n=259) in an exposure study.

Results: Altogether 37 different bands were detected in the bacterial DNA fingerprints with DGGE, of which 15 bands had at least a suggestive adjusted association ($P<.25$) with asthma. These 15 bands were sequenced for detection of specific microbes and based on the results, four qPCRs were developed or optimized. Of these four qPCRs, Clostridium cluster XI confirmed the protective association with asthma observed in the analyses of the DGGE bands. The association was dose dependent over the four quartiles of exposure (aOR 0.43 (0.22-0.84) for the fourth quartile vs first quartile) (p for trend 0.009) and independent of levels of other microbial markers. In an exposure study, environmental in addition to human sources were found to be important determinants of mattress dust levels of Clostridium cluster XI.

Conclusions: In this large international study, using non-targeted DNA fingerprinting for detection of important bacteria together with targeted quantitative confirmation, cluster XI of Gram-positive Clostridium bacteria was independently associated with lower risk of prevalent asthma. Results suggest the importance of environmental bacteria also in adult asthma, but this needs to be confirmed in prospective studies.

0174 | Perinatal probiotics decreased eczema up to 10 years of age but at 5-10 years allergic rhino conjunctivitis was increased

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Introduction: Probiotics have shown promising results in primary prevention of allergies in the first years of life but long-term effects need more evaluation.

Objectives: We conducted a randomized double-blind, placebo-controlled trial to determine whether perinatally given probiotics prevent allergic diseases in high-risk children.

1223 pregnant women were randomized to receive probiotics (LGG ATCC 53103, *L. rhamnosus* LC705, *Bifidobacterium breve* Bb99) or placebo from 36 gestational week and their infants with a high allergy risk pro-and prebiotics or placebo from birth until 6 months. At 10 years parents filled in questionnaires, based on the ISAAC-study. Our primary outcome was the prevalence of allergic diseases.

Results: Of intention-to-treat children´s parents 79.2% returned the questionnaire. The lifetime prevalence of any allergic disease (eczema, allergic rhinitis (AR) or asthma) was similar (64.4% vs 70.0%) in the probiotic and placebo group. However the lifetime prevalence of eczema was lower in the probiotic group (35.2% vs 41.7%, adjusted OR 0.74 (0.55-1.00 $P<.05$). The lifetime prevalences of any doctor-diagnosed allergic disease (eczema, AR or asthma) (49.4% vs 52.3%), eczema (33.0% vs 37.6%), AR (27.0% vs 24.5%) or asthma (14.3% vs 15.5%) were comparable between the probiotic and placebo groups, respectively.

Between age 5-10 years allergic rhino conjunctivitis occurred more frequently in the probiotic group (36.5% vs 29.3%; OR 1.39; 95% CI 1.03-1.86; $P=.03$). The prevalences of eczema (26.7% vs 31.9%) and asthma (9.4% vs 12.4%) were similar.

At age 5-10 years, Caesarean-born children in the probiotic group had less respiratory tract infections than the controls (13.0% vs 33.3%, OR 0.30; 95% CI 0.13-0.70; $P<.01$) and more often no use of antibiotics in the past 5 years (17.4% vs 6.7%, adjusted OR 0.31; 95% CI 0.10-0.98; $P<.05$).

Conclusions: Perinatally received probiotics may reduce the cumulative incidence of eczema until age 10 years, but the prevalence of allergic rhino conjunctivitis at age 5-10 years increased.

SUNDAY, 18 JUNE 2017
OAS 05
WHAT'S NEW IN INSECT VENOM ALLERGY?

0175 | Role of cytokine gene polymorphisms in hymenoptera venom allergy

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Introduction: Insect venom allergy is a potentially life-threatening allergic reaction following a bee, or wasp sting. Some individuals being stung can be develop systemic allergic reaction while most of the others do not. Genetic predisposition may be responsible from the development of anaphylactic reactions in these subjects.

Objectives: In this study, we aimed to investigate the role of polymorphisms in cytokine genes such as IL-1alpha, IL-1β, IL-1RA, IL-2, IL-4R alpha, IL-4, IL-6, IL-10, IL-12, IFN-gamma, TGF-β and TNF-alpha, in insect venom allergy. Twenty-one patients who had a history of early systemic allergic reaction after a sting were included in the study. Nineteen male beekeepers and 18 non-atopic healthy subjects who did not experience any systemic allergic reaction despite insect sting were selected as control groups. Insect venom allergy was diagnosed by using skin prick and intradermal tests and/or specific IgE measurements. Cytokine genotyping and haplotyping were carried out from genomic DNA by polymerase chain reaction with sequence-specific primers (PCR-SSP) using a commercial kit.

Results: Frequency of IL-10-1082, -819 AT* (76.2%) in allergic patients was found significantly higher than both from non- allergic beekeepers (35.3%) and healthy controls (58.8%) (*P*=.042). On the other hand, tendency of lower frequency of IL-10-1082, -592 A*A in non-allergic beekeepers (46.7%) comparing to allergic individuals (75%) was shown. Frequency of IL4-1098, -590 TT* (43.8%) in allergic patients was found higher than both from non-allergic beekeepers (30.8%) and healthy controls (13.2%). Comparison of allergic individuals with non-allergic beekeepers resulted with significant *P* value for IL-10-1082,-819 AT* (*P*=.013, frequency; 76.2% and 35.3%, respectively). However, non-allergic beekeepers showed a tendency of higher frequency of IFN-gamma +874 A genotype than in the allergic patients (*P*=.062, 94.7% and 71.4%, respectively). Gene polymorphisms did not differ between non-allergic beekeepers and healthy controls. Furthermore, the presence of IFN-gamma +874 A genotype was correlated with the lower grade of severity of systemic sting reaction (*P*=.018).

Conclusions: Our data suggest that polymorphisms which have negative effects on expression of IL-10 gene may affect susceptibility on the development of severe allergic reaction in hymenoptera venom allergy.

0176 | Polistes venom allergy: identification and immunological characterization of novel allergens

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Introduction: Allergies due to venoms of hymenoptera can cause severe anaphylaxis. In the last years, progress of component-resolution advanced the differential diagnosis of honeybee and wasp venom allergic patients. The discrimination between clinically relevant sensitization to venom of different wasp species, especially between *Vespula* and *Polistes* species, is still challenging. Both species live side to side in Mediterranean regions and the US, but with *Polistes dominula* being an invasive species, *Polistes* venom allergy is likely to evolve in northern regions of Europe. Amongst others, the diagnostic challenge is due to extensive cross-reactivity between the venoms. Additionally, for *Polistes dominula* only a small fraction of venom components is known. In this study, *Polistes* venom was analyzed for additional allergens, which were subsequently characterized in detail regarding their potential to trigger allergic reactions.

Objectives: *Polistes* venom was extensively analyzed by mass spectrometry and de novo peptide sequencing following 1D or 2D gel electrophoresis. Identified components were cloned from venom gland mRNA and expressed in insect cells. The resulting purified proteins, together with their homologues of different hymenoptera species, were characterized by immunoblotting and assessed for IgE cross-reactivity. Moreover, their capacity to activate basophils of either honeybee or wasp venom allergic patients was evaluated.

Results: Newly identified *Polistes* venom components and homologues from other hymenoptera species were successfully produced in Sf9 insect cells and thus were devoid of cross-reactive carbohydrate determinants. The analysis of sera from honeybee, *Vespula* and *Polistes*venom allergic patients revealed extensive IgE cross-reactivity between homologous proteins, independent of glycosylation. Additionally, basophil activation tests could reveal the capability of the allergens to activate basophils of venom allergic patients and also show cross-reactivity. This indicates the presence of shared IgE epitopes, probably in conserved regions of venom proteins.

Conclusions: The detailed mass spectrometry analysis of *Polistes* venom led to yet unknown venom components and serologic as well

as cellular tests demonstrated their importance in *Polistes* venom allergy. However, as the newly identified proteins show high homology on amino acid level with homologous allergens of other hymenoptera species, component-resolved analyses are affected by extensive immunological cross-reactivity.

0177 | Component-resolved allergen content of therapeutic honeybee venom extracts

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Introduction: Venom immunotherapy is the only curative treatment for honeybee venom (HBV) allergy. The amounts of different major allergens in the therapeutic extracts might have a high impact on therapy outcome in patients with particular sensitization profiles. Differences in the allergen content of therapeutic HBV extracts, derived from pure venom, might occur due to natural variations of the source material or different down-stream processing strategies.

Objectives: Polyclonal and monoclonal antibodies were generated against CCD-free recombinant allergens. The allergen-specific antibodies were used to address the allergen composition of different therapeutic grade HBV extracts which are approved for immunotherapy in different countries world-wide. Moreover, the stability of potentially labile allergens was assessed.

Results: The extracts were analyzed for their content of the major allergens Api m 1, Api m 2, Api m 3, Api m 5 and Api m 10. The use of the allergen-specific antibodies demonstrated dramatic differences in the allergen composition ranging from the presence of all allergens down to only two detectable allergens. Moreover, particular major allergens showed increased instability in solution.

Conclusions: Taken together, the variable allergen content of different therapeutic HBV extracts might have a high impact on therapy outcome and the clinical management of HBV-allergic patients with specific IgE to particular allergens. Moreover, standardization of therapeutic venom extracts by determination of the total allergenic potency might imply the intrinsic pitfall of losing information about particular major allergens.

0178 | Can tests distinguish between acute patients, VIT treated patients, and asymptomatically sensitized subjects?

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Introduction: Currently no reliable serological marker is known to predict tolerance to hymenoptera stings. Our aim was therefore to evaluate different parameters and methods (sIgE and IgG_4 levels, IgE/IgG_4 ratio, ELIFAB assay, BAT-Inhibition) in three different groups of sensitized subjects to identify possible differences.

Objectives: We included 41 subjects with acute systemic sting reactions grade 2 or higher. The subgroup of VIT treated patients consisted of 37 patients, and 40 asymptomatically sensitized subjects were included. We measured baseline levels of sIgE and $sIgG_4$ to bee venom, wasp venom, rApi m 1 and rVes v 5 immediately after acute sting reactions and before sting challenges in VIT treated and asymptomatically sensitized subjects. To determine the time courses of sIgE and $sIgG_4$ levels, blood was taken one month after the acute sting or sting challenge. Additionally, the inhibitory activity was determined by using the ELIFAB assay and BAT-Inhibition-tests.

Results: Baseline sIgE levels to bee venom, wasp venom, rApi m 1 and rVes v 5 did not differ between the groups. IgG_4 levels proofed to be significantly higher in VIT-patients ($P < .001$), while IgE/IgG_4 ratios where consequently lower ($P < .001$). At baseline, IgE/IgG_4 ratios in acute patients and asymptomatically sensitized subjects tended to be higher in acute patients, although the difference was not statistically significant. The ELIFAB assay correlated with the low IgE/IgG_4 ratios in VIT patients showing markedly higher allergen-blocking capacity ($P < .001$).

Four weeks after the sting, sIgE and IgG_4 levels increased in acute patients and asymptomatically sensitized subjects, but not in VIT patients. A clear inhibition of basophil response could be shown for patients treated with wasp venom, whereas inhibition failed in patients treated with bee venom.

Conclusions: Baseline IgG_4 values of all parameters were significantly higher ($P < .001$) in VIT-patients, which reflects the immunological effect of VIT. In addition to this, the ELIFAB assay confirmed the results of the IgE/IgG_4 ratios in VIT patients. BAT inhibition was a promising tool at least to monitor VIT with wasp venom. Different responses of IgE and IgG levels after bee and wasp stings, and differences in the BAT inhibition implied that bee and wasp venom allergy may be immunologically different.

0179 | CD63 and CD203c expression during specific immunotherapy (SIT) for wasp venom allergy using basophile activation test (BAT): Results after 3 years and correlation to sting challenge test

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Introduction: SIT is an established therapy for wasp venom allergy. The aim of our work is to investigate the progression of surface antigen CD63 and CD203c expression during SIT using BAT.

Objectives: We included 71 patients (61 wasp, 10 honey bee; 19 aborted) in our study, which was approved by the institutional ethical review board. Here we report on 40 adult patients with SIT against wasp venom after 3 years of SIT. Blood samples were collected before and 3 days (3d), 2 weeks (2w), 6 months (6 m) and repeatedly every 6 months until 3 years (3y) during SIT. For all samples we determined CD63 and CD203c expression using BAT after stimulation with various wasp venom concentrations. We evaluated the relative proportion of activated basophile granulocytes at 57 μg/l venom concentration (a2) and the calculated concentration c50 to stimulate 50% of total activatable basophile granulocytes. Clinical evaluation of outdoor stings during SIT and final sting challenge tests after 3 years was obtained from patient charts.

Results: CD63 expression (and inversely c50) (n=36) decreased in 27 and increased in 4 patients, while it was constant in 5 cases. Median changes to baseline at 3y were a2=−79% ($P<.01$) and c50=722% ($P=.02$). CD203c expression (and inversely c50) (n=40) decreased in 25, increased in 9 and did not change in 6 patients. Median changes to baseline at 3y were a2=−52% ($P<.01$) and c50=435% ($P<.01$). None of the patients that underwent outdoor stings (n=5) and took part in clinical proven sting challenge tests (13) reported clinical symptoms after stings. BAT shows increased c50 (outdoor sting: CD63 80%/CD203c 60%; sting challenge test: CD63 100%/CD203c 85%) and decreased a2 (outdoor sting: CD63 60%/CD203c 60%; sting challenge test: CD63 85%/CD203c 54%).

Conclusions: In spite of different individual progression of CD63 and CD203c in BAT over 80% of patients show a decreased expression of surface proteins over time. Statistical significance can be demonstrated for CD63 and CD203c expression after 3 years. Clinical sign of recovery is associated with increasing c50 and decreasing a2 in patients with further stings during SIT.

0180 | Understanding health care system settings and funding structures in European countries in management of patients with Hymenoptera venom allergy

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Introduction: Healthcare systems vary between countries but the effect of this on implementation of guidelines in Europe has not been previously explored.

Objectives: To recognize differences in health service delivery across European countries which may influence diagnosis and treatment of Hymenoptera venom allergy.

Results: A link to an online questionnaire was sent to a practicing allergy physicians or representative of each of the 36 national allergy societies associated to EAACI. Data collection is ongoing. Twenty two responses have been received so far (61%). First aid following field insect sting is delivered in most countries in emergency department [ED] (77%), ambulance (68%), or by general practitioners [GPs] (59%). In over 50% of countries surveyed, help received in ED or ambulance is free of charge. In about 1/3 of countries GPs and ED staff are allowed to prescribe adrenaline autoinjector. The price of an autoinjector ranged between 40-116 across the surveyed countries, but in 23% of countries it is either free or fully reimbursed. In almost all countries (96%) GPs refer patients to an allergy specialist, although in 46% patients can self-refer to specialists. Waiting times for a first appointment with an allergy specialist varied considerably between 1 week and 6 months. Diagnostic blood tests are performed at primary (55%) or secondary/tertiary level (70% both) whereas skin tests are performed mainly at tertiary level (82%). In over 50% of countries diagnostic procedures are either free (46%) or fully reimbursed (9%). Venom immunotherapy (VIT) is available in most countries (86%) and is free in over 42% of them, regardless of venom extract. Both incremental and maintenance dose of VIT are usually delivered on tertiary level (90%, 84%, respectively). A vial of venom extract costs between 15-595. In about half of the countries there are national guidelines for venom allergy.

Conclusions: Our interim analysis found considerable heterogeneity in the health care systems across Europe. Referral systems, drug

costs, reimbursement options and training programs appear to vary between countries and this can have implications for HVA diagnosis and management in these countries. Such discrepancies may have considered while drawing up EAACI clinical guidelines.

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SUNDAY, 18 JUNE 2017
OAS 06
UPDATES IN ALLERGIC RHINOCONJUNCTIVITIS

0181 | Conjunctival redness at allergen provocation with photodocumentation: influence of missing values on the therapeutic effect

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Introduction: Diagnostic efficacy of the conjunctival provocation test (CPT) was shown in recent years, exploring the severity of allergic reaction by rhinoconjunctivitis in immunotherapeutic, placebo controlled dose-finding and safety studies. The CPT is based on the eye mucosa challenge, instilling the allergen solution onto the conjunctival sac. Assessing the allergic symptoms, which occur after the conjunctival provocation with experimental allergen, severity of the allergic rhinoconjunctivitis and success of the immunotherapy is evaluated. Among the allergic symptoms only redness can be documented for further reassessment by different methods.

Objectives: To estimate an influence of missing values in assessment of the conjunctival redness severity during the hyposensitisation of the allergic rhinoconjunctivitis.

Results: Current analysis processes the data, collected in five prospective, multicenter, double-blind, placebo-controlled, randomized, immunotherapeutic hyposensitization studies in inhalative allergies. The CPT was an essential diagnostic method to assess the therapeutic effect in all considered studies. After an assessment of the allergic reaction on the conjunctival provocation, the study investigators documented the high-resolution digital photos of the challenged eyes. The allergic redness, represented on the eyes photos, was first assessed by an external observer, using the grading score. Then the imaging software was applied to assess the eye redness independently from the investigators subjectivity. Missing values, which appeared because of incompleteness or bad quality photodocumentation, were imputed by multiple imputations and compared with the original data set. The therapeutic effect uncertainties, induced by missing values, were estimated. Diagnostic performance of the redness, estimated by the imaging software and external observer, was assessed with the receiver operating characteristic (ROC) curves and correlation analysis, before and after the missing value imputation.

Conclusions: Multiple imputations of the photodocumentation missing values were successfully applied to reveal an assessment accuracy of the conjunctival redness during the immunotherapeutic hyposensitization studies with conjunctival provocation challenge.

0182 | Conjunctival transcriptome in vernal keratoconjunctivitis

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Introduction: Vernal Keratoconjunctivitis (VKC) is a chronic disease with unknown pathogenesis. The study of the human transcriptome may help to identify the genes involved in this disease.

Objectives: To investigate the extent of the human transcriptome that can be quantified from conjunctival impression cytology samples. The aims are to determine if sufficient RNA can be isolated from a patient's conjunctiva, to identify differences in gene expression between VKC and normal subjects (CT), and to identify potential disease or disease activity biomarkers. Conjunctival cells were collected by impression cytology (Eyeprim) from 23 VKC patients and 8 CT. Based on prior optimization of all assay steps, RNA was isolated from the samples using a Qiagen RNeasy Plus Mini Kit. The RNA integrity number (RIN) was assessed with an Agilent Bioanalyzer. Samples were then assayed with the NanoString human immunology code set to assess the expression levels of 579.

Results: The inflammatory NanoString assay on 579 genes showed that 188 genes were significantly overexpressed in VKC compared to CT samples. Considering the three different phenotypes of the disease, the number of significantly differentially expressed genes (at 5% on raw *P*-value + FC>2) vs CT were 216 in tarsal VKC, 149 in limbal VKC and 191 in the mixed form of VKC. The comparison between limbal and tarsal forms showed 120 significantly differently expressed each other. Of the most overexpressed genes in the VKC group, to be noted were CCL3, CCL4, CCL18, CCL22, CCL24, CXCL1, CARD9, CLEC4E, CLEC7A, CD45RB, CSF3R, FN, SOCS3, CARD9, CIITA, ICAM1-2-4, IL-1, IL-6, IL-13, IL-23, IRAK1/2, ITGA4, ITLN1, KLRC3, LILRB, MAF, MUC1, NOD2, PTAFR, TGFbeta1, TLR4, TLR8 and ZAP70.

Conclusions: Conjunctival impression cytology can be used to collect sufficient RNA from conjunctival surface cells that, when processed optimally, allows successful transcriptome-wide expression analysis. Factors involved in both innate and adaptive arms of the immune system were found over-expressed in VKC samples. The increased expression of several chemotactic factors and co-stimulatory signals required for T cell activation and survival, confirms that VKC is mostly a cell-mediated pathology. While the present transcriptome analysis used a limited number of patients, larger studies with different types and severities of ocular allergy should reveal

significant gene expression trends that can then be targeted to improve ocular allergy treatments.

0183 | Expert interpretation of the allergic rhinitis clinical decision support system (AR CDSS): results of a survey monkey of allergic rhinitis experts

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Introduction: The allergic rhinitis Clinical Decision Support System (AR CDSS) was recently published, and will be incorporated into a MACVIA-ARIA app for healthcare providers (*Allergy Diary Companion)*. This app collects AR patients' visual analogue scale (VAS) score, type of AR, current treatment(s), and allergen exposure, and provides a treatment recommendation based on the AR CDSS.

Objectives: The objectives of this study were to (i) access AR expert consensus on the AR CDSS step-up, and step down treatment strategies, (ii) formulate specific treatment recommendations for each step and (iii) improve robustness of the AR CDSS. A survey monkey was sent to 70 AR experts. The first part posed questions about the AR CDSS approach itself; when to start, step-up and step-down treatment. The second part asked in depth questions on specific AR treatment recommendations at each step. For each scenario, respondents indicated their level of agreement on a VAS ranging from 0 (strongly disagree) to 100 (strongly agree).

Results: 35 experts responded (50.0%). They agreed that AR treatment should be stepped up for treated AR patients with a VAS score ≥5. For those with VAS score ≥2 to <5, treatment should be continued for patients with intermittent AR or continued/stepped-up for those with persistent AR. Treatment should be stepped down for those with VAS score <2. There was also good agreement on specific treatment recommendations. On average, experts agreed that patients on treatment step 1 (i.e. anti-histamines (AH, oral/intranasal), leucotriene receptor antagonists, chromones or eye drops) should be stepped-up to intranasal corticosteroids (INS) OR INS + azelastine (AZE), and those on INS stepped-up to INS + AZE. Short course oral corticosteroids could be added here if necessary, but patients referred if VAS score remained ≥5. Experts considered that those on multiple therapies should be stepped up to INS + AZE. Stepping down was essentially the same in reverse, with the proviso that patients with nasal congestion should be stepped down to an INS-containing regimen in preference to AH. Step-up and step-down strategies remained the same irrespective of immunotherapy status.

Conclusions: Experts endorsed the AR CDSS approach, with good agreement achieved on treatment recommendations. The AR CDSS and expert treatment recommendations will be incorporated into the *Allergy Diary Companion*. When using this app healthcare providers benefit from the combined wisdom of world experts in AR.

0184 | A novel method of measuring nasal specific IgE in local allergic rhinitis patients

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Introduction: Prior methods used for measuring nasal specific IgE (NsIgE) in local allergic rhinitis (LAR) have shown a variable sensitivity: 22% for *D. Pteronyssinus* (DP) using the Greiff/Grünberg method and lower with Naclerio method.

Objectives: In this study a novel method of detection of NsIgE in patients with confirmed LAR to DP was evaluated.

Methods: Sixteen LAR (positive nasal allergen provocation test to DP (NAPT-DP), negative skin testing/sIgE to DP), 10 allergic rhinitis (AR) as positive control (positive NAPT-DP and skin testing/sIgE to DP), and 12 healthy controls as negative control (negative NAPT-DP and skin testing/sIgE to DP) were recruited. DP-ImmunoCAP solid phase was applied directly in the lower turbinate of each nostril for 10 minutes before and 24 hours after NAPT-DP and analyzed following the manufacturer´s instructions. ROC curves were performed to obtain the optimal cut-off point of nasal sIgE value to calculate sensitivity (S) and specificity (SP), and outcomes were compared with NAPT-DP result (gold standard test). Study was approved by local ethics´ committee.

Results: All LAR and AR subjects had a positive response to NAPT-DP, and none in the healthy control group. At 24 hours after NAPT-DP, mean NsIgE values were 0.119 kU/l in LAR, 1.600 kU/l in AR and 0.115 kU/L in healthy controls. ROC curves using NsIgE values obtained 24 hours after NAPT-DP were performed. In LAR subjects, the area under the curve (AUC) was 0.7277, *P*=.0054. The optimal cut-off point to discriminate LAR subjects from controls was 0.135 kU/l, obtaining a S=20.31% and SP=88.09%. In AR (positive control group) the AUC was 0.9798, *P*=<.0001, and the optimal cut-off point was 0.17 kU/l with S=95% and SP=100%.

Conclusions: Measurement of NsIgE by direct application of DP-ImmunoCAP in LAR shows similar sensitivity to other methods and good specificity, with the advantage of being non-invasive, easier to perform and faster. FIS PI14/00864.

0185 | Frequency of local allergic rhinitis diagnosed by high dose nasal allergen provocation

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Introduction: Local allergic rhinitis (LAR) describes a phenomenon where patients with symptoms of allergic rhinitis who are negative in allergy testing with skin tests and specific IgE determination (sIgE) react positively to nasal allergen provocation (NPT). LAR has been described and confirmed by several groups but considerable uncertainty remains about its incidence and the correct methodology for its diagnosis.

In our department, NPT is performed routinely following the recommendations of the German Society for Allergology and Clinical Immunology. A combination of symptom scores and rhinomanometry is used to determine the outcome. As opposed to others we challenge with high and increasing doses of allergen.

Objectives: The aim of this study was to analyze the frequency of LAR in our patient population. We performed a retrospective analysis of routine nasal allergen provocations that were performed between 1990 and 2013 in our allergy clinic. Only patients with complete data sets were included. Allergens from one manufacturer in concentrations of 5.000 BE/ml, 25.000 BE/ml, and 50.000 BE/ml - corresponding to 0.22 μg, 1.1 μg, and 2.2 μg major allergen of D. farinae per application - were used.

Results: 942 patients with complete data were extracted. 789 patients had a positive allergy test (either skin test or sIgE or both). In this group 58.1% (458/789) had a positive NPT and 41.9% (331/789) a negative NPT.

153 patients had negative allergy tests. Of these, 71 patients were negative in both sIgE and skin test. 3 patients were sIgE negative (and had no skin tests), 79 patients were skin test negative (and had no sIgE test).

21.6% (33/153) of the patients with negative allergy tests had positive results in NPT. In 15.2% (5/33) one of the two higher allergen concentrations was needed to obtain positive NPT results.

In the group of patients where both skin test and sIgE were negative 18.3% (13/71) had positive NPT results. 24.1% (19/79) of the skin test negative patients without sIgE results had positive NPT results. 1 of the 3 patients with negative sIgE and no skin test had positive NPT results.

Conclusions: Approximately every fifth patient with negative routine allergy tests in our department where NPT was performed during clinical routine fulfilled the criteria of local allergic rhinitis.

These results confirm the clinical relevance of LAR in patients with symptoms of allergic rhinitis and underline the importance of NPT in routine allergy diagnosis.

0186 | Probiotics-impregnated bedding covers in house dust mite allergic rhinitis patients: A double-blind, randomised, placebo-controlled, crossover clinical trial

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Introduction: House dust mites are one of the most relevant indoor allergy triggers, causing allergic rhinitis and allergic asthma in many patients worldwide. As current evidence for existing house dust mite avoidance measures is low, new methods are being developed.

Objectives: To explore the effect of a probiotics-based textile treatment on symptoms and quality of life of patients with allergic rhinitis to house dust mite.

Methods: A double-blind, randomised, placebo-controlled, crossover trial was conducted at Ghent University Hospital, Belgium. The pilot trial included 20 adult patients with allergic rhinitis to house dust mite. The trial consisted of an 8-week period with untreated (placebo) covers and an 8-week period with probiotics-impregnated covers in random order, with a washout period in between of at least four weeks. Der p 1 concentrations were measured in dust samples collected from mattresses and pillows. Symptoms and quality of life were assessed through self-reported questionnaires, including Visual Analogue Scales (VAS), Rhinoconjunctivitis Quality of Life Questionnaires (RQLQ), and Nocturnal Rhinoconjunctivitis Quality of Life Questionnaires (NRQLQ).

Results: There was a comparable and significant reduction of Der p 1 levels with both the probiotics-impregnated covers and the untreated covers. Several symptom and quality of life scores improved significantly with the probiotics-impregnated covers, whereas no significant changes were observed with the untreated covers. The effects of the probiotics-impregnated covers on symptoms and quality of life scores however were not significant compared to the placebo covers (except for a subscore *NRQLQ sleeptime)*.

Conclusions: This pilot study suggests that probiotics-impregnated bedding covers may improve symptoms and quality of life of patients with allergic rhinitis to house dust mite. A large-scale study is warranted to further investigate this promising probiotics-based method. *Prior to patient enrolment, the trial was registered at clinical.trials.gov (NCT01997606).*

SUNDAY, 18 JUNE 2017
OAS 07
IMMUNE DEFICIENCY AND AUTOIMMUNITY

0187 | PGM3-deficiency associated hyper IgE syndrome: from molecular basis to intervention approaches

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Introduction: Recurrent bacterial sinopulmonary and cutaneous infections associated to eczema and elevated serum levels of IgE are features of the hyper-IgE syndrome (HIES), a complex primary immunodeficiency disorder. Heterozygous dominant-negative STAT3 mutations are associated with autosomal dominant HIES and homozygous mutations in DOCK8 account for the large majority of autosomal recessive HIES.

Objectives: Herein, we report on the molecular basis of the novel PGM3-deficiency associated to a rare autosomal recessive HIES and the assessment of related intervention approaches.

Results: We identified in two consanguineous tunisian families with multiple affected individuals, who were wild type for STAT3 and DOCK8, two distinct homozygous mutations in Phosphoglucomutase 3 (PGM3). PGM3 catalyzes the reversible conversion of N-acetyl glucosamine 6P to N-acetyl glucosamine 1P required for biosynthesis of uridine diphosphate N-acetylglucosamine an essential precursor for protein glycosylation. PGM3 deficiency is likely to impair glycan mediated processes such as cell-cell recognition or immune signalling. Functional studies of the two mutations show that they lead to an aberrant glycosylation pattern in leucocytes with reduced (bi-), tri-, and tetra-antennary N-glycan branching in leucocytes from affected individuals. The mutations are one in-frame deletion and one amino acid substitution, both of them permit at least some expression and translation of the variant PGM3. Furthermore, these hypomorphic mutations did show dose-dependent increase of enzyme activity in vitro, so we hypothetized that supplementing with an excess of substrate might improve residual enzymatic activity in vivo and clinical condition of patients, at least partially. Two PGM3 deficiency patients suffering eczema with extremely severe itching were treated with GlucNAc supplement and results are discussed.

Conclusions: This is a novel and rare primary immunodeficiency due to a congenital disorder of glycosylation. The proof of principal to correct a glycosylation defect by supplementation, is available since e.g. individuals deficient in mannose-6-phosphate isomerase (Fru-6-P to Man-6-P conversion) lack sufficient Man-6-P for complete physiologic N-glycosylation and daily supplements of mannose can correct this glycosylation deficiency. In PGM3 deficient patients supplementation with excess enzyme substrate or by a compound that bypasses the block could be a therapeutic approach to improve, at least, their severe eczema.

0188 | Serum free light chains as a possible diagnostic tool in primary hypogammaglobulinemia: a multicentric study on 344 CVID patients

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Introduction: Serum free light chains (sFLC) are widely used as a prognostic marker in B cells lymphoproliferative disorders; in these conditions a raise of either kappa or lambda chain represents a marker of clonality. Furthermore, polyclonal increase in serum FLC has been identified in many autoimmune conditions as a marker of disease activity. Diagnosis of Common variable immunodeficiency (CVID) is an exclusion process. In particular, differential diagnosis between CVID and lymphoproliferative disorders or nonsecreting multiple myeloma may be challenging in adult patients. We recently suggested a role of sFLC in the diagnostic work-up of CVID, in a small single-center cohort of adult patients.

Objectives: sFLC levels were determined in 344 adult patients with CVID, enrolled in 5 different Italian referral centers for Primary Immunodeficiencies. B cell phenotype was available, according to EUROCLASS Study, of a subgroup of 120 patients. All CVID patients fulfilled the ESID-PAGID diagnostic criteria.

Results: Mean age was 51 (range 18-82); diagnostic delay was 10.6 years, on average. In 255 out of 344 patients (76.3%) a reduction of either kappa or lambda light chains or both was observed. 166 patients (49.7%) showed a decrease of both k and l chains; 82 patients (24.5%) presented a reduction of k chain only, with normal l levels; 7 patients (2.1%) presented a reduction of l chain only, with normal k levels. According to our previous study, we defined "$\kappa^-\lambda^+$" "$\kappa^+\lambda^-$" and "$\kappa^-\lambda^-$" as "CVID-like patterns". Interestingly, in a subgroup analysis of 120 subjects, 47.5% of patients with CVID-like pattern presented switched memory B cells %<2%, while the % of SmB cells was normal in 81% of normal FLC patients. Moreover, CD21lo cells resulted increased in 35% of CVID-like pattern patients vs 14.3% of normal sFLC patients.

Conclusions: A CVID-like sFLC pattern is present in more than 75% of our CVID cohort. This confirms our previous data and the promising diagnostic value of sFLC in the initial work-up of primary hypogammaglobulinemia. Finally, the subgroup analysis suggests the

need for further investigations on the relationship between a CVID-like pattern and the impairment of B cell function, in order to assess a putative prognostic role of sFLC determination.

0189 | Sustained natural killer cell expansion in virologically suppressed HIV+ individuals on antiretroviral therapy: a phenotypic study

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Introduction: The introduction of antiretroviral therapy (ART) has dramatically improved the course of HIV infection. The immune reconstitution following ART initiation is different in each HIV+ individual, according to several factors (1). Like T cells, Natural Killer (NK) cells undergo significant changes, with a redistribution of the different NK subpopulations according to disease stage (2). We noticed that almost 10% of our patients undergoing ART have increased numbers of circulating NK cells and this expansion is sustained over time, with no associated clinical feature.

Objectives: We analyzed the redistribution of NK cell subpopulations in HIV+ patients undergoing ART with stable expansion of NK cells (≥27%) and suppressed viral replication (HIV-RNA< 40 cp/ml) for 2 consecutive years (NK-exp); we then compared both clinical and laboratory features of NK-exp to age-matched HIV+ patients without sustained NK cells expansion (Non-exp) and a HIV-negative control group (CG). Flow cytometry analysis was performed on peripheral blood mononuclear cells with the following antibodies: CD3, CD56, CD57, CD16, CD158a and CD158b (KIRs), CD159a (NKG2A), CD159c (NKG2C), CD94.

Results: NK-exp showed a decreased number of circulating CD8+ T cells compared to Non-exp group (22.8 vs 40.9%, $P<.0005$) while CD4+ T cell counts showed no significant differences. A marked increase in $CD56^{dim}CD16+CD57+$ cells in the NK-Exp group was seen (62.7 vs 29.35%, $P<.05$), with increased expression of NKG2C (33.6 vs 13.2%, $P<.05$), KIRs (74.8 vs 47.6%, $P<.05$) and lower NKG2A/NKG2C ratio ($P=.03$) compared to Non-exp. Besides, other NK subpopulations like $CD56^{bright}CD16+/-$ cells were diminished in NK-exp compared to Non-Exp and CG groups (1.52 vs 5.2 vs 6.1%; $P<.05$). No differences between NK-exp and Non-exp groups were observed in either T CD4 + cell count nadir and disease duration.

Conclusions: In NK-Exp individuals there is a marked decrease in circulating CD8 + T cells, along with an increased number of mature $CD56^{dim}CD16+CD57+$ NK cells. These cells show a different activation status compared to age-matched individuals that have been linked to CMV replication control and immunosenescence (5). Since no significant differences in clinical history or disease severity could be observed between the two groups, we hypothesize a different NK immune reconstitution profile in a small subset of HIV+ patients. Further studies on $CD56^{dim}CD16+CD57+$ cells function in the NK-expanded group are needed.

0190 | Dysfunctions of neutrophilic granulocytes in immunocompromised children with viral coinfections: chronic herpes-viral and recurrent respiratory viral infections

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Introduction: Present data show the very important role of neutrophilic granulocytes (NGs) in the antiviral defense. At the same time it is known that viruses can affect different functions of NGs.

Objectives: We have studied 27 children (14 boys and 13 girls aged from 5 to 8 years old), suffering from chronic viral coinfections: herpes-viral infection (HVI) and recurrent acute respiratory viral infections (rARVI). The control group consisted of 20 healthy children. The cytoflowmetric method with mAbs panel to CD64, CD32, CD16, CD11b molecules on surface membrane of NGs was used. The phagocytic function and oxidative microbicidal activities (ROS) of NGs were tested. The PCR and the serological methods were used.

Results: All patients had clinical signs of immunodeficiencies (ID). The frequencies of acute episodes rARVI and chronic recurrent HSVI and HSVII were from 9 to 20 a year, they have also EBV, CMV, VHG6 infection. All patients suffered from mono-or mixed HVIs. Different disturbances of NGs were found: the phagocytic activity was decreased ($P<.001$). The processes of absorption, killing, digestion were impaired ($P<.05$). The levels of the spontaneous and induced activities of NADPH-oxidase were not changed ($P>.05$). The number of $CD64^{+}$NGs and its' MFI didn't change in ($P>.05$). The number of $CD16^{+}$NGs did not differ from control ($P>.05$). The density of expressed CD16 in 10 patients (group A) was decreased vs control ($P<.001$). In group B (15 patients) the density of expressed CD16 was increased (MFI) vs control, $P<.05$). The phagocytic, killing and digestive activity NGs in the group A was lower than in group B ($p1<.05$; $p2<.05$). There was significant decrease the number of $CD32^{+}$NGs in group C (9 patients) vs in control ($P<.001$). In group D (18 patients) it didn't change. MFI in group C and D didn't differ ($P>.05$), but in group C and D the levels of induced activities ROS were differed ($P<.05$). Patients of group C had high frequency of mixed HVIs—in 87.5% of cases with high levels of replication. The

number of CD11b$^+$NGs was as in the control group (P>.05). MFI CD11b$^+$NG was more higher in patients with viral coninfections than in healthy children (P<.01).

Conclusions: The studied immunocompromised children with viral coinfections (HVI, rARVI) had different affected functions of NGs in 96% of cases: the different remodeling of phenotype, impair of ROS activities, phagocytic disturbances.

0191 | Different serum cytokine profiles reflect anti-neutrophil cytoplasmic antibodies (ANCA)-specificity in patients with anca-associated vasculitis

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Introduction: Evidence supporting the classification of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) based on ANCA type is accumulating [1].

Objectives: To evaluate serum cytokine profiles in patients with AAV classified by ANCA type (proteinase 3 (PR3)-ANCA vs myeloperoxidase (MPO)-ANCA) or by clinical diagnosis (granulomatosis with polyangiitis (GPA) vs microscopic polyangiitis (MPA)) or by clinical phenotypes.

Methods: A panel of 30 cytokines was tested in patients with active AAV at inclusion in the Rituximab in ANCA-Associated Vasculitis (RAVE) trial as previously described [2-3]. We analyzed the association of levels of these cytokines with ANCA specificity, clinical diagnosis, and distinct clinicopathologic phenotype categories derived from the BVAS/WG items recorded at the time of enrollment (capillaritis, granulomatous manifestations, renal involvement and alveolar hemorrhage; new diagnosis and relapsing disease), as described [4].

Results: All cytokines tested except for RANTES, ACE, bFGF and VCAM-1 were significantly increased in the RAVE cohort when compared to healthy controls (P>.05). Within ANCA type, levels of 9 mediators were significantly higher in PR3-AAV (IL-6, NGFb, GM-CSF, IL-15, IL-18, IL-18Bb, sIL-2Ra, IL-8, TARC), compared to 5 different cytokines that were higher in MPO-AAV (sIL6R, NGAL, sICAM-1, VCAM-1, sTNFR II). In contrast, only 4 (GM-CSF, IL-15, IL-18, sIL-2Ra) cytokines were higher in GPA than MPA, and 1 (NGAL) was higher in MPA than GPA. The association of the majority of cytokines was stronger with ANCA specificity than with clinical diagnosis (ANCA type: association with $P \leq .001$ for 5 cytokines, association with $.001 \leq P \leq .01$ for 3 cytokines, and with $.01 \leq P \leq .05$ for other 6 cytokines. AAV diagnosis: association with $.01 \leq P \leq .05$ for 5 cytokines). Similarly, the defined clinical phenotypes were also not separated by cytokine signatures as clearly as the ANCA specificity was (*data not shown*).

Conclusions: Cytokine signatures separate patients more clearly by ANCA specificity than by clinical diagnosis, suggesting important differences in underlying pathophysiology and validating stratification of patients by ANCA specificity for treatment trials.

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0192 | Gestational age at birth and the risk of type 1 diabetes

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Introduction: Preterm birth may obscure immune system development and is e.g. associated with increased risk for infections and asthma. Type-1 diabetes (T1D), an autoimmune disease, may as well occur more often in those born at a lower gestational age. We aimed at studying this in young adults born in Finland, a country with a record high T1D prevalence.

Objectives: We assessed whether a shorter gestation increases the risk of T1D.

Results: We followed all 235 622 children born in Finland from Jan 1987 to Sep 1990. The national unique person identity number linked our data (Medical birth register: best estimated gestational age (GA), The Finnish Care Register for Health Care HILMO: ICD-9 and ICD-10 Diagnoses and dates of hospitalizations (1987-2015) and specialized care outpatient visits (1998-2015), Population Register: time of death and emigration, Statistics Finland: education). For adequate power, we ran Cox's regression analysis in six GA categories (Table).

A total of 2422 individuals were coded as T1D in HILMO during the total 6.3 million person years of follow-up. We observed no pooled effect of all preterm births (Table). However, early term birth categories with GA either 37 or 38 full weeks associated with a 13% to 35% increased hazard of developing T1D. Parental T1D clearly increased offspring's risk but left, also in model 3 with parental education, the effects of early term birth relatively unchanged.

Conclusions: In conclusion, those born early at term develop T1D more often. This may indicate that development towards autoimmunity may occur during fetal and perinatal periods. Infancy and childhood conditions, such as birth intervals and infections, may also play mediating roles.

	Model 1			Model 2			Model 3*		
	HR	95%	CI	HR	95%	CI	HR	95%	CI
Mother's Age (years)	1.00	0.99	1.01	1.00	0.99	1.01	1.00	0.99	1.01
Male Sex	1.23	1.14	1.34	1.24	1.14	1.34	1.24	1.14	1.34
Gestational age (weeks) Below 37, n=12 007	1.09	0.91	1.31	1.01	0.84	1.22	1.01	0.84	1.22
37, n=11 435	1.36	1.15	1.61	1.28	1.09	1.51	1.28	1.09	1.51
38, n=29 838	1.16	1.04	1.31	1.13	1.01	1.27	1.13	1.01	1.27
39 to 41, n=169 459	Ref			Ref			Ref		
42 or more, n=9688	0.91	0.74	1.13	0.92	0.74	1.14	0.92	0.74	1.14
Unknown, n=3040	1.12	0.79	1.58	1.08	0.76	1.52	1.08	0.77	1.53
Mother's T1D				4.29	3.42	5.39	4.29	3.42	5.38
Father's T1D				4.76	4.05	5.59	4.76	4.05	5.59

T1D, Type 1 Diabetes.
*Model 3 includes parental longest achieved education.

SUNDAY, 18 JUNE 2017
OAS 08
FOOD ALLERGY EPIDEMIOLOGY: A WORLD OF DIFFERENCE

0193 | Prevalence of childhood food allergy among older order mennonites (OOM) in Western New York, introduction of foods and breastfeeding

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Introduction: A growing number of studies, including ours in the OOM community in Western New York, have found that farm life is protective against the development of allergic rhinitis, atopic dermatitis, and asthma. OOM lifestyle includes living on farms, consumption of unpasteurized farm milk, home births and large families. However, there is little evidence of the farm effect being protective against food allergy (FA).

Objectives: A total of 500 surveys were distributed to OOM families. Surveys queried FA status of the children as adapted from the National Health and Nutrition Examination Survey (NHANES). Further phone contact determined if the child was also avoiding the food to assess the prevalence of likely or possible food allergy. Breast milk was collected from 41 OOM and 25 Rochester non-OOM mothers at 1-2 months, and IgA to foods measured by ELISA.

Results: A response rate was 30.8%. Among 644 OOM children, the rate of self-reported FA was 4.96% (95% CI, 3.10–6.83%), compared to NHANES 2007-2010 rate of 6.50% (95% CI, 5.70 - 7.30) (P=.4). When not counting the children who were reported to eat the suspected foods, the prevalence of likely/possible FA among OOM children was 2.67% (95% CI, 1.29 - 4.06), which was significantly lower compared to 5.89% (95% CI, 5.11 - 6.67) in NHANES (P=.0057). Among foods asked, OOM introduced peanut late, at average 21 months of age, whereas yogurt and kefir were introduced early at 7.1 and 8.3 months. At 6 months, 96% of OOM were still breastfeeding, compared to only 56% of New York State and 49% of the U.S. infants (CDC Breastfeeding Report Card 2014). OOM breast milk had higher levels of IgA1 to beta-lactoglobulin (P=.028) and ovalbumin (P=.016) than Rochester milk.

Conclusions: OOM have low rates of FA despite delayed introduction of highly allergenic foods such as peanut. Factors that may be protective against FA include long periods of breastfeeding, high specific IgA in breast milk and early introduction of yogurt and kefir, typically homemade. This study provides rationale for future mechanistic studies using OOM community as a model population with a low rate of FA.

0194 | Evaluation of the financial costs of healthcare services for people with peanut allergy vs matched controls in the United Kingdom

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Introduction: Allergies such as those to various foods are likely to result in increased costs of health care. Determination of this marginal cost allows for an appreciation of the potential cost saving of preventing the disease or condition, or by reducing its severity.

Objectives: Our aim was to evaluate the difference in resource use and the related financial costs of treating people with peanut allergy (PA) in the United Kingdom, compared with the costs of treating people who did not have a diagnosis of PA.

Results: Of 15 483 people with a diagnosis of PA, it was possible to match 13 609 cases in Cohort-1 and 9320 in Cohort-2.

The annual, mean cost of primary care contacts for cases and controls in Cohort-1 was: £288 per patient year (PPY) vs £183 PPY, respectively (D=£105, P<.001).

In Cohort-2, the costs were £263 vs £196 PPY, respectively (D=£67, P<.001).

The annual cost of hospital inpatient care for cases and controls in Cohort-1 was: £335 per patient year (PPY) vs £268 PPY, respectively (D=£67, P<.001).

In Cohort-2, the costs were £327 vs £207 PPY, respectively (D=£120, P<.001).

The annual, mean cost of drug prescribing for cases and controls in Cohort-1 was: £146 per patient year (PPY) vs £48 PPY, respectively (D=£98, P<.001).

In Cohort-2, the costs were £124 vs £53, respectively (D=£71, P<.001).

Conclusions: Albeit a conservative estimate, treating people with PA resulted in an annual, average increase in direct, financial costs to the health service of between £270 (»318) and £258 (»304) for those simple matched and atopy-matched respectively.

0196 | Prognosis of symptoms and IgE reactivity to cow's milk in Swedish children from early life to adolescence

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Introduction: Few studies have prospectively followed children with cow's milk-associated symptoms beyond school age, although tolerance is thought to occur by this age. We examined the prognosis of symptoms, including timing of onset, as well as repeated measures of Immunoglobulin E (IgE) reactivity to milk, focusing on gender differences from early life to adolescence in the BAMSE population-based birth cohort.

Objectives: This study included 2320 children with data on milk-associated symptoms in early life (to 4y), and at 8y and 16y. Children with parent-reported, doctor-diagnosed lactose intolerance (n=188) were excluded. Timing of onset was defined as persistent (early life and/or 8y and 16y), transient (early life and/or 8y only), or late onset (16y only). At 16y, information on symptoms involving the gastrointestinal, dermatological, respiratory and/or cardiovascular/neurological systems was collected. At this age, multiple symptoms were defined as involvement of two or more systems. Data on milk-specific IgE were available at 4, 8 and 16 y for children who were IgE positive (≥0.35 kU_A/l) to the food mix fx5 at the respective ages. Data were analysed using descriptive statistics, Chi-square, Fisher exact tests and t-tests.

Results: Milk-associated symptoms were most prevalent in early life (17.1%), but decreased at 8y (1.5%) and 16y (3.1%). Symptoms were equally distributed between genders until 16y, when more girls than boys were affected (4.0% vs 2.3%; *P*=.002). Regarding timing of onset, transient cases were most common (16.5%), followed by persistent (4.5%) and late-onset (3.0%). Compared to boys, late-onset symptoms were more common in girls (61/87; 70.1%), amongst whom the majority reported gastrointestinal symptoms (e.g. vomiting or stomach pain) as the only symptom. Multiple symptoms were common amongst persistent cases (24/34; 70.6%), but rare amongst late-onset cases (1/87; 1.1%). Sensitisation to milk-specific IgE at 16y was uncommon in all groups, although most prevalent among persistent cases (6/31; 19.4%), followed by transient (16/416; 3.9%) and late-onset (1/62; 1.6%) cases.

Conclusions: Cow's milk-associated symptoms affect nearly 20% of children in early life. Many outgrow their symptoms. However, adolescent girls disproportionately dominate late-onset cases, who have only gastrointestinal symptoms and are not sensitised to milk. Our study gleans insights into the prognosis of milk-associated symptoms in a population-based birth cohort.

0197 | Lipid transfer protein allergy in UK adults—characterisation and comparison to adults with pollen-food syndrome

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Introduction: In the UK manifestations of fruit and vegetable allergy are usually triggered by cross-reactions between pollen antibodies and PR-10 plant food allergens, known as pollen-food syndrome (PFS). Non-Specific Lipid Transfer Protein (LTP) allergy, a more severe type of plant food allergy seen mainly in Mediterranean countries, has been shown to exist in Northern Europe, but it is not clear how similar it is to PFS in terms of presentation.

Objectives: To compare the symptoms, trigger foods, skin prick tests, sensitisation to aeroallergen and food components and quality of life of subjects with LTP allergy, and those with diagnosed PFS.

Results: A total of 50 subjects were recruited; 35 subjects (80% female, mean age 37 years) born and residing in the UK, with a positive Pru p 3 and diagnosis of LTP allergy, and 15 with diagnosed PFS (60% female, mean age 34 years). All subjects completed general allergy questionnaires and the validated Food Allergy Quality of Life Questionnaire - Adult Form (FAQLQ-AF). They also underwent skin prick testing (SPT; ALK Abello) to a panel of aeroallergens and foods and had a serum sample analysed using the ISAC microarray (ThermoFisher). There were no differences in atopic history or foods reported to cause reactions, although the PFS group were more likely to report reactions to raw fruits and vegetables (*P*<.001). Those with LTP allergy also reported significantly more severe symptoms, were more likely to carry adrenaline and have had an emergency hospital visit than those with PFS (*P*<.001). A positive SPT to peach, walnut, barley, mustard, cabbage, raspberry and lettuce occurred more frequently (*P*<.001) in LTP allergic subjects, who were also more likely to be sensitised to the LTP allergens in mugworth (Art v 3), plane tree (Pla a 3), peach (Pru p 3), peanut (Ara h 9), hazelnut (Cor a 8) and walnut (Jug r 3) (*P*<.001). Having LTP allergy also had a greater impact on most domains on the FAQLQ-AF.

Conclusions: LTP allergy in the UK manifests with severe symptoms and affects quality of life. Whilst no key trigger foods could be identified to differentiate between the two types of plant food allergy, those with PFS were more likely to react to raw plant foods only. Skin prick testing to LTP-enriched peach reagent was universally negative in the PFS group, and together with other foods provides a robust diagnostic tool should testing to LTP component allergens not be available.

0198 | Prevalence of food allergy in Vietnam: the first population-based study

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Introduction: There has been an increase in the number of people suffering from food allergy in Vietnam. Although numerous studies have investigated the prevalence of food allergy in general Asian populations, none have been conducted in Vietnam.

Objectives: We conducted a nationwide, cross-sectional, population-based study to evaluate the current prevalence of food allergy and the distribution of offending food groups in this populous country. The survey was implemented in 5 different provinces across Vietnam and targeted towards two different age groups: children of age 2-6 years old from local kindergartens and university students, aged from 16 years old (adult group).

Results: A total of 17659 responses were collected from 8127 children and 9532 adult participants. The average response rate in the two groups was 69.9% (children: 77.9%; adults: 66.2%). There were more female participants (59.3%) answering the questionnaires than male ones (40.7%). There were 47.3% participants reported adverse reactions due to food consumption, but only 12.4% sought medical services for diagnosis. In 1477 (8.4%) doctor-confirmed food allergy participants, crustacean (55.3%), fish (25.4%) and molluscs (20.3%) were the leading causes of food allergy, followed by beef (12.4%) and milk (10.7%). Hives was the most typical clinical symptom in food allergic patients in this study, after nausea and diarrhea. Adult participants demonstrated a higher risk of allergy to beef (17%) as compared to children (3.6%). In contrast, allergy to egg was more frequent in children (12.2%) than adults (7.7%).

Conclusions: This is the first population-based study ever conducted on food allergy in Vietnam. The prevalence of food allergy in Vietnam from this study is about 5.9%. We discovered that seafood is the predominant cause of food allergy while peanut and tree nut allergy are much less frequent. Furthermore, the occurrence of beef allergy is surprisingly high. Food allergy was shown to manifest differently in different age groups in this population.

SUNDAY, 18 JUNE 2017
OAS 09
ENVIRONMENT AND ALLERGIC DISEASES IN CHILDREN

0199 | Development of atopic sensitization in Finnish and Estonian children—a latent class analysis in a multicenter cohort

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Introduction: The prevalence of atopy is associated with a Westernized lifestyle as illustrated by the Karelia studies, which compared two neighboring regions with different socioeconomic backgrounds. However, the distribution of the underlying atopy phenotypes and their association with environmental factors and disease development remain unclear.

Objectives: To define phenotypes of atopic sensitization in early childhood and to examine their association with allergic diseases in Finland and Estonia.

Methods: The analysis included 1603 Finnish and 1657 Estonian children from the DIABIMMUNE multicenter young children cohort. Specific IgE levels were measured at age 3, 4 and 5 respectively, and categorized into three CAP classes. Latent Class Analysis (LCA) was performed with the statistic software package poLCA in R.

Results: Both populations differed in terms of socioeconomic status, environmental determinants, and prevalence of allergic diseases. Nevertheless, we found similar latent classes (LC) in both populations: an unsensitized LC, a food LC, two inhalant LCs, differentiating between seasonal and perennial aero-allergens, and a severe atopy class. The latter was characterized by high total and sIgE levels and strongest associated with wheeze (odds ratio 5.64 [3.07-10.52] and 4.56 [2.35-8.52]), allergic rhinitis (22.4 [11.67-44.54] and 13.97 [7.33-26.4]) and atopic eczema (9.39 [4.9-19.3] and 9.5 [5.2-17.5], for Finland and Estonia, respectively). The seasonal inhalant class was additionally related to genetic risk of type 1 diabetes in Estonia (2.5 [1.2-5.1]) as determined by HLA-DQ genotypes.

Conclusions: LCA revealed similar patterns of atopic sensitization in countries with different environmental determinants. Atopic disease was related to a different type of atopy than the genetic risk of type 1 diabetes.

0200 | Changes in sensitization pattern in allergic brazilian children: first results from 2 cross-sectional studies 12 years apart (proal I & II)

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Introduction: Allergic sensitization is our initial clue to link clinical reactivity to etiology. Establishing this relation, clinical practice is started. The first Brazilian Project of Allergy (PROAL I) was established in 2004 in order to assess the prevalence of sensitization of the main allergens among Brazilian allergic children. This knowledge allowed us to evaluate the best management practices for the allergic diseases of our country. Primary, secondary and tertiary prevention had been developed more effectively.

Objectives: After a 12-year interval, a new assessment was conducted to estimate the prevalence trends (PROAL II). This time, with advancement of technology, including molecular and structural biology through the availability of allergen molecules. Eleven Brazilian Pediatric Allergy centers enrolled 470 individuals (mean - 40 children by center, randomly selected; age range 6 m-18y). Identical design, diagnostic criteria and in-vitro analytical methods were used in both surveys (ImmunoCAP®, positive >0.35 kUA/l).

Results: The samples were similar for number, gender and age in the two surveys. There were significant increase in sensitization between the studies (PROAL I vs PROAL II) for cat (12.3% vs 29.9%), peanut (14.7% vs 20.5%), corn (10.9% vs 16.9%), milk (20.4% vs 31.9%), fungi (3.1% vs 13%), grass (10.7% vs 22.6%) dog (8.1% vs 40.3%), cow (11.4% vs 29.9%) and horse (4.6% vs 13.2%) ($P<.05$). No increase was seen for *D. pteronyssinus* (67.8% vs 63.9%); *D. farinae* (66.5% vs 63.9%); *B. tropicalis* (57.1% vs 57.4%); *P. americana* (34.4% vs 31.2%); egg (24.5% vs 29.4%); wheat (20.1% vs 23.4%) or soy (12.3% vs 15.8%).

Conclusions: Sensitization to peanut and furry animals but not to mites, increased over 12 years' time in our multicenter, cross-sectional study in Brazil. Life style changes in dietary habits and indoor exposure to pet might have contributed to these changes. The

knowledge about the major allergens involved in the sensitization of allergic patients is the first step to achieve a precision treatment for them.

0201 | Indoor microbiota and the development of asthma by age 10.5 years: a birth cohort study

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Introduction: Early-life indoor microbial exposure, and lately bacterial richness, has been associated with the risk of asthma development but the roles of specific microbial genera and communities are poorly understood.

Objectives: To identify individual early-life bacterial/archaeal or fungal genera in indoor microbiota which are associated with the development of asthma.

Results: Dust samples from 395 living rooms were collected at 2 months of age. Illumina MiSeq sequencing of bacterial/archaeal and fungal DNA amplicons were performed. Children were followed up until the age of 10.5 years.

Low bacterial/archaeal richness and low amount of house dust were associated with increased risk of developing asthma. The first two axis scores of principal coordinate analyses of the weighted bacterial/archaeal beta-diversity matrix were inversely associated with asthma. The first axis mainly represented the relative abundance of *Firmicutes* and *Proteobacteria*, and the second *Actinobacteria* abundance and diversity. Among the most abundant 139 genera, 41 bacterial genera correlated ($r>|.4|$) with either axis score. Twelve genera were inversely ($P<0.10$) and *Lactococcus* genus (aOR 1.36, 95% CI 1.13-1.63 per IQR change, $P=.001$) positively associated with the risk of asthma. Only the association with the *Lactococcus* genus was independent of the other taxa, and also independent of the bacterial/archaeal richness, and the amount of dust. The *Lactococcus* finding was also emphasized when using an alternative statistical approach ((Multivariate Association with Linear Models (MaAsLin)). No association with asthma development was found with between fungal diversity and only weak associations were observed with fungal genera.

Conclusions: Our data suggest that the risk of childhood asthma is affected by bacterial composition of the early-life home dust microbiota while fungal microbiota has less impact. The asthma risk was positively associated with relative abundance of *Lactococcus* genus.

0202 | Differences in the immune profiles and asthma risk in urban and rural children

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Introduction: Asthma is common affecting children and environmental exposures may alter the risk of asthma development. Many studies have shown that rural environment has a protective effect against the development of asthma and allergies but the exact protective mechanisms are not clear.

Objectives: To characterize the differences in the immune profiles of urban and rural school children.

Results: In an initial screening of 18 000 children aged 4-8 years from Hong Kong (urban) and County of Conghua (rural southern China), asthma and allergies is 2-3 times more prevalent in Hong Kong than in rural Conghua. We then proceed to conduct a case-control study among 146 subjects from Hong Kong (47 controls vs 32 cases) and Conghua (47 controls vs 20 cases) to determine their immune profiles. Blood samples were collected for regulatory T (Treg) cell count, gene expression and cytokine studies. The adaptive stimuli induced ex vivo cytokine production, with more remarkable amplitude in Conghua controls and cases compared with that in their Hong Kong counterparts. Significantly higher concentrations of Th1-related cytokines such as IFN-γ, IL-1β and TNF-α were detected in samples from the Conghua group ($P<.01$). The percentage of Treg cells was higher in unstimulated PBMC of the Hong Kong group. Moreover, Treg cell markers including FOXP3, GITR and CTLA4 were expressed at much higher transcription levels in Conghua controls as compared with Hong Kong controls (by fold change, $P<.05$). Gene expression of Th17 marker IL-23R was significantly different between Hong Kong cases and Conghua cases (by fold change, $P<.0001$), a similar pattern also seen in Conghua controls vs Conghua cases.

Conclusions: Environmental exposures in Conghua potentially affect cytokine secretion and gene expression of Treg cell and Th17 markers through adaptive stimulation. The very low prevalence of asthma in Conghua children may be partially explained by a propensity to Th1 immunity and increased functional capacity of Treg cells.

0203 | Rural-urban differences in the prevalence and comorbidity of asthma and allergic disorders in Belarus and Poland.

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Introduction: Many children with asthma suffer from additional allergic diseases. It is not clear if the prevalence of allergic disease among children with asthma is consistent between locations. This knowledge could help understand phenotype patterns which may impact management.

Objectives: The aim of the analysis was to compare the prevalence of asthma and allergic conditions among children and their associations with living in rural and urban area.

This was an international, cross-sectional study of randomly selected children aged 6-14 years living in urban and rural areas of Grodno (Belarus) and Silesia Region (Poland).

Physician-diagnosed asthma as well allergic diseases and symptoms were ascertained using the ISAAC questionnaire completed by the parents.

Results: A total of 8055 children aged 6-14 years participated in the study (response rate=76.87%). In the rural and urban areas, asthma prevalence in Belarus was 1.4% and 1.5%, respectively while in Poland it was 3.5% and 4.1%. Any allergy was lower ($P<.05$) in Belarus than Poland [Belarus rural: 10.7%, urban: 17.7% ($P=<.05$); Poland rural: 16.1%, urban: 24.3% ($P<.05$)]. Any allergy present in children with asthma in rural and urban areas was 41.4% and 80.0% in Belarus ($P<.05$) and 48.3% and 66.2% in Poland ($P<.05$). After controlling for age and gender, among children with asthma, rural living was associated with a reduced risk for having any allergic condition (Belarus: OR=0.18, 95% CI=0.06-0.55; Poland: OR=0.52, 95% CI=0.27-0.94). Such differences in prevalence ($P<.05$) and similar associations (ORs) were observed between each individual allergic condition and living area in children with asthma.

Conclusions: Our findings revealed that significant differences in comorbidity of asthma and allergic conditions between Belarus and Poland as well as between rural and urban areas. Allergic conditions are less frequent and are less strongly associated with asthma in children living in rural as in urban area.

0204 | The association between asthma and type 1 diabetes in children and adolescents—a case-cohort study in Finland

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Introduction: Based on Th1/Th2-paradigm, the association between allergic diseases and type 1 diabetes, two immune-mediated diseases, has been suggested to be inverse. However, evidence from observational studies is inconsistent: inverse, direct and null associations have been reported.

Objectives: The aim of the present study was to assess the association between asthma and type 1 diabetes in Finnish children and adolescents taking into account the time sequence of disease diagnoses and potential confounders in a large case-cohort study. Among the initial cohort of all children born between 1.1.1981-31.12.2008, those who were diagnosed with asthma (n=81473) or type 1 diabetes (n=9541) up to 16 years of age by the end of 2009 were identified from the Central Drug Register maintained by the Social Insurance Institution of Finland. A 10% random sample from each birth year cohort was selected as a reference cohort (n=171138). The association between asthma and type 1 diabetes were studied using multistate modelling approach to estimate transition rates between healthy and disease states, asthma and type 1 diabetes. As the rates for a disease were modified similarly at different ages by the appearance of the other disease, a single hazard ratio with 95% confidence intervals (CI's) was calculated to represent the average change in transition rate between the disease states.

Results: Altogether 80871 children with only asthma, 8939 children with only type 1 diabetes, and 602 with both diseases were identified. After adjusting for sex and birth decade, prior diagnosis of asthma increased the risk of subsequent type 1 diabetes on average by 41% (95% CI's 1.28-1.54), while prior diagnosis of type 1 diabetes decreased the risk of subsequent asthma on average by 18% (95% CI's 0.69-0.98).

Conclusions: Results from the present study's novel approach to the association between asthma and type 1 diabetes indicate that the direction of the association between asthma and type 1 diabetes may depend on the sequential occurrence of the diseases. Thus, the association between the diseases is complex, not just inverse. Additional studies that take into account the sequential appearance of the diseases are warranted to further clarify the association between asthma, other allergic diseases and type 1 diabetes. Elucidation of biological mechanisms underpinning the association between asthma and type 1 diabetes may give further insights into pathogenesis of both of these diseases.

SUNDAY, 18 JUNE 2017
OAS 10
DRUG ALLERGY: FROM PATHOMECHANISM TO MANAGEMENT

0007 | Elevated expression of Ikaros Family Zinc Finger 1 (IKZF1) exacerbates mucocutaneous inflammation

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Introduction: Stevens-Johnson syndrome (SJS) involves an acute inflammatory vesiculobullous reaction of the skin and mucosa present on the ocular surface, in the oral cavity, and in genitals. In patients with extensive skin detachment and a poor prognosis, the condition is known as toxic epidermal necrolysis (TEN). About 40% of SJS/TEN patients diagnosed by dermatologists develop severe ocular complications (SOC). Cold medicines including multi-ingredient cold medications and nonsteroidal anti-inflammatory drugs (NSAIDs) were implicated in SJS/TEN with SOC and about 80% of our patients developed SJS/TEN with SOC after taking cold medicines a few days before disease onset (CM-SJS/TEN with SOC). Our genome-wide association study documented an association between CM-SJS/TEN with SOC and Ikaros Family Zinc Finger 1 (*IKZF1*). Few studies examined biological and pathological functions of *IKZF1* in mucosal immunity. We hypothesized that IKZF1 contributes to the mucocutaneous inflammation of CM-SJS/TEN with SOC.

Objectives: To investigate the function of IKZF1 in mucocutaneous inflammation. Human skin and conjunctival tissues were obtained for immunohistological studies. Primary human conjunctival epithelial cells (PHCjECs) and adult human epidermal keratinocytes (HEKa) also used for gene expression analysis. We also generated K5-Ikzf1-EGFP transgenic mice (Ikzf1 Tg) by introducing the Ik1 isoform into cells expressing keratin 5, which is expressed in epithelial tissues such as the epidermis and conjunctiva, and then examined them histologically. Moreover, Ikzf1 Tg were induced allergic contact dermatitis.

Results: We found that human epidermis and conjunctival epithelium expressed IKZF1, and in PHCjECs and HEKa, the expression of IKZF1 mRNA was up-regulated by stimulation with polyI:C, a TLR3 ligand. In Ikzf1 Tg, we observed dermatitis and mucosal inflammation including the ocular surface. In contact dermatitis model, inflammatory infiltrates in the skin of Ikzf1 Tg were significantly increased compared with wild type.

Conclusions: Our findings support the hypothesis that Ikaros might participate in mucocutaneous inflammation.

0008 | Genetic polymorphisms of costimulatory molecules and maculopapular eruption induced by antituberculosis drugs

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Introduction: Maculopapular eruption (MPE) induced by antituberculosis drugs (ATD) is the most frequent adverse reactions requiring the discontinuation of the scheduled treatment. Genetic susceptibility to ATD-induced MPE is not well determined yet, although CYP2C19 and CYP2C9 genetic polymorphisms were reported to be significantly associated with the risk of developing ATD-induced MPE. Since costimulatory molecules play crucial roles in the activation of lymphocytes, we examined if the polymorphisms in costimulatory molecules (CD28, CTLA-4, CD40, and CD40L) are associated with ATD-induced MPE.

Objectives: We enrolled 72 patients with ATD-induced MPE and 238 ATD-tolerant subjects who were treated with the first line ATDs including isoniazid, rifampicin, ethambutol and pyrazinamide. After enrollment, DNA was isolated from whole blood of the subjects and genotyped for the single nucleotide polymorphisms (SNPs) in *CD28*, *CTLA4*, *CD40 and CD40LG*. Genotype frequencies of SNPs and haplotypes were compared between patients with ATD-induced MPE and ATD-tolerant patients.

Results: In the comparisons of genotype frequencies of SNPs of *CD28* (rs3116496), *CTLA4* (rs5742909, rs231775, rs3087243, rs17268364), *CD40* (rs1800686, rs1883832) and *CD40LG* (rs3092952), there was no significant difference between the patients with ATD-induced asthma and ATD-tolerant controls. Next, the haplotypes frequencies of *CTLA4* and *CD40LG* genes were not different between case and control groups.

Conclusions: Genetic polymorphisms of costimulatory molecules (CD28, CTLA-4, CD40, and CD40L) were not associated with ATD-induced MPE. These findings suggest that genetic variations of costimulatory molecules do not confer susceptibility to ATD-induced MPE.

0009 | Biotinylated clavulanic acid as a tool for identifying serum proteins target of haptenation by clavulanic acid in the context of allergy studies

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Introduction: Clavulanic acid (CLV) is a betalactam (BL) which inhibits betalactamases activity and is frequently administered combined with amoxicillin (AX). Both BLs can be independently involved in allergic reactions. Indeed, selective immediate allergic reactions to CLV have recently been reported in 30% of patients allergic to AX-CLV combination. Protein haptenation with BLs is known to be necessary to activate the immune system and currently there are no tools, such as a monoclonal antibody against CLV, which allows the study of protein haptenation with CLV.

Objectives: The objective was to study haptenation by CLV and develop tools to identify CLV target proteins. To achieve this aim, CLV was derivatized with biotin, then incubated with either human serum albumin (HSA) or sera and, finally, the resulting protein adducts were analyzed by different techniques.

Results: Biotinylated CLV (CLV-B) was synthesized by means of introducing an oxygenated linker between CLV and a biotin moiety.

HSA was incubated with increasing concentrations of CLV or CLV-B. The resulting conjugates were purified by filtration and characterized by MALDI-TOF MS. Data showed that HSA was modified by both CLV and CLV-B (Dm=279.8 and Dm=543.0 respectively, compared with HSA control). The modification was proportional to the amount of CLV or CLV-B used during incubation. Results were in agreement with the grade of biotinylation in HSA-CLV-B, determined by electrophoresis using biotinylated BSA as calibrator.

Sera were incubated with CLV-B and proteins separated by 2D electrophoresis followed by HRP-streptavidin detection. This showed spots corresponding to HSA, transferrin and heavy and light chains of immunoglobulins as candidate targets of CLV-B haptenation.

Competition experiments between CLV and CLV-B for HSA haptenation were performed by HSA incubation with CLV-B, after a preincubation with increasing concentrations of CLV. Aliquots of the incubation were subjected to SDS-PAGE and transferred to a PVDF membrane. The incorporation of CLV-B, assessed by detection with HRP-streptavidin, showed that pre-incubation of HSA with an excess of CLV moderately reduced the incorporation of CLV-B.

Conclusions: Our results show that CLV-B could be a valuable tool for the identification of CLV targets with high sensitivity. The elucidation and comparison of CLV and CLV-B reactivity during conjugation deserve further study to finally understand the activation of the immune system by CLV.

0010 | Serum procalcitonin for differentiating dress syndrome from bacterial infection

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Introduction: Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome is a serious drug-induced adverse reaction. The major clinical manifestations are fever, skin rash, and the involvement of several internal organs, which are non-specific and observed in the course of bacterial infection. Procalcitonin (PCT) level determination has been reported to be more useful markers for bacterial infection than C-reactive protein (CRP), and for differentiating bacterial infection from non-infective causes of inflammation such as autoimmune disease and drug hypersensitivity.

Objectives: We investigated to assess the usefulness of serum PCT levels in differentiating DRESS from bacterial infection. Eighty DRESS syndrome patients who were admitted at Dong-A university hospital and 328 bacterial infection patients including 202 sepsis patients were collected by retrospectively. Serum PCT, CRP were measured, and the highest levels of each markers during the disease course were compared.

Results: The PCT and CRP levels were significantly higher in bacterial infection and sepsis than DRESS syndrome (PCT, 39.25±57.82 ng/ml vs 51.93±65.57 ng/ml vs 1.17 + 2.12 ng/ml, $P<.05$; CRP, 19.88±9.78 mg/dl vs 21.0±9.09 mg/dl vs 6.91±9.85 mg/dl, $P<.05$). The area under the receiver operating characteristics curve for PCT was higher than that for CRP (0.926, 95% CI 0.894-0.958 vs 0.898, 95% CI 0.849-0.947) in differentiating DRESS syndrome from sepsis. The best cutoff value for PCT was 1.16 ng/ml (sensitivity 90.2%, specificity 71.9%), and for CRP 10.04 mg/dl (sensitivity 87.7%, specificity 78.1%). In the DRESS patients, the PCT levels were the most highest in nonsteroidal anti-inflammatory drugs, followed by allopurinol and antibiotics.

Conclusions: Serum PCT may be more useful than serum CRP for differentiating DRESS syndrome from sepsis (severe bacterial infection).

0011 | Desensitization for drug hypersensitivity (allergen immunotherapy-like)

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Introduction: Drug hypersensitivity reactions (DHRs) affect about 7% of the population. Nearly 15% of them have an underlying adaptive immunological mechanism, ranging from immediate to delayed hypersensitivity. Importantly, DHRs often imply the treatment withdrawal, which represents a major issue, particularly if the therapeutic compound is either life-saving or irreplaceable. In the case of DHR, rapid desensitization has proven to be effective in preventing treatment discontinuation. Indeed, rapid desensitization induces tolerance through the slow infusion of the offending drug, intravenously at increasing doses. Yet, tolerance is not sustained. Thus, a permanent modification of the administration scheme is required.

Objectives: We propose a novel approach of desensitization for DHR that can be called allergen immunotherapy-like desensitization (AILD).

Results: *Rituximab*: All patients completed the AILD without major adverse reactions. 6 patients (11%) experienced local immediate reactions (itchy wheals, diameter <10 cm) at the site of the injection. 28 patients (52%) developed late local reactions at the site of injection (erythema and oedema). After AILD, 40 patients (76.9%) received the planned dose of Rituximab with the previous treatment administration scheme, without adverse reactions. Only 7 patients (13%) cannot be readmitted to normal Rituximab treatment (desensitization failure).

Cytarabine: Both patients completed the AILD. No major adverse reactions were observed during the desensitization course. Only one patient developed modest transient infiltrated nodules at the site of injection.

After AILD, both patients received the planned dose of cytarabine with the previous treatment administration scheme, without adverse reactions.

Conclusions: AILD is effective, safe and can be pursued for both immediate and delayed DHRs. AILD prevents the treatment withdrawal and, importantly, provides sustained tolerance allowing treatment resumption.

0012 | Sixteen day desensitization protocol with oral chemotherapeutics for non-immediate hypersensitivity reactions: experience of a single centre

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Introduction: Although desensitization in immediate type hypersensitivity reactions due to chemotherapeutics is well described and standardized for many drugs, common protocols in delayed type hypersensitivity reactions are not standardized.

Objectives: To evaluate the effectiveness of the previously published slow desensitization protocol in the nonimmediate type hypersensitivity reactions (HR) induced by oral chemotherapeutics (OCs).

Method: Using our previously published slow desensitization protocol with capecitabine, we desensitized the patients who gave non-immediate hypersensitivity reactions to different OCs. The protocol was started with the 1/100 of the culprit drug; then the doses increased slowly in order to complete the desensitization in 16 days. Demographic and clinical features of the patients were appraised.

Results: Six patients (mean age was 54.5±15.75 years, 4 female) were enrolled. The culprit drugs were lenalidomide (n=3), pazopanib (n=1), nilotinib (n=1) and temozolomide (n=1). The types of HR were maculopapular eruption (MPE, n=5) and eczematous rush (n=1) and the mean reaction time was 6.6±2.1 days. The desensitization was successfully completed in 16 days in 2 patients. In 4 patients MPE developed in an average of 11.25±3.3 days. In three of them desensitization was completed with a more slowly dose incrementation and the targeted doses were achieved in the average of 29±5.5 days. However in one patient, only ¾ of targeted nilotinib dose could be given. Because this amount was under the therapeutic goal, treatment plan was changed.

Conclusions: Although 16-day desensitization with OC can work in some patients, longer periods can be necessary in some others. Desensitization plans should be tailored for each patient.

SUNDAY, 18 JUNE 2017
OAS 11
LYMPHOCYTES

0014 | Th22 cells form a distinct th lineage from Th17 cells in vitro with unique transcriptional properties and Tbet-dependent Th1 plasticity

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Introduction: Interleukin (IL)-22 is a member of the IL-10 family of cytokines and is expressed in mucosal tissues during microbial infections and chronic inflammatory diseases (e.g. asthma and COPD). Th22 cells are a major source of IL-22 and have been identified in inflammatory infiltrates in patients suffering from asthma, cystic fibrosis, COPD, and RSV infection. However, their molecular characteristics and functional roles remain largely unknown because of our inability to generate and isolate pure populations.

Objectives: To develop a novel Th22 differentiation assay and to generate dual IL-22/IL-17A reporter mice to isolate and compare pure populations of cultured Th22 and Th17 cells. To further determine the transcriptional regulation and plasticity of cultured Th22 cells and lastly, to validate our findings *in vivo*.

Results: Using dual IL-22/IL-17A reporter mice we have identified a novel differentiation assay to generate large numbers of Th22 cells *in vitro*. Transcriptional profiling and *Il17a* fate-mapping provide evidence that Th22 cells have never expressed IL-17A, suggesting that they are potentially a distinct cell lineage from Th17 cells under *in vitro* culture conditions. Interestingly, Th22 cells also expressed granzymes, IL-13, and increased levels of Tbet. Using transcription factor-deficient cells, we demonstrate that RORγt and Tbet act as positive and negative regulators of Th22 differentiation respectively. Furthermore, under Th1 culture conditions *in vitro*, as well as in an IFN-γ-rich inflammatory environment *in vivo*, Th22 cells displayed marked plasticity toward IFN-γ production. Th22 cells also displayed plasticity under Th2 conditions *in vitro* by upregulating IL-13 expression. Finally, transcriptional comparison with Th22 cells purified from the lungs of infected dual IL-22/IL-17A reporter mice confirms our *in vitro* findings.

Conclusions: Our work has identified conditions to generate and characterize Th22 cells *in vitro*. Further, it provides evidence that Th22 cells develop independently of the Th17 lineage, while demonstrating plasticity toward both Th1- and Th2-type cells.

0015 | The cannabinoid receptors agonist WIN55,212-2 promotes anti-inflammatory responses in dendritic cells: implications for allergic diseases

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Introduction: The endocannabinoid system has been shown to play different functions in the context of allergic diseases. In humans, the mRNA expression levels of the cannabinoid receptor 1 (CB1) were upregulated in allergic patients, but the functional significance of such observation remains elusive. Human dendritic cells (DCs) express CB1 and cannabinoid receptor 2 (CB2), but their actual role in allergy and the underlying immunological mechanisms are not fully understood.

Objectives: To study the role played by cannabinoids receptors in the regulation of human DCs as potential therapeutic targets for immunotherapy of allergic diseases.

Results: We showed that human monocyte-derived DCs (hmoDCs) express CB1. The CB1/CB2 agonist WIN55,212-2 down-regulates the expression of HLA-DR, CD86 and CD83 as well as the production of IL-6, IL-1β, IL-8, and TNF-α in hmoDCs stimulated with LPS without altering cell viability. Pharmacological inhibition experiments with specific antagonists for CB1 and CB2 demonstrated that both CB1 and CB2 contributed to this inhibition. Similarly, WIN55,212-2 inhibits cAMP levels induced by LPS in hmoDCs. Human DCs activated in the presence of WIN55,212-2 promoted the generation of IL-10-producing T cells. Initial experiments demonstrated that PI3K/Akt/mTOR and NF-κB signalling pathways might well be involved in the mechanisms by which WIN55,212-2 induces anti-inflammatory responses. Interestingly, our data with a specific fluorescent chemical probe revealed that both human myeloid and plasmacytoid DCs from peripheral blood and tonsils express significant levels of CB1 on the cell membrane.

Conclusions: The synthetic cannabinoid WIN55,212-2 promotes the generation of tolerogenic human DCs with potential anti-inflammatory properties. These findings might well have important implications for future therapeutic strategies not only in allergy but also in other immune tolerance related diseases such as autoimmunity, cancer, organ transplantation rejection or chronic infections.

0016 | Circulating CCR10+ ILC2 frequencies and CCL27 plasma concentrations reflect asthma severity

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Introduction: We previously showed that patients with severe allergic asthma have higher numbers of ILC2s expressing the chemokine receptor CCR10 in the blood compared to allergic subjects without asthma or healthy controls.

Objectives: To confirm and extend these results, we analyzed circulating $CCR10^+$ ILC2s by flow cytometry not only in allergic but also in non-allergic asthmatic patients (n=42). Phenotypic and functional properties of human $CCR10^+$ and $CCR10^-$ ILC2s were further characterized by multiparametric flow cytometry and intracytoplasmic staining of cytokines. The role of $CCR10^+$ ILC2s in asthma pathophysiology was addressed in a mouse model of allergic asthma induced by birch pollen.

Results: $CCR10^+$ ILC2s are significantly enriched in the blood of both allergic and non-allergic severe asthmatic patients. These cells are less activated than their $CCR10^-$ counterpart, with a lower expression of CD25 and KLRG1 and a higher expression of CD27. This subset exhibits ILC1-like properties including a capacity to produce IFN-y. Surprisingly, the depletion of lung $CCR10^+$ ILC2s by anti-CCL27 and anti-CCL28 antibodies exacerbates the airway hyperreactivity of mice challenged with birch pollen. We confirmed that $CCR10^+$ ILC2s are present in human lung samples. In agreement with the aforementioned results, plasma concentrations of the CCR10 ligand CCL27 are also significantly increased in severe asthmatics compared to non-asthmatic individuals.

Conclusions: Frequencies of circulating $CCR10^+$ ILC2s and CCL27 plasma concentrations are increased in relationship with asthma severity, independently from the allergic status of the patients. The functional analysis of $CCR10^+$ ILC2s in human and mice suggests that these cells could have a beneficial role in asthma control.

0017 | Type 2 ILC2 in the blood of asthma and copd patients share characteristics of tissue resident cells

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Introduction: In recent years the importance of innate lymphoid cells (ILCs) in the regulation of immune responses has become evident. These cells are enriched at barrier surfaces and can rapidly respond to pathogens by producing cytokines and interacting with other cell types. Murine studies have defined ILCs as tissue resident cells that exert their function locally. However, these cells are also present in the circulation and elevated numbers of ILC2s have been found in the blood of asthma patients.

Objectives: We compared ILC2s from nasal polyps and peripheral blood phenotypically and functionally to identify differences between circulating and tissue resident cells. Based on this we analysed the expression of different activation and tissue homing markers on ILC2s in the blood of severe asthma patients and patients suffering from COPD as compared to those from healthy controls. The effects of cytokine stimulation on the regulation of these markers was also studied.

Results: Nasal polyp ILC2s differed from blood ILC2s in their expression of CD45RO instead of CD45RA, lack of CD62L, but they do express CD69 and CCR10. These cells were more activated compared to blood ILC2s as they already produced IL-5 upon culture with only IL-2. In vitro we were able to reproduce this activated phenotype upon culture of blood ILCs with specific stimulating cytokines. In the blood of severe asthma patients we observed a population of ILC2s that displayed this activated phenotype. The same was observed for ex-ILC2s with an ILC1 phenotype in the blood of COPD patients. In this manner these cells resembled their tissue counterparts. Interestingly, in mild to moderate asthma patients we did not observe a population with this activated phenotype.

Conclusions: Taken together this is indicative that in severe forms of pulmonary inflammation there is also a systemic component which affects the ILCs. We propose that the activation state of peripheral blood ILCs can be used as a marker for disease severity.

0018 | Increases in group 2 innate lymphoid cells are inhibited by glucocorticoid treatment in asthma patients via stat signalling pathways

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Introduction: Group 2 innate lymphoid cells (ILC2s) were closely associated with asthma. However, the effects of glucocorticoid on ILC2s in patients and the mechanisms are not well understood.

Objectives: The purpose of this study was to perform a prospective study and evaluate the ILC2 levels and function after systematic glucocorticoid therapy in asthma patients and the potential mechanisms.

Results: Glucocorticoid was used to treat the isolated ILC2s *in vitro* and the possible signalling pathways involved were also investigated. The patients were well-controlled and the ILC2 levels were significantly decreased at 1 and 3 months. ILC2s were successfully sorted out from the asthma patients and were confirmed to be the predominant source of the large amounts of IL-5 and IL-13 in response to IL-25, IL-33 plus IL-2, and glucocorticoid significantly decreased their levels. Furthermore, p-STAT3 and p-STAT6 levels were highly increased in Lin^- cells with the stimulation and were reversed with the glucocorticoid administration.

Conclusions: The data suggested that glucocorticoid administration could be effective in treating asthma by regulating ILC2s via STAT3 and STAT6 signalling pathways. This will provide an efficient new idea for clinical medicine application in regards of allergic diseases.

SUNDAY, 18 JUNE 2017
OAS 12
AIT BIOMARKERS AND PERSISTENCE OF IMMUNE RESPONSE

0019 | Interference into JAK/stat signalling—a concept for improved allergen-specific immunotherapy?

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Introduction: Allergen-specific immunotherapy (AIT) is the only curative treatment for type-1 allergies. It is able to reduce allergic symptoms and to maintain its efficacy, but sometimes shows limited therapeutic response as well as local and systemic side effects. This limited control of local inflammation and severe patient symptoms anticipate the widespread use of AIT in severe conditions like allergic asthma.

Objectives: Our aim was to evaluate whether AIT could be more effective in the suppression of local inflammation, if it is applied in combination with a short-term non-specific immune-modulator, which interferes with the JAK pathways.

Results: Human naive CD4+ T cells were differentiated *in vitro* into induced regulatory T cells and the effect of Tofacitinib (TOFA, a FDA-approved JAK inhibitor) and other immune-modulators like Rapamycin and Cyclosporine on T helper cell subset differentiation was assessed via flow cytometry. In C57BL/6J mice, an *in vivo* model of ovalbumin (OVA)-induced allergic airway inflammation and allergen-specific immunotherapy was combined with the administration of TOFA from 48 hours prior to 48 hours after therapeutic OVA-injection. Plasma- as well as broncho-alveolar lavage samples were investigated on cellular as well as on cytokine level. TOFA enhanced the induction of human $FOXP3^+CD4^+$ T cells in contrast to other tested immune-modulators. *In vivo*, AIT combined with short-term TOFA administration was significantly more effective in suppressing total cell numbers and eosinophil infiltration into the lung, as well as local cytokine production including IL-1β and CXCL1. A trend for the reduction of IL-4, IL-13, TNF-α and IL-6 compared to AIT alone was also observed. Furthermore, TOFA co-administration was able to significantly reduce the level of systemic IL-1β, IL-6, and OVA-specific IgE and induced IgG1 to the same extent as AIT alone.

Conclusions: This proof of concept study shows that JAK inhibition via TOFA did not inhibit tolerance induction *in vitro* and *in vivo*, but amplified the positive effects of experimental AIT at the level of local inflammation. The improved control of local inflammation might extend the use of AIT in more severe conditions such as polysensitization, asthma and high-risk patients.

0020 | IgG4, but not IgG1, repolarizes pro-allergic M2a macrophages to a tolerogenic M2b-like phenotype: implications for allergen immunotherapy-mediated immune tolerance

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Introduction: The induction of allergen-specific IgG4 antibody is a hallmark of allergen-specific immunotherapy (AIT), but the mechanism how IgG4 could be involved in the induced immune tolerance is still unknown. We investigated here whether IgG4 could convert M2a macrophages, which are predominant in allergy, into an immunoregulatory M2b-like phenotype as new concept of AIT-mediated immune tolerance.

Objectives: Because macrophages, in the presence of immune complexes, can be polarized *in vitro* into M2b, we investigated whether it would make a difference whether the allergen would be complexed by IgG1 or IgG4.

Methods: Monocyte-derived macrophages (MDMs) were treated with M-CSF, followed by IL-4/IL-13 to induce the M2a allergic phenotype. To mimic immune complexes, culture plates were either directly coated with myeloma IgG1 or IgG4, or first coated with grass pollen major allergen Phl p 5 (for control Bet v 1 from birch), followed by incubation with recombinant human Phl p 5-specific IgG1 or IgG4. Finally, M2a polarized macrophages were added and grown for 72 h. Changes in cellular markers and cytokines were detected by flow cytometry and ELISA, respectively.

Results: M2a cells expressed significantly ($P<.0001$) higher level of IL-10 when co-incubated with myeloma IgG4 than with myeloma IgG1. Furthermore, only upon incubation with the IgG4 myeloma,

M2a cells secreted CCL-1 and IL-6 into supernatants, accompanied by a down-regulation of CD163 and CD206, and an up-regulation of CD86 expression, indicative for a shift to an M2b-like phenotype. In analogy, preliminary results obtained with the recombinant human anti-Phl p 5 antibodies demonstrate a significant (P=.0014) reduction of CD163 expression only when the IgG4 subclass was used in comparison with IgG1. Analyses of Fcg receptors (FcgRs) on macrophages demonstrated no modulation of FcgRIII expression by IgG4 treatment in comparison with IgG1, and failed to demonstrate a clear modulation of FcgRI or FcgRII by IgG4.

Conclusions: Our results indicate that the IgG4 subclass can redirect the pro-allergic M2a macrophages to an immunosuppressive phenotype, characterized by IL-10. IL-6, and CCL-1, secretion and down-regulation of activation markers. We propose that this implies the involvement of macrophages in tolerance induction and could be a novel mechanism of AIT.

0021 | Identification of novel biomarkers in the assessment of grass pollen slit response

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Introduction: Allergic disease prevalence in developed countries is estimated between 10-30% of population with significant increase in progression and severity in last decades. Sublingual administration of *Phleum pratense* allergy immunotherapy (SLIT) tablets represents one of the most recent approaches of tolerance induction by way of the oral mucosal route aiming to a long-term allergen-specific immunomodulation in patients with allergic rhinoconjunctivitis, with or without moderate asthma. We have documented a "disease-modifying" effect of SLIT-tablets for at least two years after stopping the treatment.

Objectives: We now aim to elucidate the mechanisms responsible for these effects and to identify novel biomarkers useful for follow up and efficacy measurement.

Results: 32 patients allergic to grass pollen, were enrolled in a double blind clinical trial for two years based on either placebo or immunotherapy treatment using a standardised grass SLIT-tablet. Serum samples and Peripheral blood mononuclear cells (PBMCs) from all the patients were used to perform metabolomic and transcriptomic analysis. Samples for metabolomics were analysed using high throughput Liquid Chromatography—Mass Spectrometry (LC-MS) technique. The gene expression profile of all the samples was analysed using the GeneChip WT PLUS Reagent Kit and two specific softwares. Results from metabolomics showed there were statistically significant differences between the two groups after two years of immunotherapy. However, the time was the most significant factor observed in the trial. Significant metabolites were tentative identified using online databases. Transcriptome analysis suggests significant differences in gene clusters associated with immunological response between placebo and active group.

Conclusions: These findings in relation to immune response metabolism were joined to the transcriptomic outcomes in order to have more information that may elucidate the biological changes implied. In the light of the above, this is the first study of its kind that showed first insights into the effect of immunotherapy in patients allergic to pollen using SLIT- tablets.

0022 | Persistent induced regulatory T-cell response to grass tablet sublingual immunotherapy is linked to lower specific immunoglobulin-e and clinical benefit

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Introduction: Sublingual administration of Phleum pratense allergen immunotherapy (SLIT) tablets is a clinically efficient treatment for grass pollen-induced allergic rhinoconjunctivitis. However, kinetics of immunological changes in patients undergoing grass tablet SLIT treatment has not been adequately studied.

Objectives: A 5-yr prospective clinical trial was designed, including a 3-yr grass tablet SLIT treatment period followed by 2 additional years after SLIT discontinuation. Systemic effects of SLIT on both humoral and cellular immune responses were evaluated in peripheral blood samples, in order to try to identify key immunological parameters that could explain sustained clinical benefit.

Results: Thirty patients completed the 5-yr protocol. Grass tablet SLIT administration induced a 2-phase systemic humoral and cellular response that mostly persisted 2-years after therapy discontinuation. The Th2 response was initially exacerbated, detected as an increase in both allergen-specific IgE (sIgE) levels and IL-4 producing cells; followed by downregulation of the Th2 response, with a shift toward a Th1 cytokine profile, measured as an increment in IFN-gamma producing cells. A progressive increase in specific IgG4 (sIgG4) levels and IgE-facilitated allergen binding (FAB) blockage, as well as

reduced grass-pollen-season eosinophil counts, were also relevant findings. sIgE/sIgG4 ratios at the end of trial were lower than preimmunotherapy in 70% of patients, meanwhile IgE-FAB remained lower in 83% of them. At the same time, a progressive development of a regulatory T cell response was observed in two thirds of the patients. Interestingly, there was a statistical association between this regulatory response, the maintenance of lower eosinophil counts during grass pollen seasons, and sIgE titers lower than before immunotherapy treatment, being the last ones significantly associated with clinical response.

Conclusions: Our results suggest that the maintained clinical improvement observed in patients 2-years after finishing a 3-yr treatment period with grass tablet SLIT is linked to acquisition of a regulatory T cell response, which in turn is associated with lower eosinophil counts and sIgE levels.

0023 | Changes in T-regulatory cells activity during dermatophagoides pteronyssinus immunotherapy

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Introduction: Specific immunotherapy is currently the only allergen-specific treatment with long-lasting effects and efficacy against allergens like house dust mites (HDM). Subcutaneous specific immunotherapy (SCIT) has shown to modify the HDM allergic response characterized by a Th2 pattern, towards Th1 patterns, with a generation of specific Treg cells that may produce IL-10 and TGF-β, both important in tolerance induction. However, many works have reported no changes in Treg percentage, indicating the regulatory effects produced during immunotherapy can be due to modifications in their regulatory activities.

Objectives: We aimed to determine changes on Treg functionality and the mechanism involved during HDM-SCIT in patients who had a clinical improvement. To achieve this goal, 16 HDM-SCIT allergic patients and 8 non-treated allergic patients were included in the study. HDM-specific IgE and IgG4 were determined by ImmunoCAP from serum at both before and 12 months after HDM-SCIT. Treg ($CD4^+CD25^+CD127^{LOW}FoxP3^+$) and T effector cells ($CD4^+CD25^-$) before and after 12 months of HDM-SCIT were isolated. Cells were cocultured in different combinations, without and with α-IL-10, α-TGF-β and both inhibitors (10 μg/ml) in presence of nDer p 1 (10 μg/ml). Cellular phenotyping, proliferative response and inhibition assays were evaluated by flow cytometry.

Results: Treg cells obtained after 12 months of HDM-SCIT increased Th1 ($CD4^+IFN\gamma^+$) proliferation (*P*=.004) and decreased Th2 ($CD4^+CD27^-CD294^+$) and Th9 ($CD4^+CD294^-IL\text{-}9^+$) proliferation (*P*=.04 and *P*=.04). Moreover, Treg after 12 months enhanced IL-10 producing cells ($CD4^+IL\text{-}10^+$) (*P*=.028) and diminished IL-4 and IL-9-producing cells ($CD4^+IL\text{-}4^+$ and $CD4^+IL\text{-}9^+$) (*P*=.033 and .049). The presence of α-IL-10 blocking antibody (both alone or combined with α-TGFβ) inhibited the Treg activity only on those obtained after 12 months of HDM-SCIT. Finally, the increase of HDM-specific IgG4 after 12 months was correlated with the increase of Treg percentage (*P*<.0001) and the IL-10 production (*P*=.023) and Treg percentage was correlated with IL-10 production (*P*=.036).

Conclusions: One year of immunotherapy induced changes in Tregs activities. These Tregs could be mediated by the generation of IL-10 suppression cytokine and can modify the immunoglobulin generation pattern. All the changes play an important role in the induction of tolerant response against the allergen leading to a clinical improvement of the symptoms.

0024 | Suppression of allergen-induced basophil activation upon treatment with subcutaneous house dust mite immunotherapy: a prospective study

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Introduction: The effect of allergen-specific immunotherapy (IT) on basophils remains incompletely understood. Several studies have shown an early suppression of basophil activation, and a few studies have reported long-term suppression in response to SIT. Most studies involved venom IT, some involved grass, birch or cat IT. To our knowledge, no previous studies investigated the effect of house dust mite (HDM) IT on basophil activation.

Objectives: To study the effect of HDM IT on allergen-stimulated basophil activation and to evaluate its relation with clinical response to treatment.

Methods: Patients with HDM allergic rhinitis undergoing conventional HDM subcutaneous IT (HDM SCIT, n=23) and patients not undergoing IT (HDMA, n=10) were studied prospectively. Basophil activation experiments were performed before start of IT (*visit 0*), halfway initiation (*visit 1*), at the end of initiation (*visit 2*), after 4 months of maintenance (*visit 3*), and after one year of treatment (*visit 4*). The same time line was followed for the HDMA subjects. Whole blood samples were stimulated with various concentrations (0.0023-22.7 ng/ml) of Dermatophagoides pteronyssinus extract. Activated basophils were identified with flow cytometry by expression of CD63. Dose-response curve metrics (EC50, CD-sens, and Area Under the Curve) were determined as measures for basophil allergen-sensitivity.

Results: Basophil activation of at least 15% upon stimulation with the highest allergen concentration was observed in all patients. IT induced a significant decrease of basophil activation over time ($P<.001$; change observed from *visit 2*, most marked at *visit 3*, maintained at *visit 4*). No decrease in basophil activation was observed in the patients not undergoing IT ($P=.523$). The difference between both groups was significant ($P=.005$, HDM SCIT vs HDMA). After one year of treatment with IT, three patients reported "no change", five patients "a little improvement", six patients "improvement", and nine patients "much improvement" of allergy symptoms. None of the patients reported worsening of symptoms. Within the HDM SCIT group, the change in allergen-stimulated basophil activation was not correlated with the change in patient-reported symptom control (VAS score, $r_s=.26$, $P=.24$).

Conclusions: House dust mite immunotherapy induced a long-term decrease of allergen-stimulated basophil-activation. The degree of change in basophil activation was not correlated with the degree of symptom improvement.

SUNDAY, 18 JUNE 2017
OAS 13
MANAGEMENT OF ATOPIC DERMATITIS

0025 | Burden of illness in adults with atopic dermatitis: Analysis of national health and wellness survey data from France, Germany, Italy, Spain, and the UK

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Introduction: There are limited data on the burden of illness in adult patients with atopic dermatitis (AD) in Europe.

Objectives: To measure the burden of illness in adult patients with AD in Europe.

Methods: Data were from 80 600 adult participants of the 2016 National Health and Wellness Survey from France, Germany, Italy, Spain, and the UK. Patients with self-reported diagnosis of AD were 1:1 propensity-score-matched with non-AD controls on their demographic characteristics. The matched groups were compared on their health-related quality of life (HRQoL) (Short Form-36v2 [SF-36v2] Health Survey mental and physical component summary scores and SF-6D health utilities), work productivity and activity impairment, frequency of atopy-associated comorbidities (asthma and nasal allergies/hay fever), mood (anxiety and depression) and sleep disorders, and healthcare utilization.

Results: After propensity-matching, demographics between the matched samples (both n=1860) were comparable. AD patients vs matched non-AD controls had significantly reduced HRQoL on mean SF-36v2 mental component summary score (42.0 vs 44.2) and physical component summary score (49.4 vs 51.2), and health utilities (0.66 vs 0.70), all $P<.001$. Activity impairment was greater for AD vs non-AD controls (31.8% vs 26.5%, $P<.001$) and among employed respondents, AD patients reported a higher level of overall work productivity impairment (mean 27.0% vs 23.7%, $P=.009$). Frequencies of asthma (20.9% vs 7.3%), nasal allergies/hay fever (50.5% vs 20.8%), anxiety (31.9% vs 14.4%), depression (25.8% vs 12.9%) and sleep disorders (22.7% vs 12.6%) were significantly higher in AD patients vs non-AD controls (all $P<.001$). The number of healthcare provider visits (mean 7.4 vs 4.5) and the incidence of at least one emergency room visit during the prior 6 months were significantly greater (21.6% vs 16.5%; $P<.001$) in AD patients vs matched non-AD controls, respectively.

Conclusions: In adult patients from France, Germany, Italy, Spain and the UK, AD is associated with significant impairments to HRQoL, work productivity and everyday activities, and increases comorbidities and healthcare utilization. These results reveal the high humanistic, economic and societal burden associated with AD in the European adult population.

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0026 | Use of topical treatment of eczema among adolescents - data from a population-based birth cohort (BAMSE)

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Introduction: Adolescents have reported that one of the most difficult aspects of having eczema is to maintain the daily treatment with emollients and/or with topical glucocorticoids. However, knowledge about how adolescents treat themselves and how prescribed topical glucocorticoids are dispensed is sparse.

Objectives: To explore how adolescents with eczema use emollients and topical glucocorticoids in relation to sex and eczema severity. In addition, to assess how topical treatment are dispensed from the pharmacy.

Method: The study population consisted of adolescents participating in the BAMSE birth cohort 16-year follow-up (n=3108). Eczema (atopic dermatitis) was defined as dry skin in combination with itchy rash on typical localizations the previous 12 months. Severity of eczema was assessed according to BAMSE Eczema Severity Score (BESS) based on self-reported symptoms. Information about dispensed topical glucocorticoids was obtained from The Swedish Prescribed Drug Register.

Results: In total, 10% (n=297) fulfilled the study definition of eczema. According to BESS, 73% had mild, 17% moderate and 10% severe eczema. Almost all adolescents used emollients, 75% regularly and 21% less than 1 month a year, whereas only 55% used topical glucocorticoids. The latter were used more often among those with moderate or severe eczema than in those with mild eczema (84%, 84% and 44%, respectively). The use of topical glucocorticoids did not differ between sexes (girl 53% vs boy 61%, $P=.179$). The odds to treat with glucocorticoids increased when the adolescents had symptoms of current eczema, OR5.96 (95% CI 1.92-18.5) adjusted for self-rated severity of eczema, gender, family history of eczema, IgE

sensitization, socioeconomic status and adolescents smoking. Of those with eczema, 24% (n=70) had any topical glucocorticoid (i.e. weak to potent) dispensed the last year. In the group with doctor's diagnosis of eczema ever (n=169), 32% had any topical glucocorticoid dispensed; 75% with a package of 100 grams and 25% with a smaller amount.

Conclusions: Almost all adolescents with eczema used emollients regularly. Girls and boys treated themselves equally with topical glucocorticoids, but one out of five adolescents with moderate or severe eczema had not used any topical glucocorticoid. Of adolescents with doctor's diagnosis of eczema, 7 of 10 had not had any glucocorticoid dispensed.

0027 | Immuno-modulatory effects of prebiotics, probiotics and active microbial structures on human primary epithelial cells

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Introduction: The human skin is one of the largest immunologic organs and represents the interface between the body and the environment. To achieve an effective defense barrier against environmental insults a crosstalk between epithelial and immune cells as well as the skin´s microbiota has to be maintained. Besides direct cell-cell contact also indirect contact via the exchange of soluble mediators is used to transfer information. The release of these mediators is assumed to be potentially influenced by "beneficial intervention factors" such as prebiotics, probiotics or active microbial structures. However, the underlying mode of action as well as a direct contribution to skin health is not clear to date.

Objectives: To gain deeper insight in this field we investigated whether pre-/probiotics or active microbial structures have a direct effect on immune regulation as well as barrier function of human primary epithelial cells. For this purpose human primary keratinocytes and nasal epithelial cells from healthy or atopic donors were stimulated with a specific mixture of non-digestible short-chain galactooligosaccharides (GOS) and long-chain fructooligosaccharides (FOS) alone or in a combination with either lactic acid bacteria or lactocepin. Subsequently, the immunomodulatory effect of epithelial cell supernatants on dendritic cells (DCs) was tested by analyzing changes in DC cytokine release. Furthermore, prebiotics were tested regarding their direct effect on epithelial barrier function in an air-liquid interface model.

Results: Results revealed that the presence of GOS/FOS decreases in epithelial cells the secretion of pro-inflammatory cytokines such as IP-10, CCL-2 and CCL-5 while combining GOS/FOS with probiotic bacteria leads to an even more pronounced effect. The strongest effect, however, could be observed in the presence of lactocepin. In contrast, the release of Galectin 9 was enhanced in response to GOS/FOS in human keratinocytes. In addition, a dampening of the decrease of TEER after stimulation with IL-4 and IL-13 could be induced by GOS/FOS. Finally, it could be observed that GOS/FOS conditioned supernatants of epithelial cells led to an increase in the IL-10/IL-12 cytokine release ratio by DCs.

Conclusions: Taken together the current study shows that pre-/probiotics as well as active microbial structures can indeed influence inflammatory processes and barrier function in human epithelial cells and can act as regulatory compounds.

0028 | Optimizing outcome on azathioprine treatment in patients with atopic dermatitis and/or chronic hand/foot eczema by co-prescription of allopurinol

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Introduction: Azathioprine (AZA) is frequently used in patients with moderate to severe atopic dermatitis (AD), but recent daily practice studies show high rates of AZA treatment discontinuation, due to ineffectiveness or side effects. AZA is converted in the liver into a variety of pharmacological active metabolites. The most important metabolites are 6-thioguanine nucleotide (6-TGN, with immunosuppressive effect) and methylated 6-methylmercaptopurine (6-MMP). Highly elevated 6 -TGN concentrations are associated with the development of myelotoxicity; highly elevated 6-MMP concentrations are associated with hepatotoxicity. Studies in patients with inflammatory bowel disease using AZA led to strategies to reduce the risk of toxicity and optimize efficacy and safety by adding

allopurinol. This co-prescription can shift the metabolism towards the production of 6-TGN, resulting in an alleviation of thiopurine hepatotoxicity and an increase of clinical efficacy.

Objectives: To Investigate the co-prescription of allopurinol during AZA treatment in patients with AD and/or chronic hand/foot eczema on AZA metabolite levels (6-TGN, 6-MMP) , side effects and clinical efficacy.

Methods: In this prospective mono-center study in the department of Dermatology (University Medical Center Utrecht, the Netherlands), AZA metabolite levels were analyzed in adult patients with AD and/or chronic hand/foot eczema during AZA monotherapy and after co-prescription of allopurinol. Clinical efficacy (measured by the Investigator Global Assessment) and side effects were registered.

Results: Fifteen patients were enrolled. Reasons for adding allopurinol were clinical inefficacy, side effects or a skewed metabolism (low 6-TGN levels with raised 6-MMP levels). After addition of allopurinol, 6-MMP values decreased and 6-TGN levels increased significantly ($P<.001$ and $P=.021$ respectively). Before the addition of allopurinol, four patients (26.7%) were classified as responder, compared to seven patients (46.7%) after the addition of allopurinol ($P=.013$). Hepatotoxicity, measured in one patient before the addition of allopurinol, normalized after the co-prescription. After the addition of allopurinol, a leukocyte decrease was measured in two patients.

Conclusions: Co-prescription of allopurinol during AZA treatment can improve clinical outcome in patients with atopic dermatitis and/or hand/foot eczema due to increasing the 6-TGN levels and decreasing the 6-MMP levels.

0029 | Efficacy and safety of dupilumab in adult patients with atopic dermatitis and an inadequate response, intolerance, or contraindication to cyclosporine: pooled analysis of two 16-week phase 3 trials

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Introduction: Patients (pts) with atopic dermatitis (AD) refractory to topical treatment are candidates for systemic treatment. Cyclosporine (CsA) is approved in this indication, however its clinical use is limited primarily due to safety concerns including hypertension and impaired renal and hepatic function. Dupilumab, a fully human anti-interleukin (IL)-4 receptor alpha monoclonal antibody, potently inhibits both IL-4 and IL-13 signalling. Dupilumab has been reported to improve AD outcomes while having an acceptable safety profile in 2 identically designed phase 3 trials of pts with moderate-to-severe AD (SOLO 1: NCT02277743; SOLO 2: NCT02277769).

Objectives: To evaluate the efficacy and safety of dupilumab vs placebo in two subsets of the pooled SOLO 1 & 2 population: pts

	Subset A (n=255) Inadequate response or intolerance to CsA			Subset B (n=1124) Remaining study population		
	PBO (N=78)	Dupilumab 300 mg Q2W (N=94)	Dupilumab 300 mg QW (N=83)	PBO (N=382)	Dupilumab 300 mg Q2W (N=363)	Dupilumab 300 mg QW (N=379)
Patients achieving EASI 75, n (%)	10 (12.8)	36 (38.3)	29 (34.9)	51 (13.4)	182 (50.1)	203 (53.6)
Patients achieving IGA 0-1, n (%)	6 (7.7)	28 (29.8)	23 (27.7)	37 (9.7)	141 (38.8)	147 (38.8)
Patients achieving a ≥4-point improvement in peak pruritus NRS, n/N1 (%)†	7/78 (9.0)	30/89 (33.7)	28/82 (34.1)	40/355 (11.3)	138/349 (39.5)	142/379 (40.9)
	Subset C (n=288) Inadequate response, intolerance or contra-indication to CsA			Subset D (n=1091) Remaining study population		
	PBO (N=88)	Dupilumab 300 mg Q2W (N=104)	Dupilumab 300 mg QW (N=96)	PBO (N=372)	Dupilumab 300 mg Q2W (N=353)	Dupilumab 300 mg QW (N=366)
Patients achieving EASI 75, n (%)	10 (11.4)	42 (40.4)	34 (35.4)	51 (13.7)	176 (49.9)	198 (54.1)
Patients achieving IGA 0-1, n (%)	6 (6.8)	32 (30.8)	27 (28.1)	37 (9.9)	137 (38.8)	143 (39.1)
Patients achieving a ≥4-point improvement in peak pruritus NRS, n/N1 (%)†	7/88 (8.0)	33/98 (33.7)	33/91 (36.3)	40/345 (11.6)	135/340 (39.7)	137/338 (40.5)

†Analysis was performed for patients with baseline peak pruritus NRS ≥4. N1 stands for number of patients with baseline NRS score ≥4. EASI-75, 75% improvement in EASI from baseline; IGA, Investigator's Global Assessment; NRS, numerical rating scale.

who had a documented history of inadequate response or intolerance to CsA (subset A; n=255) and the remaining study population (subset B; n=1124). Pts (N=1379) with moderate-to-severe AD whose disease was not adequately controlled by topical medications or for whom topical treatment is medically inadvisable were randomized to receive subcutaneous injections of placebo (PBO) or dupilumab 300 mg every 2 weeks (q2w) or weekly (qw) for 16 weeks.

Results: Subset A pts had, on average, more severe disease than subset B pts, as assessed by Eczema Area and Severity Index (EASI; mean baseline±SD EASI score 37.2±14.69 vs 32.0±13.29; nominal $P<.0001$). In both subsets, dupilumab treatment increased compared with PBO the proportion of pts reaching a 75% improvement in EASI; achieving Investigator's Global Assessment 0-1; or reporting a ≥4-point improvement in peak pruritus numerical rating scale (Table). The most common treatment-emergent adverse events in these studies were nasopharyngitis, AD exacerbations, and injection site reactions. Conjunctivitis rates were numerically higher in the dupilumab groups than in the PBO group (subset A: 3.9%, 6.3%/8.4% PBO, dupilumab q2w/qw; subset B: 0.5%, 5.4%/4.6). Similar efficacy (Table) and safety results were observed in a subset of pts including subset A pts and pts who had been considered for CsA, based on AD severity, but had not received CsA treatment as it was contraindicated or inappropriate (subset C; n = 288; conjunctivitis rates subset C: 3.4%, 5.7%/8.3%; subset D [remaining study population; n=1091]: 0.5%, 5.5%/4.4%).

Conclusions: Dupilumab 16-week monotherapy significantly improves signs and symptoms of AD regardless of a documented history of inadequate response or intolerance to CsA.

0030 | Efficacy and safety of dupilumab in adult patients with atopic dermatitis and a history of inadequate response, intolerance, or contraindication to cyclosporine: subgroup analysis from a 1-year trial

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Introduction: Atopic dermatitis (AD) is a chronic inflammatory skin disease that may persist for decades requiring systemic therapy for extended periods of time. Cyclosporine (CsA) provides a rapid and broad immunosuppressive effect but its long-term use is limited due to safety concerns including hypertension and impaired renal and hepatic function. Dupilumab, a fully human monoclonal antibody directed against interleukin (IL)-4 receptor alpha, inhibits type 2 cytokines IL-4 and IL-13. Long-term safety and efficacy of dupilumab was investigated in the phase 3 CHRONOS trial (NCT02260986).

Objectives: To compare the efficacy and safety of dupilumab vs placebo (PBO) at 52 weeks in two patient (pt) subsets of the CHRONOS population: pts who responded inadequately or were intolerant to CsA (subset A; n=114) and the remaining study population (subset B; n=509). CHRONOS was a 1-year, double-blind, randomized, PBO-controlled, parallel-group study in adults with moderate-to-severe AD and a history of inadequate response to topical corticosteroids (TCS). Pts were randomized 3:1:3 (dupilumab 300 mg weekly [qw], every two weeks [q2w], or PBO). Pts received a standardized regimen of concomitant low and/or medium potency TCS, which could be adjusted based on clinical response. Topical calcineurin inhibitors could be used in areas considered inadvisable for TCS.

Results: Subset A pts had, on average, more severe disease than subset B pts, as assessed by Eczema Area and Severity Index (EASI) (mean baseline±SD EASI score 37.0±12.70 vs 31.6±12.77; nominal $P<.0001$ [post-hoc analysis]). At Week 52 in both subsets, dupilumab treatment increased the proportion of pts achieving a 75% improvement in EASI; and the proportion of pts achieving a ≥4-point improvement in peak pruritus numerical rating scale (Table). Treatment groups had similar treatment-emergent adverse event (TEAE) rates (subset A: 88.9%, 90.9%/87.0%; subset B: 83.5%, 87.5%/82.2%). Most common TEAEs were upper respiratory tract infections, nasopharyngitis, conjunctivitis, AD exacerbations, and injection site reaction. Similar efficacy and safety results (Table) were observed in a subset of pts that included both subset A pts plus pts who had been considered for CsA, based on AD severity, but had not received CsA treatment as it was contraindicated or inappropriate (subset C; n=126).

Conclusions: Long-term treatment with dupilumab significantly improved signs and symptoms of AD regardless of a documented history of inadequate response or intolerance to CsA.

	Subset A (n=114) Inadequate response or intolerance to CsA			Subset B (n=509) Remaining study population		
	PBO±TCS (N=52)	Dupilumab 300 mg Q2W±TCS (N=19)	Dupilumab 300 mg QW± TCS (N=43)	PBO± TCS (N=212)	Dupilumab 300 mg Q2W± TCS (N=70)	Dupilumab 300 mg QW± TCS (N=227)
Patients achieving EASI-75, n (%)	10 (19.2)	10 (52.6)	21 (48.8)	47 (22.2)	48 (68.6)	152 (67.0)
Patients achieving a peak pruritus NRS score improvement ≥4 points from baseline, n/N1 (%)[†]	6/51 (11.8)	9/19 (47.4)	16/42 (38.1)	26/198 (13.1)	35/67 (52.2)	81/207 (39.1)
Patients with at least one treatment-emergent adverse event, n/N (%)[‡]	48/54 (88.9)	20/22 (90.9)	40/46 (87.0)	218/261 (83.5)	77/88 (87.5)	221/269 (82.2)
	Subset C (n=126) Inadequate response, intolerance or contra-indication to CsA			Subset D (n=497) Remaining study population		
	PBO±TCS (N=59)	Dupilumab 300 mg Q2W±TCS (N=21)	Dupilumab 300 mg QW±TCS (N=46)	PBO±TCS (N=205)	Dupilumab 300 mg Q2W± TCS (N=68)	Dupilumab 300 mg QW± TCS (N=224)
Patients achieving EASI-75, n (%)	11 (18.6)	11 (52.4)	23 (50.0)	46 (22.4)	47 (69.1)	150 (67.0)
Patients achieving a peak pruritus NRS score improvement ≥4 points from baseline, n/N1 (%)[†]	7/57 (12.3)	9/21 (42.9)	16/45 (35.6)	25/192 (13.0)	35/65 (53.8)	81/204 (39.7)
Patients with at least one treatment-emergent adverse event, n/N (%)[‡]	54/61 (88.5)	22/24 (91.7)	46/52 (88.5)	212/254 (83.5)	75/86 (87.2)	215/263 (81.7)

[†]Analysis was performed for patients with baseline peak pruritus NRS ≥4. N1 stands for number of patients with baseline NRS score ≥4. EASI-75, 75% improvement in EASI from baseline; NRS, numerical rating scale.

[‡]N stands for number of patients included in the safety analysis set.

SUNDAY, 18 JUNE 2017
OAS 14
ASTHMA MECHANISMS

0031 | Eosinophil-derived exosomes from asthmatics modify functionality on airway structural cells

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Introduction: Asthma is a chronic inflammatory disease of great importance given the social impact and the enormous health costs. The most prominent phenotype is Th2-driven inflammation, which is mainly associated with eosinophilic inflammation. Our group demonstrated that eosinophils are capable to secrete exosomes to extracellular medium.

Objectives: The aim of this study is to investigate the effect of eosinophil-derived exosomes from asthmatic patients in structural lung cells due to important role of these cells in asthma. To develop this study, Bronchial Smooth Muscle Cells (BSMC) and Small Airway Epithelial Cells (SAEC) were cultured in presence of exosomes from eosinophils of asthmatic or healthy subjects and different studies were carried out: apoptosis, proliferation, wound healing assays, analysis of phosphorylation of different signalling factor (p-ERK, p-AKT, p-STAT3) and also, we analyzed relative gene expression of important genes in asthma pathology such as TSLP, Periostin, TGF-ß, TNF-a, VEGF, CCR3, CCL26, among others.

Results: In BSMC studies, we observed proliferation was significant increased after 72 hours of exosome addition. Due to this process can be regulated by MAPK signalling pathway (p-ERK), we determined that the relative quantity of p-ERK in cells cultured in presence of exosomes at 72 hours was higher than in cells without exosomes; and this effect was reverted when BSMC were cultured in presence of p-ERK inhibitor. Moreover, the pattern of cytokine expression was different between cells in presence or absence of exosomes. Respect to SAEC, we noted that these cells need higher time to close the wound when they are in presence of exosomes. Besides, gene expression pattern was modified due to the action of exosomes from asthmatics and this pattern was different when an artificial wound on monolayer was made. Also, alterations in phosphorylation of MAPK (p-ERK), p-AKT and p-STAT3 were observed in cells cultured with asthmatic exosomes. By contrast, when exosomes from healthy subjects was added to the culture no significant effects were observed in cell functionality.

Conclusions: Functionality of bronchial smooth muscle cells and airway epithelial cells is affected by the presence of eosinophil-derived exosomes from asthmatics.

0032 | Impaired fibrinolysis and lower levels of plasma a2-macroglobulin are associated with an increased risk of severe asthma exacerbations

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Introduction: Recently we have reported that asthma is associated with enhanced plasma thrombin formation, impaired fibrinolysis and platelet activation. Clinical relevance of this observation remains to be established.

Objectives: To investigate whether prothrombotic blood alterations in asthmatics might predispose to thromboembolic events or asthma exacerbations.

Patients and Methods: In164 asthmatics, median age 53.5 (95% confidence interval: 52.1-54.9) we assessed clinical events during follow-up and analyzed their associations with thrombin formation, fibrinolysis variables as well as platelet activation markers at baseline.

Results: Data were obtained from 157 (95.7%) of the asthma patients during follow-up (median 37, range 35-42 months). There were one death, two episodes of deep vein thrombosis (1.3%), and eight acute coronary syndromes (5.1%). Patients who experienced thromboembolic events (n=10, 6.4%, 2.1%/year) had lower α_2-macroglobulin and thrombin- α_2-macroglobulin complex formation, without differences in efficiency of fibrinolysis and thrombin generation. We documented 198 severe asthma exacerbations (64/year), which occurred in 53 subjects (34%). These patients were older (P=.004), had worse asthma control (P=.02) and lower spirometry values (P=.01), at baseline. Interestingly, this subgroup had longer clot lysis time (CLT), as well as lower α_2-macroglobulin and thrombin-α_2-macroglobulin complex formation (all, P<.05 after adjustment for potential confounders). Increased CLT and lower α_2-macroglobulin were demonstrated as independent predictors of asthma

exacerbations in multiple regression model. Arterial hypertension (P=.005), gastroesophageal reflux disease (P<.0001), age 50 years or more (P=.01), severe asthma (P<.0001) and α_2-macroglobulin <14.63 nmol/l (P=.04) determined increased risk of asthma worsening in the time. None of the laboratory parameters measured at baseline was able to predict probability of a second or a total number of asthma exacerbations during follow-up.

Conclusions: Impaired fibrinolysis and lower levels of α_2-macroglobulin might predispose to a higher rate of asthma exacerbations, suggesting new links between disturbed hemostasis and asthma.

Keywords: asthma, thrombin generation, fibrinolysis, asthma exacerbation, thromboembolic complication.

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0033 | Regulation of suppressor of cytokine signalling 1 (SOCS1) expression in asthma

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Introduction: Exacerbations of asthma are most commonly caused by respiratory viral infection; rhinovirus (RV) and respiratory syncytial virus (RSV) are frequently detected during exacerbations. Interferons (IFNs) are produced by bronchial epithelial cells (BECs) and airway immune cells in response to viral infection and are essential for antiviral immunity. However, IFN responses in asthmatics are impaired. Suppressor of cytokine signalling 1 (SOCS1) is an inhibitor of IFN induction and signalling, and has recently been shown to be increased in asthmatic BECs, as well as being induced in response to both viral infection and the Th2 cytokines IL-4 and IL-13.

Objectives: This study sought to investigate the expression of SOCS1 in atopic asthmatic BECs and airway immune cells and investigate the induction of SOCS1 in response to viruses and Th2 cytokines.

Results: SOCS1 mRNA expression in BECs and BAL-acquired cells from the lungs of atopic asthmatics was measured by qPCR. SOCS1 mRNA was induced in response to RV16 and RSV infection in BECs, with a trend toward increased induction in asthmatics. RV16 and RSV-induced SOCS1 positively correlated with number of positive skin prick tests (SPT; P<.05); RSV-induced SOCS1 was also associated with Serum IgE and serum eosinophilia (P<.01). In BAL cells, SOCS1 but not SOCS3 was induced following viral infection (P<.05). In BEAS-2B cells, combined application of IL-4 and RV were found to strongly induce SOCS1 mRNA in an additive manner. IL-4 and IL-13 were found to activate the SOCS1 promoter, as measured by luciferase reporter assay, although RV did not. Furthermore, RV was found not to enhance SOCS1 mRNA stability. Chromatin immunoprecipitation-sequencing (CHIP-seq) was used to assess histone H3K27 acetylation in BECs obtained from atopic asthmatic patients and healthy controls, which has identified a region of H3K27ac enrichment 30Kb distal to the SOCS1 gene. This region appears to function as a distal enhancer for SOCS1 and we are currently investigating this a potential mechanism for viral-induced SOCS1.

Conclusions: SOCS1 expression in asthmatics appears to be related to atopy. Virus-induced SOCS1 is enhanced by pre-treatment with IL-4 or IL-13, although virus induced SOCS1 appears to be independent of the proximal promoter, and may involve epigenetic mechanisms.

0034 | Activation of inflammatory cells during asthma exacerbations is initiated prior to their migration to the lung

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Introduction: Underlying acute exacerbations of asthma are recruitment of immune cells into lung tissue and ensuing activation of complex inflammatory gene networks. Currently, key features of this process, notably the precise location of tissue compartment(s) where activation of inflammatory cells is initiated, and the full range of gene networks involved, are incompletely understood. We hypothesised that key elements of this activation process may occur prior to trafficking of inflammatory cells to lesional sites in the airways.

Objectives: To characterise the cellular and molecular mechanisms underlying severe exacerbations of asthma and identify molecular drivers of these mechanisms.

Methods: The approach entailed comparative cellular (Flow Cytometry) and molecular (RNA-Seq) profiling of PBMC obtained from atopic asthmatic children (n=19) presenting to the emergency department during an acute exacerbation and at convalescence. Network and upstream regulator analysis (WGCNA) was employed to identify coexpression networks and infer the drivers of these networks.

Results: At acute exacerbation, 78.6% of the children were virally infected and the dominant agent was rhinovirus (71.4%). The children were characterised by leukopenia (P=.0002) and lymphopenia (P<.0001) at acute exacerbation, and the ratio of total monocytes (acute:convalescent per ml blood) was elevated (1.25±0.36;

mean±SEM). In contrast, other subsets were reduced (CD4 + Tcells 0.39±0.10, CD4 + Tregs 0.43±0.10, CD8 + Tcells 0.46±0.16, B cells 0.71±0.12, pDC 0.08±0.02 and cDC 0.59±0.17), consistent with migration from blood to infection sites. Network analysis identified 6 coexpression modules that were upregulated in association with exacerbations, 3 of which were related to myeloid activation and migration. Prominent amongst the list of upregulated genes was CCR2, the principal chemokine receptor associated with lung homing. The immunoregulator TGFB1 was the dominant molecular driver of the myeloid-associated modules and lower in rank were IFNG, TNF, IL-6, IL-10RA and IL-4.

Conclusions: Our study demonstrates that components of the inflammatory cell activation process associated with acute asthma exacerbations are triggered prior to their recruitment into the lung. This suggests that therapeutic targeting of relevant precursor cells during acute exacerbations in their tissues of origin as opposed to only after their recruitment to the airways may be a viable approach towards asthma control.

0035 | The effects of allergen derived proteases on airway epithelial cells obtained from asthmatic and healthy individuals and associated mechanisms

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Introduction: In addition to their well described immunomodulatory properties in atopic asthma, allergens can also induce other pathological processes in the airways through their protease activities. Not much is known about the consequences of the protease activities of the allergens.

Objectives: We aimed to investigate the effects of allergen proteases on the airway epithelium obtained from healthy and asthmatic individuals.

Results: The antigens used in the study were Der p1, Der p serine proteases, Bla g2, and Lolium perenne extract. Effects of allergen derived proteases on cytokine/chemokine genes expressed in epithelial cells were determined by qPCR and multiplex ELISA. Epithelial apoptosis was measured by caspase activity and annexin V/PI staining of cells. Effects on tight junctions were determined by qPCR and confocal microscopy. Degradation of surfactants was determined by western blotting.

Allergen proteases stimulated the expression and increased the release of several cytokines and chemokines including TNF-α, IL6, RANTES, GM-CSF, IL-8, IP-10, TARC, MCP-1 and TGF-b from primary bronchial epithelial cells via protease activated receptors (PARs) and patterns of stimulation were different in healthy and asthmatic epithelial cells. Der p1 and Lolium perenne extracts opened the tight junctions between the cells and all the allergen extracts tested have induced apoptosis both in healthy and asthmatic epithelium. Allergen proteases have caused degradation of surfactant proteins to varying degrees. These effects could be reversed by specific protease inhibitors.

Conclusions: Our study shows that function of allergens in the pathogenesis of asthma is not limited to their immunomodulatory properties but they also have important functions as proteases. This may have important implications with respect to both inception and augmentation of the disease process.

0036 | Maternal exposures influence asthma severity in offspring: gleaning mechanistic insights from animal models

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Introduction: The incidence of allergic asthma has been increasing in recent decades, particularly in developed nations. While there is a clear genetic component to asthma heritability, the rapid rise in incidence is inconsistent with a purely genetic etiology. Accordingly, mounting evidence suggests that environmental factors, particularly those present during a critical early life developmental window, increase risk of developing asthma. This "developmental window" includes the prenatal period, as maternal environmental exposures, maternal diet and maternal asthma status (more so than paternal asthma status) strongly influence a child's risk for developing asthma.

Objectives: Herein, we explore the capacity of in utero exposures to 1) the common aeroallergen House Dust Mite (HDM) or 2) a cocktail of antibiotics (Ampicillin, Gentamicin and Vancomycin—the most commonly utilized antibiotics in the perinatal period) to influence later development of allergen-induced allergic asthma in an experimental model of allergic asthma.

Results: We demonstrate that compared to animals exposed to PBS in utero, in utero HDM exposure resulted in more severe AHR, airway inflammation, Th2 cytokine production, and immunoglobulin levels following induction of experimental asthma. While transmission of allergen-specific immunoglobulins from mother to child was also evident, offspring of B cell deficient mothers exposed to HDM during pregnancy demonstrated similarly exacerbated experimental asthma, suggesting that transfer of maternal immunoglobulins was not required. Interestingly, while maternal exposure to antibiotics similarly increased the severity of allergen-induced AHR compared to offspring of control mothers, exacerbated AHR was not associated with an increased magnitude of cytokine production (either Th2

or Th17-associated) from T cells. This suggests that T cell-independent factors were regulating the development of more severe experimental asthma in offspring of antibiotic exposed animals.

Conclusions: Collectively, these data suggest that maternal exposure to a variety of factors known to influence asthma risk in humans (aeroallergens, antibiotics) have a profound influence on the severity of asthma that develops in offspring. These data lend support to developmental origins of allergic disease hypothesis and suggest that these observations may be successfully modeled in experimental models of allergic asthma.

OAS 15

INFLAMMATORY SKIN DISEASES: MECHANISMS AND ASSOCIATIONS

0037 | Association of atopic dermatitis with cardiovascular risk factors and diseases

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Introduction: Epidemiological studies suggested an association between atopic dermatitis (AD) and cardiovascular diseases (CVD).

Objectives: Therefore, we investigate associations and potential underlying pathways of AD and CVD in large cohort studies: the AOK PLUS cohort (n=1.2 Mio, median age 64 years), the GINIplus/LISAplus birth cohorts (n=2286, median age 15 years), and the KORA F4 cohort (n=2990, median age 56 years). Additionally, metabolomics in KORA F4 and established cardiovascular risk loci in genome-wide data on 10 788 AD cases and 30 047 controls were analyzed.

Results: Longitudinal analysis of AD patients in AOK PLUS showed slightly increased risk for incident angina pectoris (adjusted risk ratio 1.17; 95%-confidence interval 1.12-1.23), hypertension (1.04 (1.02-1.06)) and peripheral arterial disease (1.15 (1.11-1.19)) but not for myocardial infarction (1.05 (0.99-1.12) and stroke (1.02 (0.98-1.07)). In KORA F4 and GINIplus/LISAplus, AD was not associated with cardiovascular risk factors, such as BMI, blood pressure and blood lipids, and in KORA F4, no differences in metabolite levels were detected. There was no robust evidence for shared genetic risk variants of AD and CVD.

Conclusions: This study indicates only a marginally increased risk for angina pectoris, hypertension and peripheral arterial disease and no increased risk for myocardial infarction or stroke in AD patients. Relevant associations of AD with cardiovascular risk factors reported in US-populations could not be confirmed. Likewise, AD patients did not have increased genetic risk factors for CVD.

0038 | Atopic dermatitis is not independently associated with cardiovascular or autoimmune comorbidities in the general population: Results from the LIFE-adult-study

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Introduction: Atopic dermatitis (AD) was recently reported to be associated with cardiovascular, gastrointestinal and/or autoimmune comorbidities.

Objectives: Based on a random sample from the general population, we aimed to cross-sectionally determine any association of AD and selected comorbidities in a study of the Leipzig Research Centre for Civilization Diseases in Germany (LIFE-Adult-Study). The study sample included adults (mainly ≥40 years) between 2010 and 2014 in Leipzig. Age, sex, body mass index (BMI) and waist hip ratio (WHR) were documented. Subjects were asked in a computer-assisted personal interview for physician diagnosed AD, allergic rhinitis (AR), asthma, autoimmune diseases (AID), inflammatory bowel diseases (IBD), coronary heart disease (CHD), myocardial infarction (MI), smoking/pack years (PY) and stroke. Patients with AD were compared to controls without history of AD. Statistics: Fisher's exact/Mann-Whitney-Test and, univariate und multivariate logistic regressions for non-atopic comorbidities.

Results: 372/9514 subjects (mean age 54 years, range 20-79) reported history of AD. Patients with AD (53.8 years, 20-79) were significantly younger ($P<0001$) and more frequently female (57.0 vs 52.1%; $P<.0001$) than controls (57.6 years, 19-80). BMI was not

significantly different in both groups (27.0 vs 27.4). AR was reported by 33.6% of patients with AD vs 13.1% in controls ($P<.001$), asthma by 19.9 vs 7.6% respectively ($P<.001$).

There were more patients with AD having history of AID (5.4 vs 3.2%; $P<.024$), RD (7.8 vs 5.0%; $P<.022$) and IBD (1.9 vs 1.1%; n.s.). They also reported on more PY of smoking ($P<.008$), but had decreased WHR ($P<.035$) and less frequent history of MI (1.1 vs 2.6%; $P<.087$) than controls. CHD (3.3 vs 3.5%; n.s.) and stroke (1.9 vs 2.2%; n.s) showed similar frequencies in both groups. After multivariate logistic regression, only age was significantly lower in patients with AD than in controls, while all other parameters turned out to be not significantly different.

Conclusions: Data confirmed frequent association of allergic rhinitis and asthma in subjects with AD. In contrast to some previous reports, we did not find any significant association of AD with other comorbidities investigated. One limitation of our study is the restriction to patients being mainly aged ≥40 years. Also, one might speculate that patients with severe comorbidities did not follow invitation to participate in this investigation.

0039 | Is there an increased risk of non-melanoma skin cancer in patients with atopic dermatitis treated with oral immunosuppressive drugs?

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Introduction: Oral immunosuppressive drugs are used in the management of moderate to severe atopic dermatitis (AD). Due to the chronic nature of AD, long-term treatment with oral immunosuppressive drugs is often necessary to maintain adequate disease control. An important barrier to long-term use of oral immunosuppressive drugs in AD patients is the possible increased risk of the development of non-melanoma skin cancer (NMSC, including basal cell carcinoma [BCC] and squamous cell carcinoma [SCC]). Most data on the risk of developing malignancies in patients treated with oral immunosuppressive drugs are derived from transplant patients, but data on the risk of NMSC in AD patients using oral immunosuppressive drugs are lacking until now.

Objectives: To estimate the incidence of NMSC in a large cohort of AD patients treated with oral immunosuppressive drugs in the Netherlands and to compare these findings to the general Dutch population.

Methods: All adult AD patients receiving oral immunosuppressive drugs (cyclosporine A, azathioprine, methotrexate, mycophenolate mofetil and [extended release] tacrolimus) for more than 2 months in the University Medical Center Utrecht and Groningen, the Netherlands were included. Medical records were screened for patient and treatment characteristics. All patient files in the histopathology register (Pathologisch Anatomisch Landelijk Geautomatiseerd Archiefsysteem [PALGA]), a nation-wide database for pathology reports in the Netherlands with national coverage, were screened for NMSC. The standardized incidence ratio (SIR) of SCC in our cohort was calculated by comparing of our results to the general Dutch population.

Results: 557 AD patients were enrolled. NMSC after oral immunosuppressive treatment was reported in 18 patients (3.2%). The standardized incidence ratio (SIR) for developing SCC was 13.1 [95% CI 6.5-19.7]. Patients developing NMSC were older at therapy start ($P<.001$) and data lock ($P<.001$) compared to patients without NMSC. Gender, cumulative days of oral immunosuppressive drugs and follow-up differed not significantly between these groups ($P=.42$; $P=.88$ and $P=.34$, respectively).

Conclusions: The SIR for SCC in this study was increased, but for interpretation of the results it is important to include other factors, such as lack of association between treatment duration and tumor development and the long interval between treatment discontinuation and tumor development in some patients.

0040 | Intra-transcriptome interactions between interleukins and tight junctions, reveals widespread barrier dysregulation in atopic dermatitis lesions

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Introduction: Atopic dermatitis (AD) is one of the most common inflammatory skin diseases in the general population. AD lesions are characterized by defective expression of skin Tight Junction (TJ) proteins, as well as changes in filaggrin and factors of the immunologic concert. However, regulation of the whole TJ family and cytokine expression during lesion formation remains to be elucidated to understand the key events behind AD lesion formation.

Objectives: Here, we performed RNA-sequencing and subsequent transcriptome analysis in punch biopsies from 11 atopic dermatitis patients and 7 healthy controls, comparing gene expression differences between healthy skin, AD lesional skin and AD non-lesional skin samples. Differentially expressed genes between the three skin conditions were calculated, hierarchical clustering and principal

component analysis of samples were performed, along with enrichment of the involved pathways and correlations between selected gene families.

Results: Transcriptome analysis showed a global difference between AD lesional and non-lesional skin samples. The majority of tight junction genes, including claudin-1,-4,-5,-10 as well as occludin show a significant downregulation in AD lesional skin. Likewise majority of adherens junction genes are also significantly downregulated in AD lesional skin, such as cadherin-3 and cadherin related-1. Gap junctions and desmosome families show a complete signature change in AD skin. Furthermore; the cytokines of the Th2 function IL-4 and IL-13, as well as inflammatory cytokines with keratinocytes as a source, such as IL36G, IL38 and IL37 show upregulation in AD skin. Correlation analysis between cytokines of the interleukin family and junction genes revealed possible interactions previously undocumented between cytokines and barrier related genes. IL36A and IL36G, as well as IL18 show strong negative correlations with expression of major tight junction proteins in the skin, claudin-1, -4 and -5, leading to the hypothesis that these cytokines of the IL-1 family may have a role in the epidermal barrier dysregulation seen in AD patients,

Conclusions: Our results show how gene expression differences in microbiome are correlated with inflammation status and skin barrier function. Bioinformatics analysis allowed us to identify several groups of tight junction and immune related genes that differently correlated amongst each other, potentially indicating distinct positive and negative influences on lesion development in AD.

0041 | Toll-like receptor 7 and interleukin 17 are possible targets for intervention in allergic contact dermatitis

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Introduction: The prevalence of contact dermatitis to nickel as well as pollen allergy is increasing. We aimed to follow some biomarkers in these delayed and immediate type reactions using patch tests and skin prick tests as models. The antigen presenting cells in contact dermatitis, dendritic cells and keratinocytes, exert their effects partly by production of interleukins and expression of receptors. Toll-like receptors act mainly in infections and in autoimmunity as alarming signals. Mast cells and their mediators on the other hand are crucial for immediate allergic reactions. Novel biomarkers, important in allergic reactions, may serve as new targets for intervention.

Objectives: Patch tests reactions to nickel on the upper arms were followed for two weeks and positive prick test reactions to birch tree pollen on lower arms were followed for 5 h. Skin biopsies, taken at four intervals (control, day 3, day 7 and day 14) after nickel provocation were analyzed immunohistologically with respect to occurrence and distribution of CD 3 + T cells/IL 17, IL 33, langerin, toll-like receptors (TLR-5 and TLR -7) and mast cell markers tryptase and chymase. Skin prick tests were biopsied after 20 min, 1 hours and 5 hours and compared with control skin using the same biomarkers.

Results: In control skin, Langerin-immunoreactivity (IR) was found in dendritic cells in epidermis. IL 33-IR was mainly confined to keratinocytes of the suprabasal layer of epidermis and some in endothelial cells of blood vessels scattered in dermis. TLR-5-IR was found on the surface of keratinocytes in epidermis, while TLR-7-IR was very sparse. CD3 + T cells/IL-17-IR was almost nonexistent. Mast cell tryptase/chymase-IR was also absent in epidermis and found in mast cells of the deeper part of dermis.

Langerin-immunoreactivity (IR) increased twofold on day 7, occurring not only in epidermis but also in upper dermis. Also IL-33-IR and TLR-5-IR increased twofold in epidermis on day 7. A dense TLR-7-IR appeared on the cell surfaces in epidermis and in upper dermis. The increase of TLR-7 -IR was tenfold compared to control skin. The T cells CD 3 + /IL 17 were only few in control skin, their number increasing fifteen fold on day 7. The occurrence of mast cells in dermis remained unchanged for the time followed. The density of all biomarkers was mainly unchanged in prick test reactions followed over time.

Conclusions: Our findings suggest that TLR-7 and IL 17 may serve as potential targets for treatment of allergic contact dermatitis.

0042 | Evidence for a role of eosinophils in blister formation in bullous pemphigoid

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Introduction: Bullous pemphigoid (BP) is an autoimmune disease characterized by subepidermal blister formation due to tissue-bound and circulating autoantibodies to the hemidesmosomal antigens BP180 and BP230. Although eosinophils and their toxic mediators are found abundantly in BP lesions, their role in blister formation has remained unclear.

Objectives: To investigate the role of eosinophils in the pathogenesis of BP with a specific focus on blister formation and to define conditions inducing dermal-epidermal separation (DES).

Results: Following activation with IL-5 and in the presence of BP autoantibodies, eosinophils induced separation along the dermal-epidermal junction of *ex vivo* skin. DES was significantly reduced by blocking any of the following: Fcg receptor binding (P=.048), adhesion (P=.046), reactive oxygen species (ROS) production (P=.002),

degranulation ($P<.0001$), or eosinophil extracellular trap (EET) formation ($P=.048$).

Conclusions: Our results provide evidence that IL-5—activated eosinophils directly contribute to BP blister formation in the presence of BP autoantibodies. DES by IL-5 - activated eosinophils depends on adhesion and Fcg receptor activation, requires elevated ROS production and degranulation, and involves EET formation. Thus, targeting eosinophils may be a promising therapeutic approach for BP.

MONDAY, 19 JUNE 2017
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WHAT DO ANIMALS TEACH US ABOUT ASTHMA?

0043 | Pulmonary delivery In vivo if SiRNA-based therapeutic approach for PI3K-delta isoform successfully improves HDM-induced asthmatic features via the regulation of NLRP3 inflammasome

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Introduction: RNA interference (RNAi) is an almost standard method for the knockdown of any target gene of interest *in vitro*, exploring a naturally occurring catalytic mechanism. The downregulation of pathologically relevant genes in various disorders will offer novel therapeutic approaches. RNAi is mediated by small interfering RNAs (siRNA), and thus siRNA delivery *in vivo* is of critical importance for its implementation. In addition, phosphoinositide 3-kinase (PI3K)-delta-dependent Akt activation is associated with the pathogenesis of severe respiratory diseases partly through the induction of steroid resistance.

Objectives: In this study, we aimed to investigate the effects of *in vivo* delivery of siRNA targeting PI3K-delta isoform on house dust mite (HDM)-induced asthmatic features and its therapeutic potential.

Results: We found that HDM inhalation induced the typical asthmatic features in mice including increased airway inflammatory cells, airway hyperresponsiveness, and Th2 cytokine expression in lung of mice. Moreover, the significant increased PI3K-Akt pathway activation in lung tissues of mice with PI3K-delta isoform expression and NLRP3 inflammasome activation. PI3K-delta knockdown by intratracheal delivery of siRNA into the murine lung decreased HDM-induced typical allergic asthmatic features and NLRP3 inflammasome activation. Additionally, *in vitro* transfection of siRNA targeting PI3K-delta showed the decrease in PI3K-delta mRNA expression in primary cultured tracheal epithelial cells from mice. To support the content that PI3K-delta contributes to the pathogenesis of HDM-induced asthma, we used a murine model of HDM-induced asthma in wild type (WT) and PI3K-delta knock-out (KO) mice and found that PI3K-delta KO mice showed significant reduction of asthmatic features and NLRP3 inflammasome activation in the lung.

Conclusions: This study indicates the therapeutic potential of pulmonary delivery *in vivo* of siRNA targeting PI3K-delta in allergic airway inflammation via the regulation of NLRP3 inflammasome activation.

0044 | Raw cow's milk prevents the development of airway inflammation in a murine house dust mite-induced asthma model

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Introduction: Numerous epidemiological studies show an inverse relation between raw cow's milk consumption and the development of asthma. This protective effect seems to be abolished by milk processing. Evidence for a causal relationship is however still lacking and also direct comparisons between raw and heat treated milk are hardly studied. In the present study we therefore investigated the preventive capacity of raw milk and heated raw milk on the development of house dust mite (HDM)-induced allergic asthma in mice.

Objectives: Six- to seven-week-old, male BALB/c mice were intranasally (i.n.) sensitized with 1 μg HDM or PBS on day 0, followed by an i.n. challenge with 10 μg HDM or PBS on days 7 to 11. In addition, mice were orally treated with 0.5 ml raw cow's milk, heated raw cow's milk (10 min, 80°C) or PBS three times a week throughout the study, starting one day before sensitization. At the end of the study (day 14), airway hyperresponsiveness (AHR) in response to increasing doses of methacholine was measured in order to assess lung function and bronchoalveolar lavage fluid (BALF) was examined to study the extent of airway inflammation. T helper (Th) cell subpopulations were quantified in lung cell suspensions using flow cytometry and chemokine and cytokine concentrations were determined in lung homogenates and supernatants of *ex vivo* HDM re-stimulated lung cells.

Results: Sensitization and challenge with HDM resulted in AHR and pulmonary eosinophilic inflammation. Raw milk prevented both typical features of allergic asthma, whereas heated raw milk did not. Epithelial- and DC-derived mediators, IL-33, CCL20, CCL17 and CCL22, were significantly increased in the lungs of HDM-mice. Both milk types reduced the concentration of CCL17. The percentage of Th2 cells in lung cell suspensions was also significantly reduced by both milk types. Pulmonary concentrations of Th2 cytokines, IL-5 and IL-13 were increased in HDM-mice, but only raw milk prevented this increase. Upon re-stimulation of lung cells with HDM, both raw and heated raw milk were able to significantly reduce the production of IL-4 and IL-13.

Conclusions: Raw cow's milk prevents the development of asthma in a murine HDM-induced allergic asthma model. Heat treated raw milk did not show this protective effect. Besides an abundant

amount of epidemiological evidence, this study now also suggests a causal relationship between raw cow's milk consumption and the prevention of allergic asthma.

0045 | Differences of the role of NLRP3 inflammasome between two types of fungal allergen-induced asthma

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Introduction: The NLRP3 inflammasome is a molecular platform activated upon signs of cellular 'danger' to trigger innate immune defenses through the maturation of pro-inflammatory cytokines such as IL-1β. In addition to these host defenses, emerging evidence has suggested that inflammasomes exert important effector functions during the progression of human diseases including pulmonary disorders. Recently, we have reported that suppression of mitochondrial ROS generation attenuated allergic airway inflammation associated with inhibition of NLRP3 inflammasome activation and reduced IL-1β production. However, to date, the role of NLRP3 inflammasome is controversial in the pathogenesis of allergic asthma.

Objectives: In this study, to evaluate the role of NLRP3 inflammsome in the pathogenesis of fungal allergen induced asthma, we used two types of fungal allergen induced asthma model; i) *Alernaria Alternata (Aa)*-inhaled mice and ii) *Aspergillus Fumigatus (Af)*-inhaled mice and a small molecular inhibitor for NLRP3 assembly/activation, MCC950.

Results: The results showed that two types of fungal allergen significantly induced typical features of bronchial asthma; Interestingly, the NLRP3 inflammasome activation indicators, NLRP3, capase-1, and IL-1β were dramatically increased in lung tissues of both murine models. TCA assay showed that mature IL-1β levels were significantly increased in BALF from two types of fungal allergen induced asthma models. When dexamethasone was administered to these fungal allergen-inhaled mice, interestingly, *Aa*-inhaled mice showed the dramatic improvement of all asthmatic features, while the asthmatic features of *Af*-inhaled mice including NLRP3 inflammasome activation was not affected by the treatment with dexamethasone. Meanwhile, the administration of MCC950 dramatically reduced the airway inflammation, pathologic changes, bronchial hyperresponsiveness, NLRP3 inflammasome activation, and mature IL-1β in *Aa*-inhaled mice. In case of *Af*-inhaled mice, the anti-inflammatory effects of MMC950 on asthmatic features including mature IL-1β appeared to be modest.

Conclusions: These findings suggest that NLRP3 inflammasome is one of critical pro-inflammatory contributors in the pathogenesis of fungal allergen-induced asthma, however, a single targeted inhibition for NLRP3 activation or assembly has some limitation to overcome steroid-resistant severe allergic airway inflammation.

0046 | Distinct roles for the cytokines IL5 and IL13 in pulmonary inflammation and asthma pathophysiology

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Introduction: Uncontrolled Type-2 inflammation is associated with allergic/atopic diseases. Blocking drivers of Type-2 inflammation, such as IL5 or IL13, have demonstrated clinical benefit in asthma, particularly in patients displaying elevated expression of pathway specific Type-2 biomarkers such as eosinophils. Multiple factors, including the cytokines IL33 and IL25, regulate the simultaneous induction of IL13 and IL5 from various leukocytes following lung injury. While blockade of either IL13 or IL5 ameliorates lung inflammation in both pre-clinical asthma models and clinical studies, how each cytokine contributes to individual facets of lung pathology is unclear. Furthermore, although inhibition of IL33 or IL25 attenuates lung inflammation in pre-clinical models, it is unknown whether this is due to IL13 and/or IL5 vs other inflammatory factors, and whether a greater reduction in lung pathology can be achieved by combinatorial blockade of these pathways.

Objectives: We compared the contribution of IL5 and IL13 to Type-2 lung inflammation induced by *N. Brasiliensis* infection. Given the roles for IL33 and IL25 in regulating IL5 and IL13, we examined how various parameters of lung inflammation were affected by simultaneous blockade of these pathways.

Results: We found that mucin induction and mucus-related pathologies such as airway hyper-reactivity, were IL13 dependent, while eosinophilia was more sensitive to IL5 activity. Blocking IL33 in addition to either IL5 or IL13 led to a total abrogation in these Type-2 related pathologies, emphasizing the role of IL33 in regulating these factors. However, co-blockade of IL33 and IL25 in this system led to only a partial suppression of these parameters.

Conclusions: These results highlight the distinct functions of IL5 and IL13 in disease pathophysiology, but also suggest there is a level of crosstalk between pathways which ultimately manifests in a reduction in overall inflammation when a single cytokine is blocked. These results also indicate additional efficacy over single axis therapies alone in patients with severe asthma may be achievable using approaches to dampen multiple Type-2 cytokines. Given many systems can regulate IL5 and IL13 production in vivo, attention needs to be paid to the types of combinations used. Our studies indicate blocking a central regulator of Type-2 cytokines, such as IL33, in combination with a key pathological effector, such as IL13 or IL5, will lead to the greatest reduction in lung inflammation.

0047 | The metabolite D-tryptophan from probiotic bacteria ameliorates allergic airway disease and influences the gut microbiome

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Introduction: The development of asthma and allergic diseases is highly dependent on the environment. Thus, various studies have attempted to positively modulate the microbiome using probiotic bacteria in order to prevent allergic diseases. However, these initial clinical trials have given inconsistent results. This may be explained by the complexity of the crosstalk between probiotic bacteria, the host´s microbiota and immune cells. The identification a biochemically defined, bioactive substance secreted from probiotic bacteria could circumvent this difficulty.

Objectives: To identify and characterize a bioactive, probiotic metabolite for potential prevention of allergic airway disease.

Results: 37 probiotic supernatants were screened for their ability to lower the constitutive CCL17 secretion of a human Hodgkin lymphoma cell line. Immune-modulatory metabolites were isolated by chemical fractionization and characterized by mass spectrometry and NMR. Primary, naïve CD4 T cells were isolated from murine spleens and differentiated in vitro towards Th1, Th2 and Treg with 0, 10 or 50 μM DTrp. Differentiation was assessed by qRT-PCR, cytometric bead array and flow cytometry. Balb/c mice were treated with 50 mM D-Tryptophan via drinking water while allergic airway inflammation (AAI) was induced by Ovalbumin. Gut microbiota was assessed from caecum by 16S qPCR.

13 supernatants showed immunoactivity. Bioassay-guided chromatographic fractionation of two supernatants according to polarity, followed by total ion chromatograms and mass spectrometry, proton nuclear magnetic resonance and enantiomeric separation identified D-tryptophan as bioactive substance. In contrast, L-tryptophan and eleven other D-amino acids were inactive in our in vitro screening assays. DTrp significantly lowered the differentiation of Th0 to Th2 cells and slightly increased differentiation towards Tregs in vitro. Mice supplemented with DTrp during experimental asthma induction, had increased numbers of lung and gut regulatory T cells, lowered lung Th2 responses, ameliorated allergic airway inflammation and hyper-responsiveness. Further, allergic airway inflammation reduced the gut microbial diversity, which was increased by D-tryptophan.

Conclusions: We oa beneficial effect of the bacterial metabolite D-Tryptophan on allergic airway inflammation in mice. *Thus*, defined bacterial products might able to modulate the host's immune system and should be further explored to prevent asthma and allergic diseases.

0048 | Exacerbation of experimental asthma regulated by IL-17 producing NK cells depends on IL-6 but not on IL-23 or rorgt

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Introduction: Viral infections of the lung are the major cause of acute asthma exacerbations. Double-stranded RNA motifs, produced during the replication of respiratory viruses, can trigger immune responses via activation of Toll-like receptor 3 or RIG-I. We have previously shown that local application of the synthetic TLR-3/RIG-I activator poly (I:C) alone is sufficient to trigger exacerbation of experimental allergic asthma in mice, which is mainly driven by IL-17 producing NK cells.

Objectives: This study aimed at identifying regulatory mechanisms of IL-17 producing NK cells during experimental asthma exacerbation. Therefore, poly (I:C) was applied intratracheally to mice with already established experimental allergic asthma to trigger acute exacerbation. Lung function, airway inflammation, cytokine expression, and mucus production was assessed in wild-type (WT) mice and mice deficient for IL-6, IL-23p19, and RORγt. FACS staining for different leukocyte populations were performed.

Results: Poly (I:C)-induced exacerbation of experimental asthma in WT mice is characterized by aggravation of airway inflammation, mucus production and airway hyperresponsiveness, which was associated with infiltration of IL-17 producing NK cells and increased production of IL-6. Such an exacerbation of experimental asthma could also be evoked in IL-23p19 deficient mice, but not in animals lacking IL-6. It was not possible to induce experimental allergic asthmatic in RORγt deficient mice.

Conclusions: These results indicate a critical role for IL-6 but not for IL-23 in poly (I:C) induced IL-17 production in NK cells and, thus, acute exacerbation of experimental asthma. Furthermore, our study suggests IL-6 as a potential target for the therapy of acute asthma exacerbations.

MONDAY, 19 JUNE 2017

OAS 17

FACTORS INFLUENCING THE DEVELOPMENT OF ALLERGIC DISEASES

0049 | Airway microbial dysbiosis in pre-school children associates with respiratory symptoms

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Introduction: Many young children have wheezing episodes, but not all develop asthma at school age. Often these children are over- or undertreated. With the prevalence of asthma rising, this clinical problem has only become more substantial.

A growing body of research suggests that the airway microbiome plays an important role in asthma development. However to date it is unclear if children that develop asthma at school-age have microbial dysbiosis at young age. Elucidating this could help establish diagnosis early, prevent over- and under treatment and open routes to novel therapeutic approaches aimed at restoring microbial dysbiosis.

Objectives: To determine the airway microbial diversity and abundance in pre-school children we took nasal brushes of 146 children aged 0-2, 110 had 1 or more physician objectified wheezing episode, 36 were healthy controls. We analysed the microbiome by IS-pro, this is a PCR based technique that allows identification of 3740 different bacterial operational taxonomic units (OTUs) based on the length of the interspace region of the 16S-23S rDNA. With the identified OTUs we performed unsupervised clustering to identify microbial endotypes. Furthermore, bi-annual questionnaires were taken to assess respiratory symptoms. At age 6 a paediatric pulmonologist assessed the presence of asthma according to GINA guidelines.

Results: Unsupervised clustering identified 3 microbial endotypes in the nasal microbiome with a CH index of 0.20. We found several associations of these endotypes with respiratory symptoms. Endotype I and II were associated with wheezing and asthma. They contained the highest levels of *H. influenza*, *M. catarrhalis* and *Streptococcus* spp. Endotype I showed more allergic features and 64% of the children had physician confirmed wheezing episodes, compared to 33% in endotype II. Endotype II contained most children with unconfirmed wheeze. The third endotype consisted of most healthy subjects, with 40% of children that never had a parent-reported wheeze.

Conclusions: Our findings show that there are distinct nasal microbial endotypes and that these associate with clinical features and respiratory symptoms. Our data suggests that evaluation of the nasal microbiome has value as a predictive tool for asthma development and could prevent over- and undertreatment. Our future research is aimed at understanding the functional implications of microbial dysbiosis on asthma development.

0050 | Viral infections in preschool children with wheezing

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Introduction: Infections with respiratory viruses are the most common cause of wheezing in preschool children. There is a lot of evidence that asthma exacerbations in small children are predominantly associated with upper respiratory tract infections, caused by rhinoviruses. However, other respiratory viruses may play an important role as well.

Objectives: With the advance and availability of modern microbiological methods it becomes possible to detect different viral infections in clinical practice. Therefore the aim of our study was to evaluate the role of different respiratory viruses in preschool children with wheezing. We hypothesized that rhinoviruses and respiratory syncytial virus (RSV) are the most common cause of wheezing in preschool children.

Results: From 278 otherwise healthy preschool children hospitalized because of respiratory distress, at least one respiratory virus was detected with polymerase chain reaction from nasopharyngeal swab in 198 (71.2%) of subjects. Single microorganism was isolated in 131 (66.2% of all positive) samples, in 55 (27.8%) samples we proved double infection and in 12 (6.1%) samples we identified three different viruses. Wheezing was present in 70 (53.6%) subjects with monoinfection and in 41 (61.2%) subjects with coinfection (P=.36). The most common isolate was rhinovirus, followed by RSV and bocavirus. Wheezing was presenting symptom in 81.5% (75 out of 92 isolates) of rhinovirus infections, 60.6% (20 out of 33) of bocavirus infections, 50.0% (11 out of 22) of parainfluenza virus infections, 46.7% (36 out of 77) RSV infections, 36.4% (4 out of 11) of metapneumovirus infections, 35.7 (4 out of 14) of coronavirus infections and 33.3% (5 out of 15) of adenovirus infections (P<.01).

Conclusions: We confirmed rhinoviruses as the most common cause of wheezing in preschool children and a great majority of hospitalized children with rhinovirus infection actually wheezed. Wheezing was present in less than half of our RSV cases, which is less than is usually stated in the literature. Most children infected with bocavirus wheezed as well. However, in our study bocaviruses were mainly detected as a coinfection. We found adenoviral infections significantly associated with the absence of wheezing, as opposed to the prevailing concept according to which adenovirus-caused bronchiolitis is clinically indistinguishable from RSV and other respiratory viruses. Coinfections had no influence on the prevalence of wheezing, compared to monoinfections.

0051 | Eating fish as infant and living on a farm decreased the risk of allergic rhinitis at 12 years of age

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Introduction: Allergic rhinitis is one of the most common chronic childhood diseases. The prevalence has risen, but the cause of this increase is partly unknown. The aim of this study was to analyse prevalence, gender differences and risk factors for the development of allergic rhinitis in a 12-year-old population.

Objectives: Data were collected from a prospective, longitudinal cohort study of children born in western Sweden in 2003. The parents answered questionnaires from the age of 6 months until 12 years. The response rate at 12 years was 77% (3637/4777) of the questionnaires distributed.

Results: The prevalence of allergic rhinitis at the age of 12 was 22% and 57% were boys. Mean onset age was 7.8 years. Boys had a significantly earlier onset than girls (P=.005). The most common trigger factors were pollen (85%), furry animals (34%) and mites (17%). In a multivariate analysis, parental allergic rhinitis (adjusted odds ratio, aOR, 2.32; 95% CI 1.94-2.77), doctor-diagnosed food allergy in the first year of life (aOR 1.75; 1.21-2.52), eczema in the first year of life (aOR 1.61; 1.31-1.97) and male gender (aOR 1.25; 1.06-1.47) were independent risk factors for allergic rhinitis at age 12. Growing up on a farm with animals reduced the risk of allergic rhinitis at age 12 (aOR 0.51; 0.32-0.84), so did eating fish more often than once a month at the age of 12 months (aOR 0.70; 0.50-0.98).

Conclusions: Allergic rhinitis occurred in more than one out of five 12-year-olds. The risk was lower among children growing up on a farm with animals and eating fish often.

0052 | The cost-effectiveness of polyvalent mechanical bacterial lysate treatment in children with asthma

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Introduction: One of the most frequent factors exacerbating asthma in children are recurrent acute respiratory tract infections. An increasing number of studies indicates the possible preventive action of bacterial lysates both in the prevention of respiratory tract infections and the exacerbations of chronic obstructive pulmonary disease. However, there are no analyses assessing the cost-effectiveness of applying PMBL in the case of asthma in children.

Objectives: The purpose of the conducted pharmacoeconomic analysis was to compare the costs and effects of introduction of additional PMBL (Polyvalent Mechanical Bacterial Lysate) therapy in children with asthma in relation to standard asthma treatment.

Results: In order to assess the cost-effectiveness, Markov decision model was used utilising the results of prospective, randomised, double-blind, placebo-controlled, parallel group study (PMBL vs placebo). The costs have been calculated from the perspective of the payer, patient and society with the use of conversion data from the financial section of hospitals, clinics, the National Health Fund as well as the 2014-2015 market data. The cost-effectiveness is presented with the use of the incremental cost-effectiveness *ratio (*ICER), whereas the utility analysis is based on QALY. The study lasting 9 months comprised 120 families of children with chronic IgE dependent asthma in the age of 6-17 treated in allergy or pulmonary clinics in the years 2014-2015. The population included 33 girls (27.5%) and 87 boys (72.5%) in the average age of 9.6±2.6. There were no statistically significant differences between both groups, whether in terms of socio-demographic or clinical data (w_p-0.75-0.99). From the perspective of a payer, the total average unit cost/month of treatment (PMBL vs placebo) was estimated of EUR 26.26 vs EUR 23.93 (P<.01), from the perspective of the patient at the level of EUR 34.08 vs EUR 45.77 (P<.01), whereas the social costs amounted to EUR 49.07 vs EUR 66.92 (P<.01). ICER in the period of intake of the preparation amounted to 0.044 (PMBL) vs 0.066 (placebo), whereas during 6 months of observation respectively 0.09 vs 0.11. QALYs in the period of the entire study amounted to 0.60 (PMBL) vs 0.67 (placebo).

Conclusions: The results of our study show that the addition of PMBL therapy to standard asthma treatment in children with IgE dependent asthma allows cost-saving and provides better control of the disease in comparison to the placebo group.

0053 | Food diversity during first year of life and allergic diseases until 15 years: results from lisaplus birth cohort

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Introduction: Previous research suggests that higher food diversity during the first year of life could be protective against later development of allergic outcomes. Further population-based studies accounting for potential confounding by early skin symptoms are required in order to confirm the reported associations.

Objectives: Detailed data on food diversity during the first year of life as well as allergic outcomes and potential confounders up to 15 years were collected prospectively within the population-based multicentre German birth cohort LISAplus (*n*=2518, recruited in 1998-1999). 8 food groups and 48 food items were categorized into quartiles and treated as exposures. 6 atopic outcomes were considered: doctor diagnosed (1) eczema, (2) asthma and (3) allergic rhinitis, (4) nose and eyes symptoms, sensitization to (5) food allergens and (6) inhalant allergens. Longitudinal associations were analyzed using generalized estimation equations. As in the earlier study we found clear evidence for reverse causality between early skin symptoms and introduction of solids, all analyses were run for whole study population as well as stratified by whether a child had early skin symptoms.

Results: A lower prevalence of eczema and sensitization to inhalant allergens was associated with an increased diversity of food groups introduced during the first year of life (odds ratio=0.67 (95% confidence interval=0.48-0.94) and 0.61 (0.44-0.85) in the highest vs the lowest quartile, respectively). These associations were driven by the participants with early skin symptoms, whereas in participants without early skin symptoms, association with lower asthma prevalence was observed (0.21 (0.05-0.82)). Similar protective associations for sensitization to inhalant allergens were observed with food item diversity. However, in children without early skin symptoms, higher diversity of food items was associated with higher prevalence of allergic rhinitis.

Conclusions: Our results do not demonstrate clear protective effect of early life food diversity on allergic outcomes. Parents of children with early skin symptoms might have introduced solid foods more carefully in line with that time recommendations, and hence, reverse causation might have caused the previously observed associations.

0054 | Preventive effect of human milk against food allergy: New insights into butyrate activities

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Introduction: Mechanisms of the preventive effect of breast milk (BM) against food allergy (FA) are undefined. Butyrate (BUT) has a pivotal role in immune tolerance.

Objectives: To see whether BM butyrate concentrations are able to exert tolerogenic effects in human enterocytes, peripheral blood mononuclear cells (PBMCs) from children affected by FA, and in FA animal model. Mature BM BUT concentrations from 98 healthy women were assessed by gas-chromatography. Dose-dependent effects of BUT in human enterocytes (Caco-2 cells) on immune (beta-defensin-3, HBD-3) and non-immune (mucus production; mucin 2, MUC2; tight-junction proteins, zonulin and occludin) were analyzed. PBMCs from 6 children with challenge-proven FA (2 cow milk, 2 peanut, 2 hen's egg) were stimulated with100 μg/ml of b-lactoglobulin (BLG), 200 μg/ml of peanut extract (PE) or 200 μg/ml of ovalbumin (OVA) in the presence or absence of butyrate. Expression and DNA methylation rate of IL-4, IL-5, IL-10, IFN-γ and Treg-specific-demethylated region (TSDR) *FoxP3* were assessed.4-weeks-old female C3H/HeJ mice were used in FA animal model. 2 weeks before first sensitization, oral gavage with 30 mg/kg/d of BUT was started and continued during the study. Mice were sensitized orally on day 0, 7, 14, 21, 28 with 20 mg of BLG or 1 mg OVA or 12 mg PE mixed with 10 μg cholera toxin (CT) as adjuvant. Control mice receive CT only. On day 35, mice were challenged by gavage with BLG (50 mg) or OVA (5 mg) or PE (36 mg). Anaphylaxis score and rectal temperature were assessed for 1 hours after challenge; blood samples were collected to measure MCPT-1 and sIgE. After 24 hours, mice were sacrificed, colon, ileum and spleen were collected.

Results: BUT concentration in BM was 0.75 mM (SD±0.15). BUT stimulates HBD-3, mucus production and MUC2, zonulin and occludin expression with maximal effective doses between 0.75 and 1 mM in human enterocytes. PBMCs stimulation with BLG, PE, OVA resulted in a significant increase in IL-4 and IL-5 production. A significant inhibition of IL-4 and IL-5 production was observed with 0.75 mM BUT. BUT stimulated, in a dose-dependent manner, IL-10 and IFN-γ production through a demethylation of respective genes and TSDR *FoxP3* demethylation. Pre-treatment with BUT significantly reduced anaphylactic score, body temperature decrease, serum MCPT-1 and sIgE levels. BUT stimulated mucus, IL-10 and IFN-γ production and inhibited IL-4, IL-5 and IL-13 production.

Conclusions: BUT, as effective human milk component, is able to prevent FA through immune and non-immune tolerogenic mechanisms.

MONDAY, 19 JUNE 2017
OAS 18
IMMUNOTHERAPY: MEASURES AND OUTCOMES

0055 | High correlation between the total symptom score measured in a pollen chamber and the combined symptom medication score measured during the grass pollen season in grass pollen allergic patients

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Introduction: The EAACI recommends the Combined Symptom Medication Score (CSMS) as standardized clinical outcome measure in allergen immunotherapy trials for allergic rhinoconjunctivitis (ARC). During early phases of clinical development it is challenging to select a surrogate parameter to be used in a phase II study, which adequately predicts the outcomes in field studies. Recently, a phase II trial was completed to investigate the efficacy and safety of different dosages of sublingual immunotherapy (SLIT) with a liquid Phleum Pratense extract for treatment of ARC due to grass pollen allergy. A post-hoc analysis was performed to assess the correlation between the primary endpoint Total Symptom Score (TSS) as measured in an environmental exposure chamber (EEC) and the CSMS assessed during the grass pollen season.

Objectives: The study was a single-center, randomized, double-blind, placebo-controlled, parallel-group study, with a treatment duration of 10 months. In total 168 patients (18-65 years of age) suffering from grass pollen induced ARC, were randomized to treatment with one of three different dosages of a liquid Phleum Pratense extract or placebo. For this post-hoc analysis, data from the 150 patients that completed the study were analyzed. The TSS was assessed at baseline, before the start of the grass pollen season and at the end of the study in the pollen exposure chamber. For the TSS assessment, the patients were exposed to grass pollen at an average concentration of 3500±500 ppm^3 in a mobile EEC for 6 h. For this post-hoc analysis only the TSS at the end of the study was evaluated. The CSMS was assessed during the grass pollen season, which was defined based on actual pollen counts.

Results: The TSS score was assessed in the EEC at the end of the study between August and September 2016, which was 1-2 months after the CSMS which was assessed during the grass pollen season (June/July 2016). The results showed a strong positive and statistically significant correlation between the CSMS during the pollen season evaluated after 8-9 months of treatment and the TSS measured in the EEC after 10 months of treatment (r=0.62 and P<.0001).

Conclusions: These study results support that after pollen SLIT with a liquid Phleum Pratense extract, TSS during controlled exposure to grass pollen in an EEC is a predictive surrogate parameter for CSMS measured during the grass pollen season for patients suffering from grass pollen induced ARC.

0056 | A randomized clinical trial of passive immunotherapy with single-dose anti-Fel d1 monoclonal antibodies REGN 1908–1909 in cat-induced rhinoconjunctivitis: exploratory efficacy endpoints, safety, and

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Introduction: Cat allergy, a main cause of rhinoconjunctivitis and allergic asthma, is prevalent in westernized countries; available treatments have only modest efficacy. A passively administered mixture of REGN1908 and REGN1909 (REGN), high-affinity monoclonal antibodies binding distinct Fel d 1 epitopes, showed efficacy in preclinical models and met the primary endpoint in a phase 1b trial; here we report exploratory endpoints.

Objectives: Inclusion criteria were: adults 18–55 years; history of cat-exacerbated allergic rhinitis; positive skin prick test >3 mm with cat hair extract; positive allergen-specific IgE tests >0.35 kAU/l for cat dander and Fel d 1; peak Total Nasal Symptom Score ≥7 within 1 hour after nasal allergen challenge (NAC) with cat hair extract; normal lung function. Key exclusion criteria were anti-IgE therapy within prior 6 months; nasal obstruction or history of nasal/sinus surgery. Subjects were randomized to single-dose subcutaneous (SC) REGN (n=36) or placebo (PBO; n=37). NAC was performed on Days 8, 29, 57, and 85; patient-reported nasal symptom score visual analog scale (VAS; 0–100 mm) at 0–60 min was recorded. Percent change from baseline for VAS area-under-the curve over the first hour ($AUC_{0\text{-}1\ h}$) and peak VAS were compared with ANCOVA. Safety was assessed. Blood samples were taken at predefined time points for pharmacokinetics (PK).

Results: Subjects were 49.3% male, 84.9% white, mean±SD age 27.9±9.6 years, with balance between groups. Baseline VAS $AUC_{0\text{-}1\ h}$ was 51.0±15.1 for PBO and 49.8±16.1 for REGN, with percent changes of −34.4±34.9% and −58.7±44.5%, respectively, at Day 8,

and −32.9±38.6% and −60.6±45.3% at Day 29; least square mean differences were −24.1% (95% CI −43.2%, −4.9%) at Day 8 and −28.1% (95% CI −48.6, −7.5) at Day 29. Percent changes in peak VAS scores were nominally significant for REGN vs PBO at all time points except Day 57. Time to maximum serum concentration was 5.7 and 6.4 days for REGN1908 and 1909, respectively; half-lives were 31.0 and 22.4 days. There were 107 treatment emergent adverse events (TEAEs), 46 PBO (n=23; 62.2%) and 61 REGN (n=23; 63.9%). No discontinuations were due to TEAEs; two serious TEAEs (appendicitis in PBO; pyelonephritis in REGN) were deemed not treatment-related.

Conclusions: A single prophylactic SC REGN dose was significantly more effective than PBO in providing patient-reported improvements to NAC with cat dander, with responses maintained for 12 weeks; REGN was generally well tolerated.

0057 | Long-term sustained reduction of allergic rhinoconjunctivitis in children with grass pollen allergy – results from an asthma prevention (GAP) trial

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Introduction: The GAP trial was a 5-year trial investigating the asthma preventive effect of a standard grass SLIT-tablet (75 000 SQ-T) in 812 children (5–12 years) with allergic rhinoconjunctivitis caused by grass pollen. As secondary outcomes, disease-modifying effects on allergic rhinoconjunctivitis (ARC) were assessed at the end of the trial (3 years of treatment, 2 years of follow-up).

Objectives: During 2 weeks prior to the last grass pollen season (GPS) visit of the trial, the severity of ARC symptoms was recorded daily by the subject indicating a point on a visual analogue scale (VAS) between 'no symptoms' and 'severe symptoms' in response to the question 'How has your hay fever been today?'. The use of ARC pharmacotherapy was recorded daily during the same 2 weeks (antihistamine tablets, eye drops and nasal steroid). The severity of grass pollen ARC according to the ARIA classification (mild vs. moderate/severe; intermittent vs. persistent) was assessed each year of the trial at the GPS visit.

Results: During the GPS, 2 years after end of treatment, there was a 22% reduction of ARC symptoms (assessed by VAS) with the grass SLIT-tablet compared to placebo (*P*=.005). 608 children (307 placebo, 301 active) provided VAS data The use of ARC pharmacotherapy was significantly reduced with the grass SLIT-tablet compared to placebo (27%, *P*<.001). 504 children (263 placebo, 241 active) provided pharmacotherapy data.

For each of the 3 treatment years and the 2 follow-up years, the proportion of subjects with persistent symptoms and with moderate/severe symptoms was statistically significantly lower in the active group compared to the placebo group. For the follow-up year, 2 years after end of treatment, the odds ratio (OR) for grass SLIT-tablet vs placebo for having persistent symptoms was 0.57 [0.37; 0.88] (*P*=.005) and for having moderate/severe ARC 0.57 [0.34; 0.96] (*P*=.036).

Conclusions: The GAP trial showed that treatment with a standardized grass SLIT-tablet for 3 years provided sustained reduction of ARC symptoms and ARC pharmacotherapy use 2 years after end of treatment. Additionally, the proportions of subjects with persistent ARC or with moderate/severe ARC were statistically significantly lower in the active group compared to placebo. Thus, for the first time, the disease-modifying effect of allergy immunotherapy treatment has been demonstrated in a large, long-term, randomised, double-blind, placebo-controlled trial in children.

0058 | Peanut oral immunotherapy during omalizumab protection; a clinical trial on severely peanut allergic adolescents

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Introduction: Peanut OIT (oral immunotherapy) can induce desensitization but allergic reactions occur and increased tolerance is not acquired in a substantial proportion of patients. Studies suggest that patients with low specific IgE-ab levels to peanut and Ara h 2 have a better treatment outcome. Omalizumab has been reported to reduce the frequency of allergic reactions and facilitate OIT updosing.

Objectives: To study efficacy and prognostic factors in omalizumab facilitated OIT among severely peanut allergic adolescents monitored with CD-sens (basophil allergen threshold sensitivity).

Results: Open phase 2 study in which 23 severely peanut allergic adolescents started peanut OIT after 8–24 weeks on omalizumab. Omalizumab dosing was guided by CD-sens. Starting at 1 g of peanut, equivalent to 280 mg of peanut protein, the OIT dose was increased every 2 weeks up to the 2800 mg (protein) maintenance dose. After 8 weeks on maintenance dose, CD-sens was measured

and omalizumab was reduced by 50 % if CD-sens was still suppressed and the patient had nothing but mild symptoms. The same procedure was repeated every 8th week until omalizumab was discontinued. An open peanut challenge was performed >12 weeks after the final omalizumab dose.

Patients were grouped as good responders (GR); able to discontinue omalizumab and subsequently pass a 2800 mg peanut protein provocation (n=8) or poor responders (PR); still treated or drop outs. All patients reached maintenance dose in median after 10 weeks, (range 8–34) but in significantly shorter time in GR compared to PR, median 8 (8–12) vs. 11 (8–32) weeks (*P*=<.05). Median time on OIT among GR was 69 weeks (48–97) and is 119 (74–139) weeks in for those still treated (four patients dropped out). CD-sens at baseline was significantly lower among GR 0.51 (0.27–1.86) vs. 1.49 (0.24–20.5) among PR (*P*=<0.05). sIgE to peanut and Ara h 2at baseline did not differ significantly among the groups: 64 and 165 kU_A/l respectively for peanut (*P*=.08) and 40 vs.61 kU_A/l (*P*=.27) for Ara h 2.

Conclusions: Thirty-five % of the patients tolerated 2800 mg peanut protein after treatment with OIT combined with omalizumab. High CD-sens values to peanut correlate significantly with worse and prolonged treatment response. Previous studies of peanut OIT (with or without omalizumab) have reported higher rates of success. This can probably be explained by patient selection since patients in this study have a severe phenotype in terms of history and level of sensitization.

0059 | Clinical validation of environmental exposure chamber in Strasbourg with mite in asthmatic patients

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Introduction: As recommended recently by the Task Force on Environmental Exposure Chamber (EEC), allergenic and non-allergenic exposure must be better controlled in chambers as it is in the new EEC of Strasbourg.

Objectives: The objective of this study was to validate Strasbourg EEC by determining the airborne concentration of Der p1 which induces 60% of immediate and/or late phase bronchial reactions in asthmatic patients sensitized to mite. This was a single-center, randomized, double blind, 3-way cross-over study, including 2 groups: group A: 24 asthmatic patients allergic to mites and group B: 20 asthmatic patients allergic to another allergen. All the subjects were first exposed to placebo (PCB). The group A was exposed at random to 3 airborne Der p1 concentrations: 63, 76 and 105 ng/m^3 (n=45). The number of airborne particles and the particles size were also recorded on line during the exposure (n=90). The group B was exposed to the concentration of airborne Der p1 which fulfills the objective of the study determined after a first statistical analysis.

Results: The mean age of subjects was 28.2 years (±7.27) For the 3 airborne concentrations of Der p1 we obtained more than 60% of late phase reactions (LR). The mean time necessary to obtain an immediate reaction (IR) was: 78.6±56 minutes (n=69) and 304.2±115 min (n=44) for the late phase. The optimal dose to obtain an IR and LR was 70 ng/m^3 with 100% of occurrence of IR and/or LR. The mean fall in FEV1 during IR and LR was 24.53% and 18.90 % respectively. We did not observe any severe reaction during this study. No patient from group B experienced any symptoms during the airborne Der p1 exposure (70 ng/m^3) as well as no patient experienced any drop of FEV1 during PCB exposure. The Coefficient of variation (CV) inter assay of the number of particles between 0.5–5 and 5–10 μm was 19.08±1.94. The CV intra assay of airborne Der p1 was 22.12±1.32.

Conclusions: We have validated Strasbourg EEC in patients with asthma sensitized to mite. We also demonstrated its specificity. More than 60% of our selected patients had a late phase reaction. That is of interest for future clinical studies with mite asthmatic treatments.

0060 | Factors influencing compliance to specific subcutaneous immunotherapy (SCIT)

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Introduction: Allergen-specific subcutaneous immunotherapy (SCIT) is a safe and effective treatment for the allergic rhinitis and allergic asthma. It is considered that treatment compliance is less in SCIT since it requires the patient to periodically visit the hospital. Non-compliance of patients to treatment is a major barrier for achieving optimal outcomes.

Objectives: We aimed to evaluate the compliance rates of patients receiving SCIT at our allergy clinic and identify factors affecting compliance.

Results: 366 patients who received SCIT were included to the study between 2010 – 2013. 118 patients were agreed to involve in the study. They were interviewed by face to face. Data were analysed in terms of factors influencing compliance and persistence to treatment. Factors as following; age, sex, education, living in nearby location, diagnosis (allergic rhinitis with or without asthma), symptoms (perennial or seasonal), content (single or multiple allergen), therapy benefit (symptom score, drug score, visual analogue score) were evaluated. The mean age was 39.9 varying of 20–65 years. The number of patients discontinuing the treatment was found to be 31 (%26.3). The compliance to SCIT was reversely correlated with

the number of allergens administered (p:0.023). Patients who received more benefit were found to be more compliant to treatment (p:0.01). The major reason for cessation of treatment was receiving inadequate benefit (%25.81). Although statistically insignificant; having simultaneous allergic rhinitis and asthma, suffering perennial symptoms, having low educational status and living in nearby locations were found to have higher compliance rates.

Conclusions: Compliance rates in our clinic were %73.7, quite high compared to the literature. Possible reasons for this condition could be the appropriate patient and allergen selection and supplying adequate information to patients.

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OAS 19
DIAGNOSTIC TOOLS IN FOOD ALLERGY

0063 | A novel human mast cell activation test for peanut allergy

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Introduction: The basophil activation test (BAT) showed to be highly discriminative between peanut allergic and peanut-sensitized non-allergic children. As BAT requires fresh blood cells, we investigated whether the ability to elicit peanut-induced cell activation could be transferred by passive sensitization of LAD2 mast cells with patients' plasma.

Objectives: To validate the mast cell activation test (MAT) as a biomarker of peanut allergy.

Methods: Peanut allergic (PA), peanut-sensitized non-allergic (PS) or non-sensitized non-allergic (NA) patients (n=174) were studied. Whole blood BAT to peanut was performed. In the MAT, plasma samples were used to sensitize human LAD2 cells prior to stimulation with peanut extract. The expression of IgE, FceRI, FcgRII and degranulation markers on the surface of mast cells was measured by flow cytometry. The performance of MAT in the diagnosis of peanut allergy was assessed.

Results: Sensitization with plasma increased surface IgE and transferred the ability of mast cells to respond to allergen and anti-IgE. Peanut-induced activation of mast cells sensitized with plasma from PA patients was higher than that of mast cells sensitized with plasma from PS ($P<.001$) or NA ($P<.001$) patients, overall and when comparing samples with similar peanut-specific IgE levels. Following sensitization, there was no difference in surface IgE or in mast cell activation in response to the negative or positive controls between PA and PS subjects. Overtime in culture, LAD2 cells decreased the expression of FceRI ($P<.001$) and of surface IgE ($P<.001$) and the activation to peanut ($P<.001$) and anti-IgE ($P<.001$). Thus, FceRI expression was monitored and a plasma sample from a PA patient was used as an internal control in the assay. The MAT expressed as %CD63+ LAD2 cells showed 98% specificity and 73% sensitivity in the diagnosis of peanut allergy. Diagnostic performance was similar (94% specificity, 75% sensitivity) when using the ratio of peanut-activated mast cells sensitized with test plasma to peanut-activated mast cells sensitized with internal control plasma as the outcome of MAT. The results of MAT and BAT were strongly correlated (Rs=0.808, $P<.001$).

Conclusions: MAT is a functional assay that can be performed using stored plasma samples and closely resembles the whole blood BAT. The MAT showed high specificity to diagnose peanut allergy, which provides added value to serologic tests.

0061 | FABER IgE diagnostic test: the most comprehensive view on IgE sensitization to egg and poultry allergens

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Introduction: Hen's egg (HE) allergy ranks among the most frequent food allergies in children.

The Allergome database reports 25 allergens, 10 of 25 named by IUIS-WHO. 5 major HE proteins are available for routine testing: ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), lysozyme (Gal d 4) and livetin (Gal d 5). Few data on cross reactivity or individual recognition of eggs and poultry meats from different avian species are available in literature.

Objectives: To investigate the profiles of egg and poultry sensitized patients in a population of 1751 patients by means of FABER 244, a new nanobead-based IVD test for specific IgE detection.

Results: FABER test is performed using 122 molecular allergens and 122 allergenic extracts, all coupled to chemically activated nanoparticles. The 15 allergenic preparations under study and spotted on the FABER array are Hen's, Duck's, Quail's and Turkey's white and yolk extracts, 5 major HE allergenic proteins and Chicken and Turkey poultry extracts. 48 patients, the majority in their first 10 years of life, turned out to be sensitized to at least one of the 15 allergenic preparations. The sensitization prevalence of each white egg extract in those 48 patients was detected as follows: turkey 54%, hen 48%, quail 40%, duck 38%. The sensitization prevalence of each yolk egg extract was: hen 31%, quail 25%, turkey 21%, duck 13%. The sensitization prevalence of each meat extract was: hen 23%, turkey 8%. The sensitization prevalence of each hen's protein was: Gal d 1 38%, Gal d 2 13%, Gal d 3 21%, Gal d 4 2%, Gal d 5 17%.

Conclusions: The results confirm differences in sensitization towards the various tested eggs and poultry meats. Filtering the sensitization results with an accurate patient's history combined with specific oral food challenges could provide improvement to the allergic patient's diet. The specific advantage of FABER relies on the chance of simultaneously testing patients to a broad panel of avian eggs, poultry meat extracts and HE allergenic proteins, thus reducing the chance of a misdiagnosis due to the lack of all known allergenic molecules. At the same time, FABER provides further data on

sensitizations to the largest currently possible number of other allergenic sources and molecules to which the patient can be sensitized along with the avian allergens.

0062 | The variability of the egg specific cut-off IgE levels to predict clinical reactivity by total IgE, age and types of oral food challenge tests

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Introduction: Food specific(s) IgE levels are still the main tools to predict the clinical reactivity against foods in physician's practice. Previous studies suggested different cut-off values for egg-white (EW) sIgE in childhood.

Objectives: We aimed to determine the confounding factors influencing EW sIgE cut-off levels to predict clinical reactivity. In methods, IgE-mediated egg allergy was diagnosed in the presence of a consistent history of reactivity with egg exposure and positive skin prick tests, sIgE levels or oral food challenges (OFC). The predicted probability curves of EW sIgE levels for clinical reactivity were determined by linear logistic regression analysis and were created according to the age groups: <2, 2–4, >4 years, total (t) IgE levels and types of OFC tests.

Results: A total of 196 (50.3%) of 363 egg allergic children underwent challenge tests [88 (44.9%) open; 108 (55.1%) DBPCFC]. Twelve (6.1%) had tIgE≥1000 IU/l. Except EW sIgE, baseline characteristics were similar; EW sIgE levels at OFC test were significantly higher in children who underwent DBPCFC and in those with tIgE>1000 IU/l. Children who underwent DBPCFC had more frequent anaphylaxis at OFC. EW sIgE levels showing clinical reactivity with 90% probability by using predicted probability curves were found 12.8 kU/l for all ages (7 in open, 18.8 in DBPCFC); 13.5 for <2 years; 9.7 for 2–4 years in children with tIgE<1000 IU/l. When all subjects were pooled regardless of tIgE levels or type of OFC test, EW sIgE cut-off value was found as 36 in all ages and 13.2 for <2 years.

Conclusions: Our results indicated the cut-off values of EW sIgE could be different by age groups, tIgE levels and types of OFC tests in childhood. In children less than 2 years of age EW sIgE is not influenced by tIgE but in children greater than 2 years tIgE should be considered before deciding OFC test.

0064 | Re-analysis of a new luminex-based peptide assay (LPA) to identify different degrees of milk allergic reactivity

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Introduction: The majority of children with cow's milk allergy (CMA) tolerate baked-milk products. However, reactivity to fermented milk products like yogurt and cheese has not been previously evaluated. A novel LPA was used to identify IgE binding to allergenic epitopes in milk-allergic Turkish children who tolerated different forms of milk products in oral food challenges (OFCs). We sought to determine whether LPA results could distinguish patients' clinical reactivity to different forms of milk, e.g. baked-milk (muffin), yoghurt-cheese, and whole unprocessed milk.

Objectives: Milk-allergic children were identified by OFC outcome: Reactive to baked-milk (n=16), Reactive to yogurt-cheese (n=18), Reactive to whole milk (n=23), and Outgrown milk allergy (n=32). Milk, casein, and b-lactoglobulin sIgE and sIgG4 levels were determined by UniCAP; IgE and IgG4 binding to milk protein epitopes were assessed by LPA.

Results: Overall, levels of IgE-specific epitope binding were strongly associated with varying degrees of milk protein denaturation, with consistently increasing allergenic epitope binding diversity from Outgrown to Baked-milk subjects. Similar associations were observed for milk, casein and b-lactoglobulin sIgE, assayed by UniCAP. Using machine-learning techniques, we sought to develop models that could predict different degrees of CMA. Data were randomly divided in two groups with 75% of the data retained for model development (n=68) and 25% for testing (n=21). The predictive ability of LPA data was compared with that of models using serum component proteins (UniCAP), both using a random forest algorithm. All 68 children used for training were correctly classified for models using IgE or IgE plus IgG4 epitopes. The average cross-validation accuracy was much higher for models using IgE and IgG4 epitopes (84.8%), twice the performance of the serum component proteins assayed by UniCAP (41.9%). The 21 children whose data were not used for modeling, were then used to test the performance of the model on 'unseen' data. The model including IgE peptides

correctly predicted 86% of the patients (AUC=0.89) while the accuracy of IgE plus IgG4 model was 81% (AUC=0.94).

Conclusions: Using a novel high-throughput LPA, we were able to distinguish the reactivity and diversity of IgE/IgG4 binding to allergenic epitopes in varying CMA endotypes. LPA may be useful in distinguishing different degrees of CMA in clinical settings without using oral food challenges.

0065 | Using IgG4 for cow's milk proteins to define risk of eosinophilic esophagitis in the community

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Introduction: Low titer specific IgE antibodies to common foods, especially cow's milk (CM) proteins, are an important feature of eosinophilic esophagitis (EoE). Recently two groups reported high levels of food-specific IgG4 antibodies in adults with EoE. Our objective here was to analyze serum specific IgG4 antibodies to CM proteins in children with a new diagnosis of EoE and to compare the results to a group of age-similar children from an unselected birth cohort (Project Viva).

Objectives: All sera were assayed for specific IgG4 antibodies to the CM proteins Bos d 4 (alpha-lactalbumin), Bos d 5 (beta-lactoglobulin) and Bos d 8 (caseins), as well as total IgG4 using the ImmunoCAP 250 instrument (units=μg/ml). Sera were collected from 71 children enrolled at either the University of Virginia (Charlottesville, Virginia) or Nationwide Children's Hospital (Columbus, Ohio) after being diagnosed with EoE by esophageal biopsy (≥15 eosinophils per high-power field). To obtain an estimate of the IgG4 response to CM in the community (*i.e.*, a mixture of allergic and non-allergic children), sera from 210 out of 616 children (referred to as random controls hereafter) were chosen at random from an unselected birth cohort.

Results: Preliminary studies confirmed that the IgG4 assays for CM proteins are specific for the individual antigens and that they are quantitatively accurate. Despite being quite common in the community, specific IgG4 antibodies to CM proteins were dramatically higher in titer among children with EoE compared to random controls ($P<.001$ for each). In addition, ROC analysis revealed a strong association between EoE diagnosis and specific IgG4 antibodies to Bos d 4 (area under curve=0.78), Bos d 5 (area under curve=0.74), and Bos d 8 (area under curve=0.78) ($P<.001$ for each). Furthermore, the prevalence of high titer specific IgG4 antibodies ≥5 μg/ml was significantly higher in children with EoE compared to random controls for Bos d 4 (odds ratio=8.2), Bos d 5 (odds ratio=4.6), and Bos d 8 (odds ratio=7.5) ($P<.001$ in each case).

Conclusions: The titers of specific IgG4 antibodies to CM proteins in EoE shown here are likely the highest levels of specific IgG4 antibodies reported in children, and these antibodies can make a significant contribution to the total IgG4. Although the IgG4 response to CM is common in the community, high titer specific IgG4 antibodies to Bos d 4, Bos d 5, and Bos d 8 are strikingly associated with EoE diagnosis.

0066 | Skin prick test preparations for seafood allergy: a molecular and immunological assessment

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Introduction: Allergy to seafood affects over 200 million people worldwide and is one of the most common causes of anaphylaxis. Currently available diagnostic methods do not reflect the wide diversity of fish and shellfish species consumed globally and the diagnostic utility of *in vivo* methods such as Skin Prick Testing (SPT) is limited by many factors. Preparations are only available for specific seafood species and are often not well standardised, significantly impacting the predictive value of SPT results.

Objectives: We aimed to evaluate several commercial SPT preparations on a molecular and immunological level by determining (a) the presence and abundance of seafood allergens and other proteins and (b) the pattern of *in vitro* IgE reactivity in patients with confirmed seafood allergy.

Results: SPT preparations from six different manufacturers, including 20 different fish and shellfish species, were compared. The total protein concentrations varied more than 10-fold. The SDS-PAGE separated proteins were quantified by densitometric analyses and great variations in SDS-PAGE profiles were observed between preparations of the same species from different manufactures. ELISA and immunoblot experiments were conducted using mono- and polyclonal antibodies specific to the major fish and shellfish allergens (parvalbumin and tropomyosin, respectively), as well as serum of patients with confirmed seafood allergy. IgE-reactive proteins were subsequently identified and the protein composition was further analysed by mass spectrometry. Special focus was given to cod, salmon, tuna, crab, and shrimp. Besides expected species-specific allergens and antibody recognition patterns, we found a great variance between SPT preparations for the same species from different manufacturers. Commonly used cod SPT preparations, for example, showed great differences in the content of allergens, resulting in over 4-fold difference in IgE reactivity.

Conclusions: Commercial seafood SPT preparations showed great variances in protein and allergen content, resulting in considerable differences in IgE-reactivity. There is an urgent need for region-specific, standardised allergen preparations leading to better patient management. To improve current diagnostics the inclusion of purified allergens could be considered.

MONDAY, 19 JUNE 2017

OAS 20

MANAGING ASTHMA FROM INFANCY TO ADOLESCENCE

0073 | Breast-milk microbiome and risk of asthma by 6 years of age

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Introduction: Early gut-mediated microbial stimulation appears central to normal development of functional immunoregulation and may thus influence the risk of allergies and asthma. Breast-milk is an early-life source of millions of microbes per day but its relevance prospectively on child health has not been studied.

Breast-milk microbiota were characterized from 637 breast-milk samples collected by mothers of 2 month old PASTURE birth cohort children from Finland, France, Germany and Switzerland using quantitative PCR and 16S rDNA amplicon sequencing by Illumina MiSeq platform. Asthma development was followed until the age of 6 years.

Objectives: To determine whether the breast-milk microbiota may, depending on its composition, reduce or increase the risk of developing asthma in childhood.

Results: Based on preliminary data the median (IQR) total bacterial concentration in breast-milk samples was 9.5×10^4 (3.4×10^4–2.9×10^5) cell equivalents (ce) per ml with median 2 to 1 ratio of gram-positive to gram-negative bacteria. The median operational taxonomic unit (OTU) richness, at 97% sequence similarity level, was 100 (45–185). In over half of the samples 90% of all OTUs were classified into *Firmicutes* and *Proteobacteria* phylum and over one fifth to *Streptococcus* genus. The median fungal concentration was 155 (0 – 324) ce/ml.

High concentration of gram-negative bacteria was associated, independent of maternal allergy, other confounders and bacterial markers, with lower life-time risk of asthma by the age of 6 years; adjusted odds ratio (above vs below median concentration) 0.4 (0.20–0.77), $P<.007$. However, there was heterogeneity ($P<.05$) in the association between the different study centers.

Conclusions: Our data supports breast-milk as a considerable source of early-life microbial exposure and provides first indication that breast-milk microbiota may affect the risk of asthma and thus have prolonged impact on child health.

0074 | Sex-shift of respiratory multimorbidity prevalence during adolescence – pooled analyses of longitudinal european birth cohort data from medall

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Introduction: Allergy prevalence in childhood is higher in boys than girls. For the separate diagnoses of rhinitis and asthma, a shift to a female predominance in adolescence has been suggested, but evidence for patients with respiratory multimorbidity is scarce. We examined the sex-specific multimorbidity of rhinitis and asthma before and after puberty onset in European birth cohorts.

Objectives: We harmonized data from self-/parent-completed questionnaires from population-based birth cohorts (PIAMA, BAMSE, LISAplus, GINIplus, DARC and MAS) from the EU Project MeDALL to assess rhinitis, asthma (ISAAC) and puberty status (before vs after puberty onset). We used generalized estimating equations to analyse the associations of sex, age, puberty (yes/no), and possible confounders with respiratory multimorbidity (rhinitis with coexisting asthma). Additionally, we analysed IgE- and non-IgE-associated respiratory multimorbidity separately, whereby IgE-associated disease was defined by specific serum antibodies against one or more

common allergens. We combined data of all cohorts with one stage Individual Participant Data meta-analysis.

Results: We included data from 18 852 children from birth to age 14–20 years. The prevalence of respiratory multimorbidity was lower in girls before puberty onset: adjusted odds ratio (females vs males) 0.55, 95%-CI 0.46–0.64, this male predominance shifted to a sex-balanced prevalence after puberty onset (0.89, 0.74–1.07). This shift was statistically significant, interaction sex*puberty $P<.001$.

The prevalence of IgE-associated respiratory multimorbidity showed similar results with a strong male predominance before (0.56, 0.44–0.71) and no sex differences (0.91, 0.73–1.13) after puberty onset, $P<.001$. In non-IgE-associated respiratory multimorbidity, we found a slightly smaller male predominance before puberty onset (0.63, 0.47–0.85) also shifting to a sex-balanced prevalence after puberty onset (1.04, 0.71–1.53), $P=.019$. These shifts in sex differences from before to after puberty onset showed to be stronger than those from earlier analyses in the same cohort data in patients with only rhinitis or asthma, where we found no significant sex-specific changes.

Conclusions: Analysing birth cohort data we found a clear male predominance in respiratory multimorbidity prevalence before puberty and a sex-shift towards a sex-balanced prevalence in adolescence. This shift was stronger than in rhinitis or asthma only.

0075 | Patients with rhinovirus a or c species induced severe bronchiolitis are at increased risk of using asthma control medication 4 years later

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Introduction: Rhinovirus (RV) -induced bronchiolitis has been linked to increased risk of asthma. Over 160 different rhinovirus types have been identified and divided into three species: RVA, RVB and RVC. RVA and RVC have been shown to cause more severe acute illness but the relation of species to risk of future asthma remains unknown. In this study, we evaluated the association between rhinovirus species causing severe bronchiolitis and the need for asthma medication 4 years later.

Objectives: We enrolled 408 children hospitalized for bronchiolitis at age of <24 months (attending physician's diagnosis) over two consecutive winter seasons, from November 2008 to March 2010, in three university hospitals in Finland. The viruses were detected from nasopharyngeal aspirates at index hospitalization and clinical symptoms and background information were recorded. Follow-up was performed as a mailed questionnaire 4–5 years after hospitalization. Those not responding were interviewed by phone. The main outcome was current (i.e. during the last 12 months) use of asthma control medication (i.e. inhaled corticosteroid or leucotriene receptor antagonist). Analyses were repeated using a strict definition for bronchiolitis (age <12 months and first episode of wheezing).

Results: A total of 350/408 (86%) of the subjects completed follow-up (265 by questionnaire and 86 by telephone interview). Rhinovirus was found in 111/350 (32%) of the analytic cohort. Of 111 rhinovirus positive cases, RVC was found in 82 cases (75%), RVA in 25 (23%), RVB in 3 (2.7%), and RVB+RVC in one (0.9%) case. Due to small sample size, RVB cases were excluded from the analysis. In multivariable analyses adjusted for history of atopic dermatitis, use of systemic corticosteroid at index hospitalization and parental asthma, overall RV was highly associated with current use of asthma control medication 4 years after severe bronchiolitis (RV vs. non-RV, odds ratio [OR] 3.4, 95% confidence interval [CI] 2.0–5.7, $P<.001$). Compared to non-RV, both RVA and RVC were highly associated to current medication (RVC OR 3.5, 95%CI 2.0–6.3, $P<.001$; and RVA OR 3.1, 95%CI 1.2–7.7, $P=.02$, respectively). In the strict criteria group, the result with RVC remained (OR 4.2, 95%CI 1.5–12). RVA was not computable for the strict diagnosis because of small sample size (n=3).

Conclusions: Both rhinovirus A and rhinovirus C induced severe bronchiolitis are associated with increased long-term use of asthma control medication.

0076 | Rhinovirus and respiratory syncytial virus genome load and the post-bronchiolitis use of asthma controller medication: prospective 4-year follow-up study

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Introduction: Bronchiolitis is an infection of small airways that, when severe, causes breathing difficulties and hospitalization in young children. Respiratory syncytial virus (RSV) is the most frequent cause of bronchiolitis, but other viruses, especially rhinovirus (RV), are also detected. Bronchiolitis is associated with subsequent development of asthma, but whether it is the infection itself or the susceptibility of the child which primarily drive this development remains unclear. In this study, we evaluate the association between virus genome load during bronchiolitis and the use of asthma controller medication 4 years later.

Objectives: We enrolled 408 children hospitalized for bronchiolitis at age <24 months in two consecutive winter seasons from 2008 to 2010 in a prospective, 3-center cohort study in Finland. Virus genome loads were quantified by real-time rtPCR from nasal wash samples and further divided in tertiles. The parents underwent interview during hospitalization. Four years later, the current use of asthma controller medication (i.e. use of inhaled corticosteroid or leucotriene receptor antagonist during the preceding year) was asked in a structured questionnaire. Logistic regression was used for analyses.

Results: In total, 351 (86%) children completed the 4-year follow-up; of them, 111 (32%) were RV positive, 155 (44%) were RSV positive, and 94 (27%) had been prescribed asthma controller medication during the past 12 months. RV type C (RVC) was found in 82 (75%), RV type A (RVA) in 25 (23%), RV type B (RVB) in 3 cases, and RVB+RVC in one case. Overall, there was an inverse relationship between the RV genome load and the use of medication (*P*=.04). Compared to children with low RV loads, children with high RV loads used medication less often (adjusted odds ratio [aOR] 0.37; 95% confidence interval [CI] 0.14–0.995; *P*=.049); the low vs. intermediate comparison was not significant. This novel finding appeared stronger in the RVC group (low vs. intermediate RV load aOR 0.26, 95%CI 0.08–0.85, *P*=.03; and low vs. high RV load aOR 0.21, 95%CI 0.05–0.78, *P*=.02). Among children with RVA, there was no difference in medication use by genome load. Likewise, we found no association between RSV genome load and medication use.

Conclusions: Among children hospitalized for bronchiolitis, the RV genome load during bronchiolitis was associated with the use of asthma controller medication: children at highest risk for future use of medication are those with low RV genome load, especially if they have RVC.

0077 | Exercise-induced bronchoconstriction in children with asthma: an observational cohort study in Taiwan

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Introduction: The prevalence of exercise-induced bronchoconstriction (EIB) were 5 to 20% of general population and 40 to 90% of adolescents and adults with known asthma. The diagnosis of EIB was established by changes in lung function after exercise challenge. The prevalence of EIB, lung function changes after exercise challenge, and relations between EIB and airway hyperresponsiveness were not fully described in children with asthma.

Objectives: The aim of this study was to investigate the prevalence and predictors of EIB in children with asthma in Taiwan. From a cross-sectional study on children with physician-diagnosed asthma (aged 2–10 years) (N=187), a total of 149 children above 5 years of age underwent standardized treadmill exercise challenge for EIB and methacholine challenge for airway hyperresponsiveness from October 2015 to December 2016. All participants were evaluated by a modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire and an interview conducted by a paediatric allergist.

Results: Exercise-induced bronchoconstriction presented in 52.5% of children with asthma in Taiwan. Compared with children without EIB, there were more patients with atopic dermatitis in children with EIB (*P*=.038). Allergic to *Dermatohagoides pteronyssinus* and *Dermatophagoides farinae* were also found more in children with EIB (*P*=.045 and 0.048 respectively). Exercise challenge positive rate was highest in patients who were most sensitive to methacholine provocation. Maximal decrease in forced expiratory volume in 1 second (FEV_1) were highest in patients who were most sensitive to methacholine provocation (PC_{20}<=1 mg/ml). Patients, who were more sensitive to methacholine challenge (with lower PC_{20} levels), develop EIB with more decline in FEV_1after exercise challenge (*P*=.038). Among patients with EIB, airflow limitation development in patient with methacholine-induced airway hyperresponsiveness was relatively more abrupt and severe compared with patients without methacholine-induced airway hyperresponsiveness.

Conclusions: Exercise-induced bronchoconstriction presented in 52.5% of children with asthma in Taiwan. Patients, who were more sensitive to methacholine challenge (with lower PC_{20} levels),

developed exercise-induced bronchoconstriction with more decline in FEV_1 after exercise challenge (P=.038).

0078 | The influence of polyvalent mechanical bacterial lysate on immunological parameters in asthmatic children

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Introduction: The aim of the study was to assess the specific changes occurring in a panel of immunological parameters as the result of PMBL treatment in asthmatic children.

Objectives: Randomized, double blind, placebo-controlled, parallel-group study was done (EudraCT number: 2013-000737-12 and NCT0254133, EOLIA Study). The protocol included a total of 3 visits: screening/randomization visit (V1), biology visit (3 weeks; V2) and end-of-treatment visit (12 weeks; V3). A subset of 49 patients (21 with Polyvalent Mechanical Bacterial Lysate – PMBL, Ismigen tablets and 28 with placebo) treated with 1 tablet daily, 10 days per month, through 3 consecutive months aged 6–16 years with asthma were randomized. Main inclusion criteria were: Allergic asthma diagnosis with at least one perennial allergen sensitization according to the GINA 2012 guidelines prior to screening visit, already treated with SABA PRN and ICS or ICS+LABA during the previous 3 months and at least 2 exacerbations of asthma within the 12-months period before study. The results were compared between date from V3 and V1.

Results: Among peripheral blood CD3+ T cells, significant decreases of T cell activation markers were observed in the PMBL group, as both CD3+CD69+ cells (early activated) and CD3+CD25+ cells (late activated) were decreased, whereas increases occurred in the placebo group (respectively $-2.2\pm5.2\%$ vs $+2.5\pm15.3\%$, P=.014 and $-0.6\pm5.3\%$ vs $+0.7\pm4.1\%$, P=.024). Number of CD3+CD8+ cell increased in the PMBL group ($+136.6\pm324.3$) whereas decreased in placebo group (-92.7 ± 282.8)(P=.018). The absolute number of lymphocytes expressing a Treg-like phenotype was increased in the PMBL group as compared to the placebo group ($+6.5\pm37.3$ vs -10.8 ± 24.8, P=.039 respectively). The NK cells absolute count was increased in the PMBL group and decreased in the placebo group ($+19.1\pm114.8$ vs -35 ± 164.0 respectively, P=.046). Possible explanation could be that PMBL reduces the loco-regional inflammatory status secondary to infections or aeroallergen exposure. This hypothesis is supported by the observed decrease of activation markers in T cells (CD25 and CD69) and the increase of regulatory T cells.

Conclusions: PMBL tablets exert the immunomodulatory effect via stimulation of NK cells and cytotoxic CD3+CD8+ lymphocytes and can reduce allergic inflammation in the Treg-dependent mechanisms in asthmatic children.

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NEW APPROACHES TO THE DIAGNOSIS AND MANAGEMENT OF FOOD ALLERGY

0079 | Treatment of human dendritic cells with STAT6-IP inhibits proliferation of lymphocytes from peanut allergic donors and reduces TH2 cytokines: a novel approach for treatment of food allergies.

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Introduction: Food allergies affect up to 6% of children and 4% of adults. The burden of illness for patients is significant yet there has been little important advancement in treatment. Food allergies develop when TH2-type immune responses are activated by food proteins, leading to formation of allergen-specific IgE. Upon subsequent exposure the allergic cascade is activated, resulting in anaphylaxis. STAT-6 is a transcription factor that regulates TH2 gene expression. We have generated and tested STAT6-IP, a peptide that inhibits STAT-6-dependent events and is immunomodulatory in experimental allergic asthma.

Objectives: We hypothesized that, using cells from peanut allergic donors, STAT6-IP treatment during *in vitro* exposure to peanut protein would result in decreased responsiveness to food allergens. Using peripheral blood mononuclear cells (PBMC) from allergic human donors we studied STAT6-IP treatment of dendritic cells (DC) on T and B cell responsiveness to food allergen challenge *in vitro*.

Human PBMCs were obtained from peanut allergic and non-allergic volunteer donors. Monocytes, peripheral T and B cells were isolated. Monocytes were differentiated into DC. Mature DCs were then exposed to purified peanut extract (PPE)±STAT6-IP. The DCs were then co-cultured with T or B cells and assayed for proliferation and cytokine expression/production.

Results: T cells from allergic donors proliferated in the presence of PPE with associated increases in the TH2 cytokines IL4 and IL13. STAT6-IP treated cultures demonstrated markedly reduced proliferation to PPE in a dose dependent manner. Production of IL4 and IL13 was also decreased as was the frequency of IL4+ve T cells, to near unstimulated control levels. B cells were similarly affected. These data suggest that STAT6-IP treatment reduces the proliferative potential of the TH2 cells preventing the elaboration of TH2 cytokines required to propagate allergic responses.

Conclusions: We have shown human STAT6-IP treatment of DCs qualitatively and quantitatively reduces TH2 cell proliferation, cytokine production and responsiveness to peanut allergen stimulation.

Definitive management of food allergies requires prevention or redirection of the aberrant (TH2) immune responses. Treatment with STAT-6-IP in conjunction with allergen exposure reduces proliferation of B and T cells in the presence of allergen. We anticipate that this targeted immunotherapy has significant clinical translation for this devastating disease.

0080 | A hypoallergenic hydrolysate of ovalbumin with pepsin exerts preventive and therapeutic effects in a mouse model of egg allergy

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Introduction: Egg allergy is among the most common food allergies in European children below the age of three. Oral immunotherapy (OIT) using whole egg white (EW) appears as a potential treatment option for egg allergy, although its major drawback is the high risk of severe side effects. This obstacle has prompted the investigation of allergen-derived immunomodulating peptides to be used in OIT to induce oral tolerance, or even as a strategy to prevent allergic sensitisation, once assured they lack sensitising potential. In this respect, hydrolysis of egg white proteins appears as an attractive and safe alternative for a reproducible and standardized production of immune-active peptides with low associated costs.

Objectives: The aim of this work was to evaluate the preventive and therapeutic properties of ovalbumin (OVA) hydrolysed with pepsin (OP) in an *in vivo* model of egg allergy.

Results: The sensitising and eliciting capacities of OP were evaluated in BALB/c mice orally administered either EW or OP. In addition, mice sensitised to EW were used to assess the preventive and curative potential of OVA and OP, orally administered before or after sensitisation. At the end of the experimental periods, mice were orally challenged with EW and anaphylactic responses were evaluated by scoring clinical signs and rectal temperature. Blood and faecal samples were collected to study the antibody production at different time points throughout the experiments. Furthermore, the intestinal gene expression profile was analysed by RT-qPCR and T cell subsets were phenotyped using flow cytometry.

OP did not show sensitizing or eliciting potential in BALB/c mice. In addition, this hydrolysate was able to reduce the production of EW-specific IgE and IgG1 and prevent the development of clinical signs when administered either before or after sensitisation to EW. Furthermore, a significant down-regulation of Th2-related intestinal epithelial genes (IL-33, IL-25 and TSLP), as well as a marked reduction of splenic Th2 lymphocytes was observed in mice administered OP. The preventive and therapeutic administration of OP increased the expression of regulatory cytokines, TGF-β, IL-10, and IL-17 in intestinal tissues and expanded the regulatory T cell population in

the spleen. Overall, OP was more efficient than intact OVA in the prevention and treatment of egg allergy.

Conclusions: OVA hydrolysed with pepsin is hypoallergenic and exerts preventive and therapeutic effects, more efficiently than OVA, in a mouse model of egg allergy.

0081 | Phenotypical characterization of peanut allergic children with differences in cross-allergy to tree nuts and other legumes

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Introduction: Peanut allergy in children is often associated with allergies to tree nuts and/or legumes. The aim of the present study was to analyze in cluster a cohort of children allergic to peanuts and assessed for cross-reactivity to nuts and legumes and to identify different phenotypes.

Objectives: Our main objective was to identify different phenotypes of PA children with cluster analysis.

We also tried to identify possible risk factors for cross-allergy to TN and other legumes. We included retrospectively 317 children with peanut allergy evaluated at the Allergy Unit of the Saint Vincent Hospital of Lille in the last 12 years. A complete work-up for peanut allergy and nuts and legumes was carried out for each patient. A hierarchical agglomerative clustering method was used to search for clusters of individuals in the evaluated cohort.

Results: Cross-allergy to TN and/or other legumes was identified in 137 patients (43.2%), atopic dermatitis being a major risk factor (adjusted OR=16 [95%CI: 7.4–37]; $P<.001$). Three phenotypes emerged from cluster analysis. Cluster 1 (72 patients) is characterized by high level of rAra h 2, low threshold reactive doses for peanut and high proportion of asthma; Cluster 2 (93 patients) is characterized by high threshold reactive doses for peanut and the lowest proportion of cross-allergy to TN and/or legumes; Cluster 3 (152 patients) has a high risk of cross-allergy to TN and/or legumes and most patients suffer from eczema.

Conclusions: The three phenotypes highlighted by the present study could be useful to identify children with high risk of cross-allergic reaction to TNs and legumes early after PA diagnosis.

0082 | Food products and undeclared allergens causing accidental allergic reactions in daily life

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Introduction: Accidental allergic reactions to food are frequent and can be severe and even fatal.

Objectives: Analyze the food products causing accidental reactions and the levels of undeclared allergens.

A prospective cohort study was conducted with adults (n=157) with a physician-diagnosed food allergy. All patients reported accidental allergic reactions and sampled the culprit food products for 1 year. Food samples were analyzed for suspected non-ingredient allergens and quantification of risks.

Results: In 118 of 151 accidental reactions, allergic individuals were able to attribute their symptoms to a specific product. A large variety of food products was involved. More than half (53%) of the products were from a relative small/limited selection: bread (rolls), cookies, chocolates, meat products and fruits. Food samples received were analyzed for 28 different allergens, ranging from 1–15 allergens per product, and on average 5 – 6 measurements per product. Analysis confirmed up to 4 undeclared allergens in 19 (37%) of the 51 received products. Concentrations varied from 0.01 ppm to even 5000 ppm protein of the allergenic food and were highest for peanut, milk and sesame. There was no correlation with the presence or absence of precautionary warning. In most of the unexpected allergic reactions, the allergen intake by patients was in (extreme) excess of Reference Doses as proposed by Taylor et al (2014). For each product at least one undeclared allergen presented an estimated risk for >1% up to 64% of the allergic users of the products. Undeclared milk proteins posed the highest risk in the analysed products.

In part of the accidental reactions it was difficult to attribute the reaction to a specific food product or allergen. Reasons could be that meals consisted of multiple products, allergens might be not equally distributed through the food, or the wrong product was provided by patients.

Conclusions: A variety of food products and undeclared allergens cause accidental allergic reactions, for which a limited number of product categories was responsible for the majority of accidental reactions. Undeclared allergens make choosing a safe food problematic for allergic patients, especially for those with multiple allergies. As there was no correlation with the presence or absence of a precautionary warning, guidance and harmonization of precautionary allergen labelling is warranted.

0083 | Food allergens recalls on rapid alert system for food and feed (rasff) portal from the EU Commission: 2011 – 2016 analysis

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Introduction: Food allergy is a growing public health problem and food safety concern. The unintended presence of allergens impairs the compliance of dietary avoidance and can put patients at serious risk. In 1979, the EU Commission developed a rapid alert system for food and feed (RASFF) portal, which provides a continuous service to ensure that urgent notifications are reported and responded collectively between all EU countries, Norway, Liechtenstein, Iceland and Switzerland.

Objectives: Our aim was to analyse the European notifications in RASFF portal related to food allergens in the last five years, particularly before and after the entering into force of the Regulation (EC) 1169/2011.

Methods: All occurrences in allergens category reported by all RASFF members in the last five years (31 December 2011 – 31 December 2016), were searched in RASFF online portal. The main allergens hazard, product categories and notifying countries were analysed.

Results: A total of 484 notifications regarding food allergens were identified between 2011 and 2016, and the most notified countries were UK (n=77), Italy (n=44), Sweden (n=38), Slovakia (n=33) and Netherlands (n=33). The number of allergen incidents were higher in 2015 (n=138) and 2016 (n=113) than in earlier years (n=85 in 2012; n=70 in 2013; and n=78 in 2014), and the main allergen alert notifications were for milk (n=131), cereals containing gluten (n=75), eggs (n=65), nuts (n=56), and peanuts (n=51). In those five years, incidents related to cereals and bakery products (26.7%), meat and meat products (9.7%), and ices, desserts and confectionery (9.7%) appeared more frequently and the main basis of the notifications were official control on the market (44.6%), company's own check (33.7%), and consumer complaint (10.7%). As response, the actions most frequently taken were recall from consumers (35.3%), withdrawal of the product from the market (30.5%), informing recipients (7.0%), and product relabelling (5.0%).

Conclusions: In 2015 and 2016, there has been an increase in notifications of incidents with milk, peanuts, nuts, eggs, and soy, which might be related with EU legislation 1169/2011 that came into force in December 2014 and covers new disposals regarding provision of food allergens information. However, the increasing in notifications along with the notifications due to consumer complaints may indicate that substantial efforts are needed to ensure better protection of consumers suffering from food allergies.

0084 | A randomized, double-blind, placebo-controlled, pivotal multicenter trial with budesonide orodispersible tablets for treatment of active eosinophilic esophagitis (eos-1)

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Introduction: Eosinophilic Esophagitis (EoE) is a chronic-inflammatory disease of the esophagus. So far no approved therapy is available, although swallowed topical corticosteroids have indicated efficacy in the treatment of EoE.

Objectives: To prove the superiority of a novel budesonide orodispersible tablet formulation specifically designed for EoE vs. placebo for the induction of clinico-histological remission in adult patients with active EoE. Primary endpoint: Rate of clinico-histological remission. Secondary endpoints: Change in total modified EEsAI endoscopic instrument score as well as its inflammatory and fibrotic subscores and overall assessment by endoscopist.

Results: Clinico-histological active EoE patients (n=88) randomly received 6-weeks double-blind treatment with either 1 mg budesonide orodispersible tablets twice daily (BID) (n=59) or placebo BID (n=29). Budesonide 1 mg orodispersible tablets BID were highly statistically superior to placebo in achieving clinico-histological remission and improvements in inflammatory as well as fibrotic subscores in the endoscopy (see Table 1).

In 3 patients (5.1%) under budesonide vs none under placebo, suspected local fungal infections with endoscopic and clinical signs (all mild) were histologically confirmed by sensitive Grocott staining. Bolus impaction requiring an emergency endoscopy for retraction was observed in 1 patient of the placebo group. Neither serious adverse events nor clinically relevant changes in the morning serum cortisol levels were observed in any treatment group.

Conclusions: Orodispersible budesonide tablets are highly effective and safe for a quick induction of clinical and histological remission as well as endoscopic improvement during a 6-week treatment of EoE.

	Budesonide 1 mg orodispersible tablet BID (n=59)	**Placebo BID (n=29)**	***P*-value (1-sided)**
Primary efficacy endpoint			
Number (%) patients in clinico-histological remission at wk6 (LOCF)	34 (57.6%)	0 (0%)	< 0.0001
Secondary endoscopic endpoints			
Mean [95% CI] change from baseline to wk6 in total modified EEsAI endoscopic instrument score (0–9)	−2.6 [−3.1; −2.1]	−0.1 [−0.8; 0.5]	
Mean [95% CI] change from baseline to wk6 in modified EEsAI endoscopic inflammatory subscore (0–4)	−2.1 [−2.5; −1.7]	−0.0 [−0.4; 0.3]	
Mean [95% CI] change from baseline to wk6 in total modified EEsAI endoscopic fibrotic subscore (0–4)	−0.4 [−0.6; −0.2]	−0.1 [−0.5; 0.4]	
Number (%) patients with endoscopist's overall assessment of 'no signs of EoE' at wk6 (LOCF)	36 (61.0%)	0 (0%)	

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WHAT'S NEW IN AEROBIOLOGY

0085 | The inventory of the pollen monitoring networks worldwide: results of the TF-40108 (ig aerobiology & pollution)

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Introduction: It is expected that about 600 stations for pollen and other biological particles in ambient air exist worldwide (Buters 2014). However, currently no comprehensive information on pollen monitoring sites is available as the map in the "Atlas of allergy" (Buters 2014) is already outdated and/or incomplete. In fact, in many cases no information, limited information, or false information on who, where, what and how monitoring in ambient air was performed. This is a great disadvantage for networking and community services purposes. As such, we undertake the task of updating, maintaining and disseminating an inventory of pollen monitoring stations in the world (task force TF-40108 sponsored by the EAACI).

Objectives: The aim of this work is to create an updated inventory and to provide comprehensive information on the pollen monitoring networks worldwide. To document the exact location, person responsible and their contact information as well as the methodologies and type of data that each station is collecting was our major goal.

Results: A team formed by working and advisory members defined the criteria needed to be recorded. We then constructed a database with detailed information about the existing national/regional networks of capture sites in the world. An interactive map with Information on Pollen Monitoring Sites (POMS) over the World and contacts were build and will be made available online on the EAACI Website, where all the details concerning each site can be easily accessed. Currently we have about 745 stations from 53 countries from different continents in the database. Not all countries have monitoring sites.

Conclusions: This will enable quick access to local pollen data and will greatly facilitate the establishment of collaborations in the field of Aerobiology & Pollution. Due to the use of an interactive map updates can be easily implemented, as we expect corrections and additions once we go online.

0086 | Towards automatic, real-time pollen monitoring for allergic patients: need for health information services?

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Introduction: To date, high-risk pollen exposure alerts have been provided only via pollen season forecasting models and conventional monitoring methods that are laborious and not time-effective.

Objectives: The aim of this study was to disseminate airborne pollen measurements using a novel automatic, real-time pollen sampler, so as to provide timely and accurate warning alerts to allergic patients throughout the duration of the pollen season. To achieve this, airborne pollen have been monitored in Augsburg, Germany, since 2015, using a novel automatic Bio-Aerosol Analyser (BAA 500), together with a network of conventional 7-day recording Hirst-type volumetric traps. Both techniques provided measurements on an hourly scale. The yearly pollen abundance of all recorded pollen types was estimated, as well as the start, peak and end of each pollen season. Moreover, the daily pollen abundance was assessed so as to differentiate between high-risk hours within each day. Comparisons have been made between the sampling methods so as to decide on the performance of the novel automatic pollen sampler. Dissemination methods for the widest possible informing of allergic patients and relevant health organisations have been investigated for use in daily practice.

Results: It was found that both sampling means detected comparable diversity and amount of pollen yearly. In most cases, the start, peak and end of the pollen season were satisfactorily predicted, especially for the allergenic *Betula* (birch) and Poaceae (grasses). The automatic pollen sampler sometimes overestimated the amount of pollen in the air, however, this corresponded to systematic error and was possible to fix appropriately. Training of the automatic pollen sampler improved the performance of the identification algorithms and further increased the performance. All measurements have been

broadcasted real-time via the Institute's webpage (www.unika-t.de/pollenflug/) on an hourly and daily scale.

Conclusions: Automatic, real-time information on concentrations of airborne allergenic pollen will significantly contribute to the implementation of accurate, timely, personalised management of allergies in the future. Towards this direction, novel, user-friendly health service infrastructure is needed, like mobile apps providing exposure risk alerts.

0087 | School neighbouring affects lung function and the autonomic nervous system in children

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Introduction: Community environment may influence respiratory symptoms and allergic sensitization.

Objectives: We assessed the effect of school neighbouring environment and walkability on lung function, airway inflammation and autonomic nervous system in children.

Results: Seven hundred and twelve children (51.1% girls), out of 916 participants, aged 7 to 12 years old, attending 20 primary schools were included. Lung function was assessed by spirometry, airway inflammation by exhaled nitric oxide (NO) and pH was measured in exhaled breath condensate (EBC). Pupillometry was performed to evaluate autonomic nervous system activity. Land use around schools was calculated by European Urban Atlas with the geographic information system, within a circular buffer zone of 500 m. The land use composition was further simplified with principal component analysis (PCA), where the first principal component (PC1) was characterized by discontinuous urban fabric, green urban areas and water bodies; and the second principal component (PC2) by construction sites, land without current use and railways. Walkability index was calculated based on residential density, connectivity density and land-used mix diversity within each buffer zone and characterized according to tertiles (from low to high). Mixed effect models with random effect by school were used to measure the effect of school in lung function, airway inflammation and autonomic nervous system. Intra-class correlation coefficient and the proportion of explained variation were used to quantify the effect of school and the effect of individual and neighbouring environment in the school effect, respectively. Mann–Whitney test were used to evaluate the relationship between walkability and lung function, airway inflammation, pH and pupillometry parameters.

Neighbouring environment around schools explained 44.9% and 38.0% of the school effect on FEV1 (forced expiratory volume in one second) and FVC (forced vital capacity), respectively. After adjustment to individual characteristics, a negative association was found between the PC2 and levels of FVC [β=−5.09 (−10.0; −0.15)]. Baseline pupil diameter, maximum and average constriction velocity were significantly lower in better neighbourhood walkability (5.65 vs 4.90, P=.004; 5.56 vs 4.89, P<.05; and 4.12 vs 3.69, P<.05, respectively).

Conclusions: Our findings suggest that neighbouring environment around schools influence lung function. Moreover, neighbourhood walkability appeared to affect autonomic nervous system.

0088 | Effect of nitrogen dioxide on childhood airway hyperresposiveness and incident asthma

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Introduction: Nitrogen dioxide (NO_2) is associated with childhood asthma. Exposure time of NO_2 might be important for childhood asthma. However, vulnerable time of NO_2 exposure on airway hyperresponsiveness (AHR) and asthma in schoolchildren are unclear.

Objectives: To investigate the effect of NO_2 on AHR and asthma development according to several time aspects, from prenatal period to current exposure. From 2005, 3,570 elementary schoolchildren were enrolled to a prospective 4-year follow-up survey. Individual NO_2 exposure was estimated by using an ordinary kriging method from prenatal period to 7 years of age. Information on asthma diagnosis was collected by ISAAC questionnaire. Children were considered to have "new development of asthma" for a "no" response to "asthma diagnosis ever" at the first survey but "yes" at the follow-up survey. AHR was measured by methacholine challenge test at 7 years of age and defined as methacholine PC20 $\leq$ 8 mg/dL.

Results: Higher NO_2 exposure at prenatal period, 1 and 7 years of age increased the risk of AHR (aOR, 1.323; 95% CI, 1.013–1.728, aOR, 1.332; 95% CI, 1.023–1.735, and aOR, 1.417; 95% CI, 1.085–

1.850, respectively). Higher NO_2 exposure at prenatal period and 1 year of age increased new development of asthma (aOR, 2.490; 95% CI, 1.464–4.235 and aOR, 2.199; 95% CI, 1.308–3.698, respectively), but not current exposure (aOR, 1.384; 95%CI, 0.847–2.262).

Conclusions: Both early life and current exposure to NO_2 are associated with AHR. However, early life exposure is more important on the new development of asthma. Avoidance of air pollutants exposure is required from early life to prevent future asthma development.

1. Risk of airway hyperresponsiveness according to NO_2 exposure time

	a OR*	95% CI		*P* value
Pregnancy	**1.323**	**1.013**	**1.728**	**0.040**
1 year	1.332	1.023	1.735	0.034
2 years	1.092	0.838	1.424	0.515
3 years	1.488	1.137	1.948	0.004
4 years	1.174	0.898	1.535	0.241
5 years	1.385	1.061	1.807	0.016
6 years	1.333	1.022	1.740	0.034
7 years	1.417	1.085	1.850	0.010

*Adjusted for age, sex, body mass index, preterm birth, birth weight, maternal educational degree and parental history of allergic diseases.

2. Risk of new development of asthma according to NO_2 exposure time

	a OR*	95% CI		*P* value
Pregnancy	**2.490**	**1.464**	**4.235**	**0.001**
1 year	2.199	1.308	3.698	0.003
2 years	1.210	0.735	1.991	0.454
3 years	1.384	0.844	2.269	0.197
4 years	1.300	0.794	2.128	0.297
5 years	1.095	0.674	1.779	0.714
6 years	1.021	0.627	1.662	0.933
7 years	1.384	0.847	2.262	0.195

*Adjusted for age, sex, body mass index, preterm birth, birth weight, maternal educational degree and parental history of allergic diseases.

0089 | Environmental determinants of exhaled breath condensate pH in children

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Introduction: Collection of exhaled breath condensate (EBC) is a simple and non-invasive method to obtain biomarkers from the lower respiratory tract. In healthy subjects, EBC pH is thought to be mainly influenced by the airway levels of NH_4^+, CO_2 and HCO_3^-. Both neutrophils and eosinophils have been found to release inflammatory mediators that lower EBC pH. However, other causes of variability are poorly described and studies have mainly focused on other endogenous factors.

Objectives: Thus, we aimed to assess the determinants of environmental exposure, in particular of volatile organic compounds (VOCs), in EBC pH.

Results: A cross-sectional analysis of exhaled breath condensates from 211 children, aged 7–12 years, and attending 20 primary schools, was conducted. Individual assessments included lung function and airway reversibility, airway inflammation measured by exhaled nitric oxide (NO), atopy (skin prick testing to aeroallergens) and body composition (bioelectrical body impedance analysis). Data on environmental exposure included volatile organic compounds (VOC), CO2, temperature and fungi. EBC was collected (EcoScreen Turbo, Carefusion) and pH measured (pHenomenal 1100H, VWR). VOCs were grouped using a principal component analyses and a multiple linear regression model was constructed.

The best-fitting model emerging as the most significant predictor of exhaled pH resulted in adjusted R^2 of 0.175, and included environmental aldehydes concentration (0.214, P=.001), as well as individual FEV1 (0.524, P=.014), post-bronchodilation FVC variability (0.019, P=.008), exhaled NO (0.11, P=.002), and allergic sensitization to *Dermatophagoides pteronyssinus* (−0.732, P<.001), *Alternaria alternata* (−0.813, P=.019) and cat (0.500, P=.017).

Conclusions: Our study shows, for the first time, that in addition to individual determinants such as sensitization to inhaled allergens and lung function, environmental factors may influence and should be taken into consideration when interpreting EBC pH level in children.

0090 | Cross-reactivity between tropomyosin allergens from aedes aegypti and dermatophagoides pteronyssinus involves B and T epitopes

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Introduction: Tropomyosin (TM) is a pan-allergen highly conserved in the phylum Arthropoda, including the mosquito species *A. aegypti* and house dust mite (HDM) *Dermatophagoides pteronyssinus*. The molecular aspects of cross-reactivity among allergenic TMs have been studied at the level of B epitopes; however, the functional and clinical relevance of this cross-reactivity is still a matter of debate.

Objectives: To analyse the molecular and functional cross-reactivity between TM from *A. aegypti* (Aed a 10.0101 and Aed a 10.0201) and *D. pteronyssinus* (Der p 10).

Methods: Folded and biological active recombinant Aed a 10.0101, Aed 10.0201 and Der p 10 were expressed in *E. coli* and purified. Sera from 15 Austrian HDM-allergic patients sensitized to Der p 10 were tested for cross-reactivity with mosquito TMs in ELISA. Stripped basophils re-sensitized with IgE from HDM-allergic patients were incubated with TMs and CD63 up-regulation was assessed by flow cytometry. BALB/c mice were immunized by serial intraperitoneal injections of Aed a 10.0101 or Aed a 10.0201. TM-specific IgE, IgG1, IgG2a and IgG3 levels and their cross-reactivity were determined by ELISA. Murine splenocytes were stimulated with TMs and proliferation measured by means of thymidine incorporation. T cell epitope mapping was performed with 29 overlapping peptides spanning the entire Aed a 10.0101 molecule.

Results: Der p 10-sensitized patients displayed IgE-reactivity to TMs from *A. aegypti*. Incubation of the sera with Aed a 10.0101 produced more pronounced inhibition of IgE-binding to Der p 10 than incubation with Aed a 10.0201. Aed a 10.0101 was also more potent to activate basophils sensitized with Der p 10-specific IgE. Mice immunized with Aed a 10.0101 and Aed a 10.0201 produced specific IgE, IgG1, IgG2a and IgG3 antibodies which cross-reacted with Der p 10. Their splenocytes proliferated upon stimulation with all TMs. Four regions in the TM sequences were identified to contain cross-reactive T cell epitopes.

Conclusions: In addition to humoral cross-reaction, TMs from HDM and *A. aegypti* cross-react at the T cell level, involving four potential T cell-activating regions. This cross-reactivity occurs independently of natural exposure to these allergens. Our more detailed characterization of TMs will contribute to elucidate the clinical impact of TM cross-reactivity.

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MONDAY, 19 JUNE 2017

OAS 23

NOVEL MECHANISMS IN RHINITIS AND RHINOSINUSITIS

0091 | Transdifferentiated ILC2s promote neutrophilic airway inflammation in nasal polyps of CRSwNP in cystic fibrosis

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Introduction: Cystic fibrosis (CF) is an autosomal recessive disorder characterized by a dysfunction of a chloride channel. This has deleterious effects on airways and gastrointestinal tract. Up to 45% of CF patients develop chronic rhinosinusitis with nasal polyps (CRSwNP). In CF-CRSwNP, inflammation is neutrophil driven and sinus cultures typically grow *P. aeruginosa* or *S. aureus*. We have shown that the IL-5 producing type 2 innate lymphoid cells (ILC2s) are enriched in typically eosinophilic NP of non-CF CRSwNP. However, neutrophil influx in CF-CRSwNP may alter the ILC composition. We have also demonstrated that IL-1β plus IL-12 induced a conversion of NP-derived ILC2s into IFN-γ-producing ILC1s, which was reversed by IL-4. Here, we identify epithelium-derived TGF-β, IL-1 β, and IL-23 as inducers of ILC2 transdifferentiation in CRSwNP in CF into IL-17-producing ILC3 and their role in neutrophil recruitment and maintenance in the local tissue.

Objectives: We seek to characterize all ILC subsets in CF-CRSwNP and non-CF CRSwNP. We identify which cytokines govern the ILC2 plasticity towards ILC3 in CF nasal polyps and determine their source. We analyse functional consequences of the elevated IL-17 producing ILC3 frequencies in CF.

Results: IL-17 producing ILC3 are enriched in NP of CRSwNP in CF (72% of ILC), whereas ILC2 are almost absent (0.9%), in contrast to non-CF CRSwNP (22% and 62% respectively). Increased frequencies of IL-17 producing ILC3 in CF may be a consequence of ILC2 plasticity. We show that key cytokines in CF: TGF-β, IL-1β, and IL-23 govern ILC2 transdifferentiation towards ILC3. ILC2 from non-CF CRSwNP exposed to the combination of TGF-β plus IL-1β plus IL-23 produced IL-17 and displayed a down-regulation of CRTH2 expression. Conversely, ILC3 from CRSwNP in CF exposed to IL-4 restored the CRTH2 marker expression and a subsequent stimulation with TSLP/IL-33 led to IL-5 production. This was not the case for ILC3 from non-CF CRSwNP. The major source of TGF-β, IL-1 β, and IL-23 may be epithelium as co-cultures of ILC2 with *P. aeruginosa*-challenged nasal epithelium resulted in IL-17 production by ex-ILC2s. These ex-ILC2 IL17 producers may enhance neutrophil recruitment and maintenance, as ILC2/ILC3 ratio in NP correlates with eosinophil/neutrophil ratios.

Conclusions: TGF-β, IL-1β, and IL-23 govern ILC2 plasticity towards ILC3 in CF. Elevated frequencies of IL-17 producing ex-ILC2 enhance neutrophil recruitment and maintenance in the local NP tissue in CF.

0092 | Association between type 2 innate lymphoid cells increment and allergy in patients with chronic rhinosinusitis and asthma

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Introduction: Type 2 innate lymphoid cells (ILC2s) were shown to be involved in Th2-type immune responses in allergic disease animal models. ILC2s enrichment was shown in nasal polyps in chronic rhinosinusitis (CRS) patients and peripheral blood from asthma animal models; the role of ILC2s' contribution of Th2 response in CRS and asthma remains to be investigated.

Objectives: We hypothesized that there would be enhanced expression of ILC2s in peripheral blood of patients with CRS and asthma, which may show correlation with clinical features.

6 CRS patients, 5 Asthma patients 5 healthy controls were recruited in our out-patient allergy clinic, ILC2 were quantized as Lin-CRTH2+CD127+ from PBMSs of subjects by using flow cytometry. SNOT-20, VAS score and levels of IgE were also documented. IL-5 and IL-10 were measured in serum samples from patients by ELISA.

Results: There were higher IC2 ratio (number of IC2s / one million PBMC%) in CRS group (0.958±0.6209) and Asthma group (1.1191±0.2611), compared to healthy control(0.4831 ±0.0564). ($P<.05$). SNOT-20 and VAS score and levels of cytokines were also higher in patient group than healthy controls.

Conclusions: Our results showed the IL2s ratio were correlated with clinical features of allergic airway patients, type 2 innate immune responses were higher in CRS and asthma,ILC2 maybe a good marker of airway eosinophilic inflammation and peripheral blood is useful for evaluating type 2 innate immunity in human.

0093 | Increased expression of SMAD7 in persistent allergic rhinitis

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Introduction: SMAD7 is a known negative feedback regulator of Transforming growth factor-β1 (TGF-β1). Overexpression of SMAD7 enhances Th1 and Th2 cytokine production in asthma models and inhibition of SMAD7 restores TGF-β1 signalling in human inflammatory related diseases. Previously, we reported the decreased expression of TGF- β1 and SMAD2 in persistent allergic rhinitis nasal mucosa. We sought to determine the expression of SMAD7 in the local nasal mucosa of severe persistent allergic rhinitis patients.

Objectives: Specific immunohistochemical staining was performed on inferior turbinate biopsy specimens to measure the expression of SMAD7 in subjects with severe persistent allergic rhinitis (seasonal and perennial; n=46) and healthy controls (n=19).

Results: SMAD7 expression was significantly higher in the nasal submucosa of persistent allergic rhinitis compared to healthy controls (Mean $\pm$SEM/mm^2 6.25$\pm$1.91 vs 1.79$\pm$0.53; *P*=.03). A significant increase in expression was observed in perennial allergic rhinitis compared to healthy controls (Mean$\pm$SEM/mm^2 9.86$\pm$3.48 vs 1.79$\pm$0.53, *P*=.03). Expression of SMAD7 tended to be lower in seasonal compared to perennial allergic rhinitis (Mean$\pm$SEM/mm^2 2.63$\pm$1.29 vs 9.86$\pm$3.48; *P*=.06). There was no significant change in SMAD7 expression when compared between in and out of season in seasonal allergic rhinitis nasal mucosa.

Conclusions: These findings suggest that overexpression of SMAD7 might contribute to the pathogenesis of severe persistent allergic rhinitis. Targeting SMAD7 could be an important strategy in treating upper airway allergic diseases.

[Correction added on 16 October 2017, after first online publication: Name of the author "Qiu S" has been corrected to "Qiu CS".]

0094 | Targeted metabolomics reveals novel lipid and amino acid metabolism alterations in aspirin-exacerbated respiratory disease

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Introduction: Aspirin-exacerbated respiratory disease (AERD) is characterized by asthma, chronic rhinosinusitis with polyposis and aspirin/nonsteroidal anti-inflammatory drug (NSAID) hypersensitivity. To date, the pathomechanisms of AERD remain obscure, although disturbances in the metabolism of arachidonic acid seem to contribute to the disease.

Objectives: We hypothesized that AERD patients show distinct overall metabolic profiles compared to healthy individuals and aimed to identify metabolic pathways that may play a role in AERD pathogenesis. 188 metabolites were quantified in serum of 11 AERD patients and 12 healthy controls using a targeted metabolomics approach based on LC-ESI-MS/MS and FIA-ESI-MS/MS. The analysis included free carnitine, 39 acylcarnitines, 21 amino acids, 21 biogenic amines, hexoses, 90 glycerophospholipids and 15 sphingolipids. Raw data processing and analysis was conducted with MetaboAnalyst using implemented univariate/multivariate analysis methods and pathway over-representation analysis (ORA). Participants signed a written informed consent and completed the Sinonasal Outcome Test 22 (SNOT-22) questionnaire. Spearman's correlation coefficients were determined between significantly dysregulated metabolites and the SNOT-22 scores, subgrouped for physical and psychological symptoms.

Results: A total of 14 metabolites were found to be significantly up- or downregulated in the serum of AERD patients. Of those, 5-hydroxytryptophan (5-HT, serotonin), glutamate, Hydroxytetradecadienyl-L-carnitine (C14:2-OH) and three phosphatidylcholine diacyl (PC aa) metabolites were upregulated, whilst eight members of the phosphatidylcholine family (seven of which were alkyl-acyl, PC ae) were downregulated. ORA analysis indicated changes in the tryptophan (*P*=.034) and glutamate metabolism (*P*=.063), depicting mainly 5-HT enzyme activity alterations (*P*=.014). Furthermore there was a tendency towards increased total sphingomyelins (SM, +9.4%; *P*=.169) while total PC ae tended to decrease (−6.1%; *P*=.651). Significant positive correlations were found between glutamate serum levels and psychological symptom (*P*=.006; *r*=.815) as well as total (*P*=.007; *r*=0.802) SNOT-22 scores.

Conclusions: Our data show previously unrecognized alterations in lipid and amino acid metabolism in AERD and suggest an involvement of phospholipids or their metabolites (e.g. SM, ceramides) in AERD pathology. Finally, we identified glutamate as a potential biomarker for AERD severity.

0095 | TLR2 and TLR4 signalling is responsible for impaired epithelial barrier in chronic rhinosinusitis with nasal polyps

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Introduction: Epithelial barrier dysfunction plays a role in the pathophysiology of chronic rhinosinusitis with nasal polyps

(CRSwNP). Toll-like receptors (TLRs) are thought to regulate epithelial barrier integrity, though its function has not been studied in CRSwNP thus far. In this study, we aimed at investigating whether TLR2 and TLR4 signalling is involved in regulating epithelial barrier function in CRSwNP.

Objectives: Primary nasal epithelial cells from controls (n=5) and CRSwNP patients (n=5) were isolated and grown in air-liquid interface on transwell inserts for 21 days. Epithelial integrity was evaluated by measuring transepithelial electrical resistance (TER), together with the expression of occludin and zonula occludens 1. TLR2 and TLR4 expression was evaluated using qRT-PCR. Primary nasal epithelial cells were stimulated with *Staphylococcus aureus* enterotoxin B (SEB) for 4 hours and TER was evaluated. *In vitro*, TLR2 and TLR4 signalling was blocked to evaluate the contribution to barrier dysfunction. *In vivo*, wild type and $TLR4^{-/-}$ transgenic mice were used to evaluate the role of TLR activation by SEB in increasing mucosal permeability for FD4.

Results: TER of CRSwNP cultures was significantly decreased compared to controls, which was associated with decreased expression of occludin. Stimulation with SEB significantly decreased TER of CRSwNP cultures but not of controls. TLR2 and TLR4 expression was elevated in CRSwNP, which could explain the differential SEB response. Antagonizing TLR signalling prevented SEB-induced decrease in TER *in vitro*. *In vivo*, SEB increased mucosal permeability for FD4 in wild type mice by altering the expression of occludin. No increased FD4 permeability nor decreased expression of occludin was found in $TLR4^{-/-}$ transgenic mice.

Conclusions: TLR2 and TLR4 expression is increased in CRSwNP and contributes to loss-of-epithelial barrier in CRSwNP. Interfering with TLR signalling might hold a potential therapeutic approach in the treatment of CRSwNP.

0096 | The construction of related gene expression profile of allergic rhinitis and screening the pathogenic genes

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Introduction: Allergic rhinitis is a common allergic reactions and related diseases, with the incidence in the normal population generally higher. In recent years, more and more evidence suggests that the incidence of allergic rhinitis involves regulations of multiple genes. In this research, through exploring the gene expression profiles of allergic rhinitis by RNAseq technology, the differences of gene expression profiles was analyzed, providing a research basis on the mechanism of allergic rhinitis.

Objectives: To construct related gene expression profile of allergic rhinitis (AR) and screen the pathogenic genes.

Results: Compared AR group to the control group, there 89 related genes were found and 19 genes were up-regulated, 70 genes were down-regulated. Furthermore, there 2 genes (BCL6, STAT6) differentially expressed.

Conclusions: Using Allergy & Asthma PCR Array, we preliminarily constructed the aberrant gene expression profile of AR and BCL6 and STAT6 are the possible pathogenic genes of AR.

TUESDAY, 20 JUNE 2017
OAS 24
ALLERGENS AND IMMUNOTHERAPY

0097 | Development of novel class of rationally-designed hypoallergens: bet V 1 variant for the specific immunotherapy of birch pollen allergy

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Introduction: We have discovered two important structural characteristics of allergens based on the first 3D structure of a complex between an IgE antibody and allergen: (I) the structure of an IgE epitope seems to differ from an IgG epitope and (II) allergens can form dimers which increase the presentation of identical epitopes. Based on these discoveries we have developed a technology platform allowing the rational design and manufacturing of novel class of validated recombinant hypoallergens.

Objectives: The hypoallergen development is based on targeted mutations within two major surface areas of the allergens: (I) mutation(s) introduced to the IgE epitope reducing the binding of IgE antibodies to the allergen and (II) mutation(s) introduced to the monomer-monomer interface reducing dimerisation of allergen monomers bound to the IgE-FcεRI receptor complex. These mutations lead to a significant reduction in the onset of the inflammation process. The designed recombinant hypoallergens have been produced in *E. coli*, purified to homogeneity and validated with immunotechnological methods and ultra-high resolution (FTICR) mass spectrometry (MS).

Results: The accurate molecular mass of the purified hypoallergens is determined by denatured MS and the correct folding and degree of dimerisation by native MS. We have shown that the reduction of the IgE binding and dimerisation significantly reduces histamine release from basophils for the first model allergen, beta-lactoglobulin of cow's milk but more importantly also to allergens with different 3D structures including the major birch pollen allergen Bet v 1. The developed Bet v 1 hypoallergen has been shown to be immunogenic in a mouse model causing the decrease in the allergen specific IgE combined to an increase in IgG levels. The Bet v 1 hypoallergen has also been shown to be a stable protein in a stability test carried out for a period of 12 months.

Conclusions: The developed hypoallergens are genetically and structurally 99 % identical to the wild-type allergen and, thus, they are highly capable to evoke the production of protecting IgG antibodies against the allergen as in the case of traditional desensitisation treatments with natural allergens. Due to the innovative structural features the developed hypoallergens are expected to lead to an efficient protection with minimal side-effects and therefore enabling safer and most likely shorter desensitisation treatments than the current concepts.

0098 | Minor allergens in birch pollen allergen products -insights into pollen and processing

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Introduction: Allergen research commonly focuses on major allergens while knowledge on minor allergens, especially regarding their clinical relevance, remains limited. This is especially true for birch pollen minor allergens because of the undeniable central role of Bet v 1. When studying birch pollen minor allergens, the lack of knowledge already starts at the level of the pollen. So far, neither the natural heterogeneity nor the factors influencing the content of minor allergens in birch pollen have been thoroughly investigated. In addition, birch pollen allergen products are based on pools of pollen collected from thousands of trees. Little is known how pollen lot selection or pollen processing affect minor allergens in birch pollen allergen product manufacturing.

Objectives: We aim to gain a better understanding of the natural allergen composition of birch pollen and how this composition is affected during manufacturing of birch pollen allergen products.

Results: We have analyzed birch pollen from various single birch trees for their Bet v 1, Bet v 4, Bet v 6 and Bet v 7 content using allergen-specific sandwich ELISA systems. Preliminary data indicates that minor allergen content is constant over the pollination period of a birch tree. The comparative measurement of pollen extracts prepared from different birch trees is still ongoing. Regarding the effect of pollen selection on birch pollen allergen product composition, we have shown in previous studies that the utilized birch pollen lot could not in all cases be linked to fluctuations in minor allergen content between batches of a product. Furthermore, the observed differences between products of different manufacturers were found to be too great to be caused solely by the use of different pollen lots. Hence, we investigated the influence of different extraction methods on birch pollen extract composition. We compared allergen extracts prepared from the same birch pollen lot using six different commonly used extraction protocols differing in buffer composition, duration and temperature. Three independent extractions were performed per method. Despite the use of the same pollen material, great differences could be observed between the resulting extracts, both in major and minor allergen content.

Conclusions: Our findings indicate that the choice of extraction method is a main determinant of allergen composition in birch pollen allergen products.

0099 | ICH validation of the quantification of grass pollen and house dust mite major allergens by mass spectrometry

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Introduction: According to the most recent European Pharmacopoeia (version 9.0), major allergens from pollen or mite source materials must be quantified within allergenic extracts intended for allergen immunotherapy (AIT), based on an appropriate immunochemical method, namely ELISA. However the latter method may underestimate the amount of allergens present in the extract when the antibodies used are restricted to a limited number of isoallergens. To circumvent this issue, mass spectrometry (MS)-based allergen quantification can be performed.

Objectives: We sought to develop and validate MS-based assays for the comprehensive quantification of grass pollen group 1 and house dust mite (HDM) group 2 major allergens. Allergens were purified by liquid chromatography (LC) and their primary structures were characterized by LC coupled to high resolution MS. Allergen quantification was performed by LC coupled to targeted MS following proteolysis by trypsin.

Results: Proteotypic peptides suitable for MS assays were selected following isoallergen characterization of both grass pollen group 1 and HDM group 2 allergens. Subsequently, the MS quantification assays were developed and validated according to ICH topic Q2 (R1) as well as FDA bioanalytical method validation (2001). Validation data confirmed that both assays are selective, linear (R^2>0.98), accurate (recovery ~100%) and sensitive (limit of detection <0.5 ng/ml). Moreover, intra-run precision, inter-run precision and repeatability were below 20% for both assays. Besides, allergen contents quantified by LC-MS/MS were up to 100-fold higher as compared with the results obtained using ELISA.

Conclusions: Overall, MS-based assays can provide accurate, precise and robust major allergen quantification for both grass pollen and mite extracts intended for AIT. Such highly specific methods may be applied to other major allergens, especially when facing issues linked to restricted antibody specificity or reduced affinity because of allergen denaturation and/or absorption.

0100 | Improvement of symptoms and allergen tolerance in local allergic rhinitis patients treated with depigmented polymerized Phleum pratense extract immunotherapy, a double-blind placebo-controlled trial

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Introduction: Allergen immunotherapy (AIT) has demonstrated to be an effective treatment in patients with local allergic rhinitis (LAR) to house dust mites.

Objectives: We evaluated the efficacy of subcutaneous AIT with *Phleum pratense* in LAR patients.

Results: A randomized double-blind, placebo-controlled, parallel-group (DBPCPG), phase II investigator-initiated trial was conducted in 56 patients with LAR to *Phleum pratense*. All participants provided informed consent, and the Ethic Committee and Spanish Drug Agency approved the study. During the 1st year subjects were randomized to receive subcutaneous AIT with depigmented polymerized 100% *Phleum pratense* allergen extract (LETI, S.L.U. Tres Cantos, Madrid) or placebo. During the 2nd year all received AIT. Differences regarding clinical response (combined symptoms-medication score (cSMS), medication free days), Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ), titrated nasal allergen provocation test (NAPT), skin testing, serum levels of specific-IgG4 and –IgE, and safety between groups were analyzed. Patients were classified as responders (increased tolerance to the allergen in the NAPT) and non-responders (no increased tolerance). During the 1st year AIT induced significant improvements compared to placebo in cSMS (1.2±0.8 vs 2.6±1.2, *P*=.001), number of medication free days (18.1±9.8 vs 4.1±3.3, *P*<.001), RQLQ score (1.98±0.91 vs 3.67±1.27, *P*=.001), and allergen tolerance in NAPT (0.024±0.038 mcg/ml vs 0.003±0.004 mcg/ml, *P*=.010) compared to placebo. The rate of responders was 50%, and NAPT was negative in 19% of patients treated with AIT. During the 2nd year, a comparable improvement in CdSMS, RQLQ and nasal allergen tolerance was observed in both groups. AIT was well-tolerated, with only six local reactions resolved without treatment.

Conclusions: Subcutaneous immunotherapy with depigmented polymerized allergen extracts is a safe and clinically effective treatment for LAR to *Phleum pratense.*

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0101 | Aluminium hydroxide impairs tolerogenic properties imprinted by allergoids conjugated to nonoxidized mannan in dendritic cells

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Introduction: Allergen immunotherapy (AIT) is the only curative treatment for allergy, but it faces problems related to efficacy, security, duration and patient compliance. Recent studies demonstrated that glutaraldehyde-polymerized grass pollen allergoids coupled to nonoxidized mannan (PM) represent novel suitable preparations to formulate improved vaccines for AIT. Aluminium hydroxide (alum) is the most widely used adjuvant in human vaccines but its way of action is not fully understood.

Objectives: To study the immunological mechanisms by which alum condition the capacity of PM to generate human dendritic cells (DCs) able to promote healthy immune responses to allergens.

Results: Alum significantly increases the activation of NF-κB/AP-1 and the production of IL-8 induced by PM in THP1 cells. The production of IL-10 and IL-6 by PM-activated human monocyte-derived DCs (hmoDCs) is significantly reduced in the presence of alum. In contrast, alum significantly increases the production of IL-1β and IL-23 in PM-treated hmoDCs. As determined by flow cytometry, PD-L1 expression in PM-treated hmoDCs was reduced in the presence of alum, which was accompanied by decreased numbers of induced $CD4^+CD25^{high}CD127^-$ forkhead box P3 $(FOXP3)^+$ Treg cells. Accordingly, alum significantly decreases IL-10-producing T cells generated by PM-treated hmoDCs, while increasing IL-5- and IFN-γ-producing T cells. Blocking experiments suggest that Syk, Akt and mTOR might contribute to the underlying immunological mechanisms modified by alum in PM-activated hmoDCs. *In vivo* immunizations of BALB/c mice showed that alum significantly reduces the numbers of $FOXP3^+$ Treg induced by PM and increases proliferation and cytokine production in splenocytes.

Conclusions: We provide novel insights into the influence of aluminium hydroxide in the immunomodulatory properties imprinted by allergoids conjugated to nonoxidized mannan in dendritic cells, which might have important implications for future development of novel AIT protocols.

0102 | High correlation between validated Rhinoconjunctivitis Quality Of Life (RQLQ) and EAACI recommended Combined Symptom Medication Score (CSMS) as clinical outcome measure in allergen immunotherapy trial

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Introduction: EAACI recommends the combined symptom and medication score (CSMS) as standardized clinical outcome measure in allergen immunotherapy trials for allergic rhinoconjunctivitis (ARC) and calls for further validation of this score (Pfaar, Allergy 2014). The first Phase III study with a sublingual immunotherapy (SLIT) in ARC with CSMS as primary outcome was recently completed. The primary results showed a clinically relevant and statistical significant 31% improvement in CSMS following SLIT with a liquid birch pollen extract compared to placebo ($P<.0001$) (Pfaar et al. Allergy (2016); 71 (suppl.102): 45 (abstract 87). The validated Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ-S) assessed during the birch pollen season was correlated to the CSMS of this birch SLIT study and the minimal clinical important difference (MCID) for CSMS was estimated using the RQLQ-S as anchor.

Objectives: The data from the pivotal Phase III study were used after pre- and co-seasonal birch SLIT administration in 406 patients suffering from moderate to severe birch pollen induced ARC. Previously, a MCID of 23% in CSMS compared to placebo was justified based on clinical and statistical criteria and accepted by regulators. Pearson's correlation coefficient was calculated between the secondary RQLQ-S and CSMS and the MCID for CSMS was estimated using linear regression based on the consideration that an improvement of 0.5 points in the validated RQLQ-S score is accepted to be clinically relevant.

Results: A strong positive correlation was observed between the CSMS and the RQLQ-S scores during the pollen season ($r=0.68$ and $P<.0001$). Based on regression analysis using the study results, a clinically relevant 0.5 point improvement in RQLQ-S corresponds to an MICD for CSMS of 21% (95% CI: 19–23%).

Conclusions: This is the first validation of the CSMS as proposed by the EAACI as primary endpoint in a Phase III field trial. Using the validated RQLQ-S score as anchor, the post-hoc results show that a 21% improvement in CSMS may be considered clinically relevant. Moreover, these results emphasize the clinical relevance of the 31%

CSMS improvement realized after SLIT with a liquid birch pollen extract, the first pivotal Phase III study in ARC which used CSMS score as primary endpoint. Moreover, these results provide important guidance and support for the external validation of CSMS as primary outcome measure in allergen immunotherapy trials for ARC.

TUESDAY, 20 JUNE 2017
OAS 25
INNATE IMMUNE RESPONSE IN MUCOSAL TISSUES

0103 | Differences in dendritic cell allergen sampling, migration and subsequent T cell differentiation separate induction of mucosal tolerance vs sensitisation

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Introduction: The prevalence of respiratory allergic sensitisation has increased over the last decades yet there is only limited understanding of the immunological processes involved. Our previous experimental studies have shown that reduced allergen uptake by airway mucosal dendritic cells is linked to a deficient regulatory T cell response and failure to induce mucosal tolerance to innocuous allergens.

Objectives: To provide insights into the immunological processes, modulating development of tolerance vs sensitisation during natural respiratory aeroallergen exposure, we used flow cytometry, confocal microscopy and in vitro cultures to analyse phenotypes of airway mucosal dendritic cell subsets and their functional capacity to take up and present allergen in two rat strains, these rats strains are either genetically resistant or susceptible to sensitisation and chronic airway disease when repeatedly exposed to allergen.

Results: During repeated intra-nasal, adjuvant-free ovalbumin (OVA) exposures, naïve male rats from the susceptible (BN) strain experienced expansion of the airway draining lymph nodes, production of antigen-specific IgE and airway eosinophil infiltration. In the resistant strain (PVG), OVA exposure did not induce IgE production but rather IgG and no eosinophil infiltration was observed. At baseline, airways in the resistant strain contained higher proportion of CD4+ conventional dendritic cells (DC). These DCs were the most efficient subset in sampling OVA. Interestingly, allergen sampling and lymph node transportation was less efficient in the susceptible strain compared to the resistant strain. However, the OVA carrying DCs arriving in the lymph nodes in the susceptible strain presented a more activated phenotype through up-regulation of MHC-II expression compared to the resistant strain. In the susceptible strain, OVA responsive CD4+ effector T cells were generated whereas in the resistant strain, repeated OVA exposures instead led to expansion of FoxP3+ regulatory T cells in the airways.

Conclusions: The current study identifies specific differences in allergen handling, dendritic cell migration, T cell expansion and subsequent T migration that are associated with either mucosal tolerance or sensitisation. These insights will provide a blueprint for future studies aiming at manipulating allergen handling with the long-term aim of preventing allergic sensitisation.

0105 | Monocytes accumulate in the airways of children with fatal asthma

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Introduction: Activated Th2 cells are believed to play a pivotal role in allergic airway inflammation, but which cells attract and activate Th2 cells locally has not been fully determined. Recently, it was shown in an experimental human model of allergic rhinitis (AR) that activated monocytes rapidly accumulated in the nasal mucosa after local allergen challenge, where they promoted recruitment of Th2 cells and eosinophils.

Objectives: To investigate whether monocytes are recruited to the lungs in paediatric asthma.

Methods: Tissue samples obtained from children and adolescents with fatal asthma attack (n=12), age-matched non-atopic controls (n=9), and allergen-challenged AR patients (n=8) were subjected to in situ immunostaining.

Results: Monocytes, identified as CD68+S100A8/A9+ cells, were significantly increased in the lower airway mucosa and in the alveoli of fatal asthma patients compared with control individuals. Interestingly, aggregates of CD68+S100A8/A9+ monocytes obstructing the lumen of bronchioles were found in asthmatics (8 out of 12) but not in controls. Analysing tissue specimens from challenged AR patients we confirmed that co-staining with CD68 and S100A8/A9 was a valid method to identify recently recruited monocytes. We also showed that the vast majority of accumulating monocytes both in the lungs and nasal mucosa expressed matrix metalloproteinase 10, suggesting that this protein may be involved in their migration within the tissue.

Conclusions: Monocytes accumulated in the lungs of children and adolescents with fatal asthma attack. This finding strongly suggests

that monocytes are directly involved in the immunopathology of asthma and that these proinflammatory cells are potential targets for therapy.

0106 | Differential expression of RNA-binding proteins in airway epithelium in chronic lung inflammation

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Introduction: Posttranscriptional gene regulation (PTR) critically controls inflammation by modulating messenger RNA (mRNA) turnover and translation rates. RNA-binding proteins (RBP) coordinate PTR by binding to conserved sequences of targeted mRNAs. The majority of pathogenetic genes expressed in immune and structural cells in chronic lung inflammation are amenable to RBP-coordinated PTR, yet the role of RBPs in this setting remains elusive.

Objectives: The overall aim of the study is to characterize the expression pattern and activation state of a panel of oxidative stress-regulated RBPs in patients with asthma, COPD and in relevant control subjects. RBP expression was evaluated by: immunohistochemistry (IHC) with validated antibodies using standard immunoperoxidase techniques on formalin-fixed, paraffin embedded tissues obtained from bronchial mucosa and peripheral lung samples of well-phenotyped, mild to moderate stable COPD patients (n=10), compared with age/gender/smoking history-matched smokers with normal lung function (n=12); by Western blot (WB) and real-time PCR in the human airway epithelial cell line BEAS-2B stimulated with H2O2 (100 μM, 24 h, n=3); by real-time PCR in PBMC of COPD patients (n=5)/controls (n=7) characterized as for the IHC study. Study was approved by local Ethics Committees. Statistical significance was assessed by analysis of variance and Kruskal–Wallis tests.

Results: We identified by IHC three of the RBPs mainly regulating inflammatory transcripts: tristetraprolin (TTP), Hu antigen R (HuR) and heterogeneous nuclear ribonucleoprotein D (HnRNPD, also termed AUF-1) in the airways' samples of COPD patients and controls. Expression of AUF-1, an RBP functionally linked to accelerated decay of inflammatory transcripts, was significantly (P=.015) lower in bronchial epithelium of COPD samples vs. controls, while remaining comparable between groups in bronchiolar epithelium, glandular cells and alveolar macrophages. Decreased AUF-1 expression was also found by WB analysis of BEAS2B cells treated with H2O2; in PBMC, RBP mRNA levels did not differ between groups.

Conclusions: Deregulated epithelial expression of AUF-1may amplify bronchial inflammation in stable COPD patients by increased stability of epithelial-derived inflammatory transcripts. Mapping of lung-specific RBP expression and regulatory network is undergoing to probe their pathogenic and therapeutic potential in airway diseases such as allergic rhinitis, asthma and COPD.

0107 | Systemic mastocytosis associates with cardiovascular events despite lower plasma lipid levels

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Introduction: Atherosclerosis is considered to be a chronic inflammatory condition. Mast cells, prominent inflammatory cells of the innate immune system have been implicated in the development and progression of atherosclerosis in animal models. However, it is unknown whether long-term exposure to excess of mast cells is associated with cardiovascular disease (CVD) and cardiovascular risk factors in humans.

Objectives: The aim of this study was to compare the prevalence of CVD as well as cardiovascular risk factors in patients with SM and age- and sex-matched controls.

Results: Participants of this study were 50 adults diagnosed with SM according to WHO criteria and 50 age and sex-matched controls. The primary endpoint, CVD, was determined by carotid ultrasound (carotid intima-media thickness (C-IMT) and the presence of carotid plaques and CVD events.

Secondary endpoints were cardiovascular risk factors; dyslipidaemia, diabetes, hypertension, obesity and smoking.

CVD events were more prevalent in patients with SM compared to controls (20% vs. 6%, P=.04). CVD events were significantly associated with SM (P=.02) and hypertension (P=.01) in multivariate analysis The prevalence of diabetes, hypertension, obesity and smoking was similar between SM patients and controls. The prevalence of C-IMT and carotid plaques was similar between patients with SM compared to controls (54% vs 38%, P=.11 and 0.65±0.11 mm vs. 0.64±0.13 mm, P=.65 respectively).

Levels of total cholesterol and LDL-C were significantly lower in the SM patients than in the control group (5.1±1.1 vs. 5.9±0.9 mmol/l, P<.05 and 2.9±0.8 vs. 3.5±0.7 mmol/l, P<.05, respectively).

Conclusions: Despite lower plasma total cholesterol and LDL-C, the prevalence of CVD is higher in patients with SM compared to healthy controls. This study supports the evidence that mast cells contribute to atherosclerosis and CVD events in patients with SM. Therefore cardiovascular screening in patients with SM seems warranted.

0108 | RNA is the key element of the th1-promoting and allergy-protective activation of dendritic cells by the cowshed bacterium lactococcus lactis G121

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Introduction: Traditional farming environment during fetal development and in early childhood has long been known to reduce the incidence of allergies later in life. Among the potential mediators of this protective effect, several Gram-negative and Gram-positive bacterial strains have been isolated from cowsheds and successfully been used in different mouse allergy models. Knowledge of the identity of mediators of the allergy-protective effect of *Lactococcus lactis* G121, and the pattern recognition receptors involved, however, is largely lacking.

Objectives: Identification of the ligands, central pattern recognition receptors and molecular mechanisms by which *L. lactis* G121 confers allergy-protection.

Results: (Material and Methods:) *L. lactis* G121–induced cytokine release and surface expression of costimulatory molecules by human monocyte-derived dendritic cells (moDCs), bone marrow–derived mouse dendritic cells (BMDCs), and moDC/naive CD4+ T-cell cocultures were analyzed by using ELISA and flow cytometry. In addition, expression of cytokines and transcription factors in T-cell cocultures was analyzed by intracellular flow cytometry. The pathology of ovalbumin-induced acute allergic airway inflammation after adoptive transfer of BMDCs was microscopically examined.

(Results:) *L. lactis* G121–treated murine BMDCs and human moDCs released Th1-polarizing cytokines and induced predominantly Th1 T-cells. Inhibiting phagocytosis and endosomal acidification in BMDCs or moDCs impaired the release of Th1-polarizing cytokines, the expression of costimulatory molecules as well as activation of T-cell activation upon *L. lactis* G121 challenge. *In vivo* allergy protection mediated by *L. lactis* G121 was dependent on endosomal acidification in dendritic cells (DCs). Toll-like receptor (TLR) 13-KO BMDCs showed a weak response to *L. lactis* G121 and were unresponsive to its RNA. The Th1-polarizing activity of *L. lactis* G121–treated human DCs was blocked by TLR8-specific inhibitors, mediated by *L. lactis* G121 RNA, and synergistically enhanced by activation through the cytosolic receptor NOD2.

Conclusions: Bacterial RNA is the main driver of *L. lactis* G121–mediated protection against experimentally induced allergy, which requires both bacterial uptake and endosomal acidification in DCs to be operative. In mice, *L. lactis* G121 RNA signals through TLR13; however, the most likely intracellular receptor in humans is TLR8.

TUESDAY, 20 JUNE 2017
OAS 26
INSIGHTS AND ADVANCES IN CHRONIC URTICARIA

0109 | Chronic inducible urticaria (CIndU) in Europe, Central America, and South America: findings from visit 1 of the worldwide aware study

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Introduction: Chronic inducible urticaria (CIndU) is characterised by itchy wheals and/or angiooedema that only appears when triggered by a specific stimuli. The real-world rate of CIndU across Europe (EU) and Central/South America (C/SA) is currently unknown.

Objectives: To examine diagnosis, perceived disease control (measured by the Urticaria Control Test [UCT]; scores below 12 indicate poor control) and quality of life (QoL) (measured by the Dermatology Life Quality Index [DLQI]) among patients with CIndU residing in the EU and C/SA enrolled in the ongoing observational AWARE study. Data collected at enrolment (visit 1) were used. Patients were aged 18 years or older and were refractory to at least one course of H1-antihistamine treatment. The data were split into regions for comparison: Europe (United Kingdom; Nordic countries [Sweden, Norway, Denmark]; Southern Europe [Belgium, France, Portugal, Spain, Italy, Greece]; Germany; and Russia) and C/SA (Central [Guatemala, Honduras, Costa Rica, Dominican Republic, Panama]; South [Colombia, Peru, Brazil, Argentina]). Descriptive statistics are reported here overall and by region; comparisons among subregions will be presented.

Results: Overall, 26% (n=1118) of patients with chronic urticaria (N=4226) were diagnosed with CIndU at study entry. Of these patients, 31% had angiooedema at study entry or within the past 6 months and 77% had CIndU comorbid to chronic spontaneous urticaria (CSU). The rate of CIndU diagnoses was higher in C/SA than in EU (33% vs. 26%), but the rate of angiooedema and rate of CIndU comorbid to CSU was similar in both regions. Rates of light/solar, vibratory, aquagenic, and contact urticaria were low (range: 0.7%-6.2%). Patients in C/SA had a higher rate of symptomatic dermographism (53% vs. 44%) and delayed pressure urticaria (30% vs. 25%) but a lower rate of cold urticaria (10% vs. 18%) and cholinergic urticaria (8% vs. 18%) compared with EU patients. Differences were also identified within subregion comparisons. UCT scores identified poor disease control in 77% of CIndU patients (C/SA 84% vs. EU 76%). Most patients reported a moderate (25%), very large (26%), or extremely large effect (7%) of CIndU on QoL (DLQI); ratings were comparable between regions.

Conclusions: CIndU is commonly associated with CSU, is uncontrolled in most patients, and can be severely disabling (as shown by angiooedema rates and the effect on QoL).

0110 | Causal association between IgE anti-TPO and chronic urticaria

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Introduction: The association between thyroid autoimmunity and Chronic Spontaneous Urticaria (CSU) has been evaluated in multiple studies. Recently, IgE autoantibodies against thyroid antigens such as thyroid peroxidase (TPO) have been demonstrated in a group of CSU patients in higher frequency that health subjects. However if these IgE autoantibodies can trigger an urticaria reaction has not been proven.

Objectives: To investigate the underlying relationship of concomitant IgE autoantibodies against thyroid antigens and CSU.

Results: 100 patients with CSU according to EAACI guidelines, 110 healthy subjects without clinical history of urticaria and 30 patients with autoimmune thyroid disease (ATD) (Hashimoto thyroiditis) were recruited. At study entry, autologous serum skin test (ASST), complete blood cell count, erythrocyte sedimentation rate, thyroid function tests, antinuclear antibodies, IgG anti-thyroglobulin, IgG anti-thyroperoxidase (anti-TPO) antibodies, total IgE and specific IgE anti-TPO were assessed in all the subjects. To demonstrate a causal relationship between IgE anti-TPO and CSU, basophil activation test with addition of TPO were measured to prove a direct role of TPO in effector cells. Also, skin test with TPO was done to demonstrate whether this antigen could selectively induce skin reaction in the CSU population or in all patients with anti-TPO IgE.

We observed IgE anti-TPO expression in the three groups (CSU: 34%, ATD: 16,6%, healthy subjects 8.1%). Between those patients with positive IgE anti-TPO, flow cytometry showed CD203c induction with serial additions of TPO in 76,4% of CSU group and 40% ATD but not in healthy subjects. Basophil activation was stronger in

patients with CSU than in ATD patients ($P<.05$). Only CSU patients with positive IgE anti-TPO present a positive skin test with IgE anti-TPO. We observe a direct correlation between IgE and IgG to TPO in ATD (r 0,604) and CSU (r 0,584) but the concentration of IgE anti-TPO was highest in CSU

Conclusions: IgE anti-TPO is present in patients with CSU and autoimmune disease but also in healthy subjects so it is not a specific biomarker of CSU. However, we demonstrated that IgE to TPO play a pathogenic role inducing cell effector activation and skin exacerbation in a subgroup of CSU patients.

0111 | Chronic spontaneous/idiopathic urticaria patients with moderate activity have similar burden of disease as those with severe activity - results from the ASSURE-CSU study

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Introduction: The ASSURE-CSU study aimed to identify and quantify the humanistic and economic burden of chronic spontaneous/idiopathic urticaria (CSU/CIU). Here we present data on economic and humanistic burden among patients with moderate vs severe activity CSU.

Objectives: This non-interventional, multicentre study conducted in Canada, France, Germany, Italy, Netherlands, Spain, and the United Kingdom enrolled patients with CSU aged ≥18 years with disease persisting for ≥12 months and symptomatic despite current treatment. Data came from medical charts, a patient survey and a 7-day diary. Patients completed Dermatological Life Quality Index (DLQI) and Chronic Urticaria Quality of Life (CU-Q2oL) questionnaires at enrolment, UAS questionnaire for 7 days after enrolment and Work Productivity and Activity Impairment - Specific Health Problem (WPAI-SHP) questionnaire on the 8th day. For the current analysis, UAS7 scores were grouped in score bands of 0–15, 16–27, and 28–42 reflecting well-controlled to mild, moderate, and severe disease activity, respectively. All outcomes were evaluated for the 3 disease activity groups; significance tests were performed between moderate vs severe activity patients.

Results: Among a total of 673 patients, there were 182 (27.0%) and 117 (17.4%) patients in moderate [UAS7: 16–27] and severe [UAS7: 28–42] activity groups, respectively. There was no significant difference in age, gender and disease duration between moderate and severe activity patients. Compared with moderate activity patients, severe activity patients reported significantly greater impairment in quality of life based on higher mean [SD] DLQI (13.5 [6.57] vs 10.6 [6.73]) and CU-Q2oL scores (49.3 [20.87] vs 39.7 [20.76]) (both $P<.001$). However, moderate activity patients experienced non-significantly different mean [SD] % of absenteeism (7.7 [19.60] vs 9.5 [21.28]), presenteeism (34.5 [29.03] vs 40.4 [27.42]) and overall work impairment (36.3 [29.30] vs 43.6 [28.38]) as severe activity patients (all $P>.05$). The mean [SD] activity impairment was significantly higher in severe activity patients compared with moderate activity patients (52.3 [27.72] vs 40.5 [28.21]; $P<.001$).

Conclusions: Severe activity CSU patients had significantly higher quality of life impairment than moderate activity patients, but the two groups had comparable impact on their capacity to work. Overall, moderate and severe activity CSU patients tend to have similar high burden of disease.

0112 | Can basophil histamine release assay predict response and time of response to omalizumab in chronic spontaneous urticaria?

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Introduction: Omalizumab (anti-IgE) is a licensed add-on therapy for chronic spontaneous urticaria (CSU) non-responsive to H_1-antihistamines. However, no validated biomarkers predict the response and time of response to omalizumab. Basophil histamine release assay (BHRA) detects serum histamine-releasing IgG autoantibodies to high affinity receptor for IgE (FcεRI) or cell-bound IgE. BHRA is routinely performed in all severe CSU patients before starting omalizumab or immunosuppressant in our centre.

Objectives: To assess if BHRA result predicts the likelihood of response and time of response to omalizumab, a retrospective case review of our CSU patients treated with 300 mg omalizumab every 4 weeks was undertaken. Urticaria activity score 7 (UAS7) was used to monitor response, which was defined as reduction (UAS7 < 16) or

complete resolution of symptoms (UAS7=0). Fast response occurred within first week of treatment.

Results: Ninety-one patients (sixty female; mean age of 44) were treated with omalizumab between November 2015 to October 2016. Eighty-five (93.4%) patients responded: eighty-one (95.3%) were BHRA-negative and four (4.7%) were BHRA-positive. Average pre-treatment UAS7 was 36 in the BHRA-negative group and 40 in BHRA-positive group. In the BHRA-negative group, forty-three (53.1%) patients responded within a week; fourteen responded between 1–4 weeks, nine responded between 5–8 weeks, eleven responded between 9–12 weeks and four responded between 13–16 weeks. In the BHRA-positive group, one patient responded within a week, after the 2nd, 3rd and 4th injection, respectively. Median time to response in BHRA-negative patients was within 1 week whereas in BHRA-positive patients it was 7 weeks. The response rate in BHRA-negative was 97.6% (81/83) and in BHRA-positive was 50% (4/8). The response rate ratio was 1.952 (95% CI: 0.97–3.91; *P*=.0589). Of six omalizumab non-responders, two (33.3%) were BHRA-negative and four (66.7%) were BHRA-positive.

Conclusions: BHRA-negative patients appear to be more likely to respond and be fast responders to omalizumab than BHRA-positive patients. The low numbers of BHRA-positive patients in this cohort limits the strength of this interpretation and hence larger studies are required.

0113 | Omalizumab improves angioedema-related quality of life impairment in chronic spontaneous urticaria patients: results from the X-ACT study

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Introduction: Angioedema is an important symptom in many patients with chronic spontaneous urticaria (CSU) and is detrimental to health-related quality of life (QoL).

Objectives: To examine the effect of omalizumab treatment on angioedema-related QoL in the German X-ACT study (NCT01723072).

Methods: X-ACT is a phase III, double-blind, placebo-controlled study in CSU. H1-antihistamine refractory CSU patients (18–75 years) with moderate-severe disease and with frequent angioedema episodes were randomized 1:1 to receive omalizumab 300 mg or placebo every 4 weeks for 28 weeks (8 week follow-up). Angioedema-related QoL and angioedema activity were assessed at Week 0 (baseline), 4, 12, 20, 28, and 36 (follow-up visit) using the Angioedema-QoL Questionnaire (AE-QoL) and Angioedema Activity Score (AAS), respectively.

Results: Overall, 91 patients were randomized (omalizumab, n=44; placebo, n=47) and 68 (omalizumab, n=35; placebo, n=33) completed the 28-week treatment period. At baseline, the mean (SD) AE-QoL scores were 56.2 (18.7) in the omalizumab and 59.9 (19.2) in the placebo group, reflecting a strong angioedema-related QoL impairment in both groups. Subgroup analysis revealed that female patients had higher AE-QoL scores at baseline compared to male patients (*P*=.001) and a tendency towards higher disease activity (higher AAS scores, *P*=.086).

Omalizumab 300 mg treatment was associated with a rapid decrease in AE-QoL scores from week 4 (mean [SD]; omalizumab: 29.2 [20.1]; placebo: 46.9 [19.5]) and a further and sustained decrease until the end of treatment at week 28 (omalizumab: 14.1 [14.8]; placebo: 38.1 [23.6]). Improvement in angioedema-related QoL impairment significantly correlated with reduced angioedema activity (Pearson correlation of changes in AE-QoL scores from baseline with changes in AAS scores from baseline, at week 12: 0.526, $P < .001$; and at week 28: 0.501, $P < .001$). After treatment discontinuation at week 28, angioedema-related QoL impairment and angioedema activity approached placebo levels.

Conclusions: Omalizumab 300 mg treatment leads to a rapid and sustained decrease in angioedema-related QoL impairment along with a decrease in angioedema activity. Discontinuation of omalizumab treatment is associated with angioedema symptom return and recurring QoL impairment.

0114 | Exploring demographic and clinical differences among omalizumab responders and non-responders: interim results from a 48-week, phase IV study of omalizumab in chronic idiopathic/spontaneous urticaria

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Introduction: Omalizumab efficacy and safety for the treatment of chronic idiopathic/spontaneous urticaria (CIU/CSU) have been established for treatment durations of ≤24 weeks. Not all patients respond to omalizumab treatment; it would be helpful to identify patient characteristics that would assist in predicting response to omalizumab.

Objectives: To explore baseline demographic and clinical characteristics of protocol-defined responders (randomized patients) and

protocol-defined non-responders (nonrandomized patients) identified following 24 weeks of participation in XTEND-CIU.

Methods: XTEND-CIU is an ongoing, Phase IV, multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of omalizumab through 48 weeks in patients with CIU/CSU. The study included patients ≥12 years old remaining symptomatic despite standard H1 antihistamine treatment, H2 blockers, and/or leukotriene receptor modifiers and comprised 4 phases: 14-day screening, 24-week open-label, 24-week randomized double-blind (omalizumab 300 mg subcutaneously every 4 weeks or placebo), follow-up period (Weeks 48– 60). Eligibility for enrollment into the open-label phase required patients to have 7-day urticaria activity score (UAS7) ≥16 at baseline; eligibility for randomization into the double-blind phase at the end of the open-label phase required UAS7 ≤ 6 (protocol-defined responder) in the two consecutive weeks before randomization. Descriptive statistics were used to report baseline characteristics; no between-group statistical analyses were conducted.

Results: 206 patients were enrolled, 175 completed the initial 24-week open-label period. Of these, 134 met protocol-defined criteria for response and were randomized and 71 patients did not meet this criteria and were not randomized. Protocol-defined non-responders were slightly younger, had a longer duration of CIU symptoms, and were more likely to have used systemic corticosteroids to manage their CIU/CSU symptoms (Table); however none of these differences were large enough to suggest a distinguishing characteristic.

Conclusions: After examining baseline factors independently, it appears that there were no real differences between those who responded to omalizumab and those who did not. Additional classification analyses should be considered to determine if the interplay of a combination of factors might predict eventual response to omalizumab in this population of patients.

Characteristic*	Protocol-defined Responders** n=134	Protocol-defined Non-responders n=71
Age, year	45.2 (14.3)	43.6 (14.8)
Female, *n* (%)	100 (74.6)	53 (74.6)
Race, White, *n* (%)	110 (82.1)	58 (81.7)
BMI (kg/m^2)	30.2 (6.9)	30.6 (6.8)
Duration of CIU symptoms, mo	75.6 (101.3)	85.6 (124.1)
Systemic corticosteroids for symptoms, *n* (%)†	63 (47.0)	41 (57.7)
UAS7 score‡	32.5 (7.1)	31.6 (6.8)
Number of days with angioedema‡	2.2 (2.8)	2.0 (2.6)
DLQI score	15.1 (6.9)	14.1 (6.9)
ISI score	16.2 (6.7)	15.1 (7.3)
UCT score	2.5 (2.4)	2.6 (2.7)
GAD-7 score	7.6 (6.3)	7.7 (6.2)
WPAI % of Overall Work Impairment§	45.2 (29.8)	43.8 (28.5)
WPAI % Activity Impairment¶	53.6 (26.0)	48.3 (28.1)

BMI, body mass index; CIU, chronic idiopathic urticaria; DLQI, Dermatology Life Quality Index; GAD-7, Generalized Anxiety Disorder 7-item scale; ISI, Insomnia Severity Index; UAS7, 7-day Urticaria Activity Score, UCT, Urticaria Control Test. *Data given as mean (SD) unless otherwise noted. **Defined as a UAS7=6 in the 2 consecutive weeks before randomization. †In the 12 months before screening. ‡Assessed in the 7 days before baseline: randomized patients n=133, nonrandomized patients n=70.

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ADVANCES IN HEREDITARY ANGIOEDEMA

0115 | Metabolomic analysis of contact system activation in plasma from patients with hereditary angioedema

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Introduction: Comparison of the metabolite levels in plasma from patients with hereditary angioedema (HAE) type I or II collected during symptom-free periods or during an acute attack to metabolite levels present in plasma from healthy volunteers may lead to the identification of novel biomarkers and provide additional insight into the pathobiology of HAE.

Objectives: Plasma was collected in plastic sodium citrate tubes (BD Biosciences) from healthy volunteers (N=20), HAE patients during symptom-free periods (N=20), and during an acute attack (N=20). Plasma samples from the three groups were analyzed using a mass spectrometry approach to compare relative levels of metabolites (50 - ~1500 Da) by Metabolon (Durham, NC).

Results: Only a few metabolites displayed a difference in levels between attack and basal samples, which included elevation of anti-inflammatory lipid precursors during attack and reduction of corticosteroids during attack. In contrast, a random forest statistical analysis of the aggregate metabolomic data indicated good separation of healthy and HAE subjects (basal or attack) as indicated by a 78% classification accuracy. Differences in metabolites were also noted between plasma from HAE patients and healthy volunteers ($P < .05$, matched pairs t-test and Welch's two sample t-test). Metabolites significantly increased in HAE plasma compared to that of healthy volunteers included serotonin, lipid amides, methionine sulfone, and γ/β-tocopherol. In contrast, oxidative lipids (13-HODE and 9-HODE, and DiHOME) and steroids (eg, cortisol) were decreased in HAE plasma.

Conclusions: A method for the metabolomic analysis of contact system activation based on the comparison of plasma from HAE patients, a disease known to be mediated by contact system, has been proposed. The further analysis and validation using orthogonal analytical methods may lead to new insights on the pathobiology of HAE and the identification of novel biomarkers of contact system activation.

0116 | Kallikrein-cleaved kininogen as a stable and specific biomarker for bradykinin release in hereditary angioedema

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Introduction: Hereditary angioedema (HAE) patients experience recurrent tissue swelling attacks that are driven by bradykinin. New therapies targeting bradykinin production are under development, motivating development of accompanying biomarker assays. Analysis of bradykinin in plasma is technically challenging due to its small size and instability. When plasma kallikrein cleaves high molecular weight kininogen (HK), bradykinin and disulfide linked cleaved kininogen (cHK) are generated. Analysis of cHK by immunoblot previously demonstrated its clinical relevance as biomarker in HAE.

Objectives: We aimed to develop a sensitive enzyme linked immunosorbent assay (ELISA) for cHK. Ultimately, this method should be implemented as clinical diagnostic tool and function as a platform for clinical research.

Results: We developed a heavy-chain-only antibody fragment (nanobody) specific for cHK. During assay development, we established that serine protease inhibitors in the assay buffer fully prevented pre-analytical cHK generation. Furthermore, we found that addition of a polyanionic compound to the assay buffer greatly improved the assay sensitivity in plasma samples, by increasing the avidity of the binding interaction. Kallikrein activation in plasma with β-FXIIa resulted in rapid cleavage of the total pool of HK (confirmed by immunoblotting), cHK remained stable for up to 90 min. Activation of plasma with plasminogen activator streptokinase also generated cHK. Both plasmin and kallikrein share affinity for the HK R389-S390 cleavage site, which mediates bradykinin release. Further cleavage of (c)HK by plasmin eliminated nanobody recognition. Next, we went on to investigate the levels of cHK in plasma of HAE patients. We expressed cHK levels as % increase from baseline in pooled healthy control plasma (~2–6%). HAE patients in remission have a median cHK level of 13% above baseline (n=138; range 0–73%) which is higher than healthy controls (n=68; range 0–14%; $P < .0001$ by Mann-Whitney t-test). During attacks, the median cHK level was 25% above baseline (n=40; range 0–65%), higher than both healthy control and remission levels ($P < .0001$ Mann-Whitney t-test).

Conclusions: We developed a detection method for cHK and found that these levels are increased in HAE patients. We propose that cHK constitutes a promising surrogate biomarker for bradykinin release that should prove useful for HAE and potentially other bradykinin-mediated pathologies.

0117 | Longitudinal natural history of patients with type I or II hereditary angioedema: data from the icatibant outcome survey

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Introduction: Few published data are available that characterise the natural course of hereditary angioedema due to C1-inhibitor deficiency (C1 INH-HAE). The Icatibant Outcome Survey (IOS; NCT01034969) is an international observational study initiated in 2009 that monitors the safety and effectiveness of icatibant in a real-world setting. Here we report longitudinal IOS data, independent of icatibant treatment outcomes, to better understand temporal changes in disease manifestation in patients with C1 INH-HAE types I and II

Objectives: IOS is currently conducted at 53 centers in 11 countries. Patient demographics and disease characteristics including icatibant-treated attack frequency and severity, and number of untreated attacks (defined as an attack while patients are not on prophylaxis or receiving acute treatment) were obtained from data recorded at clinic visits. Descriptive retrospective analyses were performed on IOS data from July 2009–August 2016

Results: Demographic data were obtained from 353 patients with either type I (n=335, 94.9%) or type II (n=18, 5.1%) C1 INH-HAE and more than one attack during the follow-up period. Median (Q1, Q3) age at enrolment was 39.1 years (28.3, 51.8), Median (Q1, Q3) age at first symptoms was 12.0 years (5.0, 18.0) and 60.1% of patients were female. The frequency and severity of icatibant-treated attacks and the rate of untreated attacks per year from 2009–2015 are shown in Table 1

Conclusions: This first report of longitudinal data from IOS, a large database of patients with C1 INH-HAE, shows that, overall, the frequency of icatibant-treated attacks was stable over the period analysed. Patient-reported severity of icatibant-treated attacks was consistent though a decrease in rates of severe/very severe attacks was reported for 2015. The number of untreated attacks during this period declined, perhaps due to the increased awareness of HAE and the availability of therapeutic options for HAE patients

0118 | Immunogenicity profile of lanadelumab, a fully human monoclonal antibody plasma kallikrein inhibitor, for the prevention of angioedema attacks in patients with hereditary angioedema

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Introduction: Patients with severe hereditary angioedema (HAE) may be prescribed long-term prophylaxis therapy to prevent HAE attacks. Monoclonal antibodies (mABs) have emerged as safe and

	Icatibant-treated attacks			Untreated attacks			Severity of icatibant-treated attacks for attacks with available severity data, n (%)				
Year of Attack	Number of attacks	Number of patients	Attacks per patient	Number of attacks	Number of patients	Attacks per patient	Very mild	Mild	Moderate	Severe	Very severe
2009	85	44	1.9	478	41	11.7	3 (3.8)	5 (6.3)	16 (20.3)	38 (48.1)	17 (21.5)
2010	256	79	3.2	401	66	6.1	1 (0.5)	4 (1.8)	32 (14.5)	123 (55.9)	60 (27.2)
2011	310	114	2.7	552	97	5.7	1 (0.4)	19 (6.8)	93 (33.2)	104 (37.1)	63 (22.5)
2012	584	147	4.0	1029	159	6.5	-	39 (7.2)	203 (37.9)	216 (40.3)	78 (14.6)
2013	682	176	3.9	1151	179	6.4	8 (1.4)	36 (6.1)	212 (36.2)	239 (40.7)	91 (15.5)
2014	857	182	4.7	643	161	4.0	3 (0.4)	77 (10.6)	249 (34.2)	291 (40.0)	108 (14.8)
2015	595	141	4.2	559	132	4.2	-	72 (14.8)	230 (47.2)	150 (30.8)	35 (7.2)

effective treatments for a number of conditions due to their potency, specificity, and extended dosing intervals compared to conventional medications. They are increasingly being used to treat conditions associated with allergy/immunology. Immunogenicity is often associated with mABs, although this has improved with the emergence of fully human mABs. Lanadelumab is a mAB that is a potent inhibitor of plasma kallikrein to prevent bradykinin release. It is currently in Phase 3 clinical development with potential as a prophylactic treatment against HAE attacks.

Objectives: Here we describe data obtained to-date indicating the low immunogenicity risk for lanadelumab.

Results: The low risk of immunogenicity is based on: 1) Lanadalumab is a fully human IgG1, kappa light chain mAB of G1 m3(f) allotype produced in CHO cells and exhibits minimal degradation. In vitro experiments confirmed lanadelumab does not evoke ADCC or CDC responses. Non-human glycans (a-1,3Gal and Neu5Gc) have not been detected, the molecule is not pegylated, and production of lanadelumab is associated with a low impurity and aggregate profile. These product characteristics minimize the potential to trigger an immunogenic response. 2) Patients with HAE are not at a higher risk for immunogenicity based on their minimally compromised immunologic status and absence of immunomodulatory concomitant medication use. Preexisting anti-drug antibodies have not been identified in any patients to-date. 3) In a 28-day toxicology study, 2/22 (9.1%) cynomolgus monkeys dosed with lanadelumab developed anti-drug antibodies on Day 29. 4) In a Phase 1a clinical study, anti-drug antibodies were not detected in 32 healthy volunteers after a single subcutaneous dose of up to 3 mg/kg. In a Phase 1b study, patients with HAE received 2 doses of up to 400 mg; 2/24 (8.3%) patients were positive for anti-drug antibodies, which were non-neutralizing.

Conclusions: Repeated administration is a risk factor for immunogenicity. In an ongoing pivotal Phase 3 study, patients will receive up to 13 doses of up to 300 mg lanadelumab and in the follow-on open-label extension study, patients will receive up to 26 doses of 300 mg lanadelumab. The risk of immunogenicity for lanadelumab is considered to be low and will continue to be evaluated during extended treatment in these Phase 3 studies.

0119 | Subcutaneous C1-INH (SC) preparation (CSL830) in the prevention of Hereditary Angioedema (HAE) attacks: First findings from the COMPACT extension study

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Introduction: Prophylactic subcutaneous C1-INH (SC) therapy demonstrated excellent safety and efficacy for routine prophylaxis in a Phase 3 pivotal study (COMPACT) and is now being studied for long-term safety and efficacy in the COMPACT extension study (NCT02316353).

Objectives: The objectives of these post-hoc analyses were to compare the efficacy and safety results from the 2 studies for patients (pts) who continued from the pivotal study into the extension study (Data cut-off: 17 MAY 2016).

Methods: The extension study consisted of pts continuing from the pivotal study as well as C1-INH (SC) naïve pts. A total of 126 pts were randomized (1:1) to receive 40 IU/Kg or 60 IU/Kg of CSL830 in the extension study. Subjects continuing from the pivotal study were re-randomized in the extension study independent of the dose they received in the pivotal study. Dose increments of 20 IU/Kg (up to 80 IU/Kg) were permitted in case of frequent HAE attacks. Efficacy was evaluated in terms of time-normalized HAE attacks (HAE attacks/month) and the outcomes of the extension and the pivotal study were compared within-pts using descriptive statistics. Other interim objectives were to assess the safety of long-term prophylactic treatment.

Results: 64 of 126 randomized pts in the extension study continued from the pivotal study and were randomized equally between the 2 treatment doses. In the pivotal study, the median (interquartile range, IQR) HAE attacks/month in these pts were 0.29 (0.00, 1.19) and 0.29 (0.00, 0.60) on 40 IU/Kg and 60 IU/Kg doses, respectively. The median (IQR) within-pts differences for HAE attacks/month between the 2 studies (extension study minus pivotal study) were very small and not clinically relevant [40 IU/Kg: n=15, 0.02 (-0.46, 0.20); 60 IU/Kg: n=14, 0.00 (-0.10, 0.20)]. 12 of 15 (80%) pts on the 40 IU/Kg dose were maintained at the assigned dose. 3 pts were up-titrated to 60 IU/Kg and 1 of the 3 pts was further up-titrated to 80 IU/Kg. All pts assigned to the 60 IU/Kg dose were maintained at this dose. Efficacy for these pts was analyzed by the dose they were randomized to, independent of the dose they were receiving at the time of the attack. Safety results were comparable to those noted in the pivotal study.

Conclusions: Subcutaneous C1-(INH) preparation CSL830 demonstrated sustained efficacy in the COMPACT extension study at both doses. These doses were also well-tolerated and demonstrated consistent safety results.

0120 | The use of a C1 esterase inhibitor concentrate to manage hereditary angioedema attacks in children

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Introduction: Hereditary angioedema (HAE) is a rare disease characterized by episodic subcutaneous or submucosal swelling commonly caused by C1 esterase inhibitor (C1-INH) deficiency. Intravenous (IV) C1-INH concentrate (Shire, USA) is approved for the long-term prophylaxis of HAE attacks in adolescents and adults in the US and the EU, and also for on-demand treatment and pre-procedure prevention of attacks in the EU.

Objectives: We present pharmacology, efficacy, and safety data from 6 clinical trials of C1-INH concentrate to treat and prevent HAE attacks in children. These included phase 3 efficacy and safety trials (NCT00289211, NCT01005888), open-label trials (NCT00438815, NCT00462709), a phase 2 pharmacokinetic (PK) and pharmacodynamics study in children ≥2 to <12 years of age treated for HAE attacks (NCT01095510), and an ongoing phase 3 efficacy and safety study of C1-INH concentrate for the long-term prophylaxis of HAE attacks in children 6–11 years of age (NCT02052141). Fixed doses ranged from 500 to 1500U.

Results: As of April 2015, 22 children 6-11 years of age received C1-INH concentrate to treat 121 HAE attacks, 8 children (6–17 years of age) received 41 infusions for pre-procedure prevention, and 16 (6–11 years of age) children had 1206 infusions for long-term prophylaxis. Three children 2–5 years of age were exposed to C1-INH concentrate (1 for acute treatment, and 2 for prophylaxis). Overall, C1-INH functional activity and antigen levels increased. PK profiles of C1-INH doses in children and adults were generally similar. There was no C1-INH accumulation with multiple doses. Symptom relief from HAE attacks was experienced by 75–100% of C1-INH-treated children, 6–11 years of age. For 39 of 40 medical procedures, there were no HAE attacks <=72 h post-C1-INH administration. Children 6–11 years of age receiving C1-INH concentrate for prophylaxis had a 79–88% reduction in the number of HAE attacks. Results were similar for children 2–5 years of age. C1-INH concentrate was generally well tolerated and most treatment-emergent adverse events (TEAEs; headache, nausea, pyrexia, and infusion site erythema) were mild or moderate in severity. No TEAEs possibly related to the study drug were severe or led to drug discontinuation.

Conclusions: IV C1-INH concentrate had an expected PK profile and was effective and well-tolerated for acute treatment, pre-procedure prevention, and long-term prophylaxis of HAE attacks in children.

TUESDAY, 20 JUNE 2017
OAS 28
MANAGING FOOD ALLERGY IN CHILDREN

0121 | Understanding predictors for severe allergies in pediatric food allergy natural history registry

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Introduction: Despite affecting up to 8% of children nationwide, the natural history of food allergy is not well understood. To learn more about the course of food allergy, we developed a prospective pediatric food allergy registry.

Objectives: The goal of this study was to investigate what factors best predict severe peanut and tree nuts allergies. We recruited families with one or more children with a food allergy from allergy clinics across Chicago into a registry. Data from children's medical records and caregiver surveys were analyzed. Logistic regression analyses were conducted to investigate the relationship between severe allergies and predicting factors. Severity was defined as history of reactions that involve at least two body systems.

Results: Children of respondents (N=158) were mostly male (65.2%), white (76.6%), with an average age of 6.4 years. About 34% of children were currently allergic to five or more foods. Peanut (67.1%) and tree nuts (65.2%) were the most prevalent allergens. Among 103 children with peanut allergy, 34.0% had a history of severe allergic reactions, and age was a statistically significant predictor of severity (OR=1.15, *P*=.0189). Among 98 children with tree nuts allergy, 30.0% had severe reactions, and number of current allergies (OR=0.65, *P*=.02), child's asthma history (OR=3.58, *P*=.012), father's food allergy status (OR=5.43, *P*=.016), and father's eczema status (OR=4.088, *P*=.04) were found to be statistically significant predictors of severity.

Conclusions: For peanut allergy, increase in age increased the odds of severity. History of asthma and father's history of food allergy or eczema increased the patient's odds of severe tree nuts allergy while each additional food allergy decreased the patient's odds of severe tree nuts allergy.

0122 | Amino acid-based formula including specific synbiotics modifies the gut microbiota and reduces clinical symptoms in non-IgE mediated cow's milk allergic infants

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Introduction: Cow's milk allergy (CMA) is associated with aberrant gut microbiota development in early life. Non-IgE mediated CMA is less well understood and clinical symptoms are complex. Previously we have shown that the gut microbiota composition of non-IgE mediated CMA infants was close to that of healthy breastfed infants after an 8-weeks intervention with an amino acid-based formula (AAF) including specific pre- and probiotics (synbiotics) [FAAM2016].

Objectives: This prospective, randomized, double-blind controlled study (NTR3979), investigated the effects of an AAF including synbiotics (oligofructose, inulin, *Bifidobacterium breve* M-16V) on the gut microbiota and explored clinical symptoms in infants with suspected non-IgE mediated CMA. The infants received AAF (control; n=36) or AAF including synbiotics (test; n=35) for 8 weeks. After wk 8, use of AAF (study product) was continued if advised by clinician. At baseline and wk 8, 12 and 26, faecal samples were collected, clinical symptoms recorded and concomitant medication use reported. Faecal % of bifidobacteria and *Eubacterium rectale/Clostridium coccoides* group (*ER/CC*) were determined by fluorescent *in situ* hybridization. Multi-system clinical symptoms were explored including SCORing Atopic Dermatitis (SCORAD) and GI-related symptoms such as vomiting, spitting up, and flatulence were monitored via parent diaries.

Results: At inclusion mean age (±SD) of CMA infants (n=71) was 6.00±2.98 months and 82% was using a hypoallergenic formula. Subjects presented predominantly GI symptoms (90%) and dermatological symptoms (10%); stratification was based on these manifestations. Using the intention-to-treat (ITT) data set and ANCOVA, mean% of bifidobacteria at wk 8, 12 and 26 were significantly higher in test (36, 42, 41%) *vs* control group (15, 16, 15%) (all $P < .001$) and *ER/CC* was significantly lower in test (12, 9, 18%) *vs* control group

(27, 28, 31%) (all $P \leq .001$). At baseline mean GI-symptoms were in the lower half of severity score, but further decreased in both groups. During the study fewer subjects in the test group used dermatologicals (17.1%) *vs* control group (45.7%; *P*=.019).

Conclusions: As reported for wk 8, higher bifidobacteria and lower ER/CC were observed at wk 12 and 26 in infants receiving AAF including specific synbiotics *vs* control group. Whilst clinical symptoms were relatively low at baseline, possibly due to hypoallergenic formula use at study entry, assessed clinical symptoms further reduced in both study groups.

0123 | Peanut epitope-specific IgE binding in the first 2 years of life can predict clinical peanut allergy

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Introduction: About 20% of young children reacting to peanut will outgrow their allergy, but there is no laboratory test that accurately identifies this subset. Utilizing a cohort of 240 subjects from the CoFAR natural history study (Sicherer et al, 2010), we monitored the development of IgE and IgG4 binding to sequential allergenic peanut epitopes in high risk infants from 3–15 mos to 8 yrs of age and determined their utility to predict clinical peanut allergy at age 5 years.

Objectives: A novel high-throughput luminex-based assay (Genisphere LLC) was used to quantitate IgE and IgG4 antibody binding to 50 sequential epitopes found on Ara h1-3. Sera from 240 subjects were evaluated for IgE and IgG4 epitope-specific antibodies at baseline, 2 and 5 yrs. At 5 yrs of age, 126 subjects were non-allergic whereas 48 had confirmed allergy and 66 had food-specific IgE>15 kU_A/l. Epitope-specific binding (EB) scores were obtained from fluorescence intensities. Machine learning algorithms were used to build models that based on EB profiles (EBPs) at 2 years of age could predict whether an individual would be allergic to peanut or not at age 5 years.

Results: While the baseline EBPs of peanut allergic children at 5 years was no different from those without allergy, significant differences in EBPs were evident at 2 years and profound differences were observed at 4–5 years of age; 5 y/o subjects with peanut allergy had a marked increase in IgE epitope binding while non-allergic did not. One hundred eighty subjects were randomly selected for "model development" and 60 for "testing." Of strategies evaluated, the random forest algorithm performed best, accurately predicting the allergy status of all 180 children; with an average AUC>88.3% (Accuracy>81%, Balanced Accuracy>81%) in cross-validation. Models combining EBPs at baseline and Yr-2 together were not more predictive than models using Yr-2 alone. The model obtained during model development was then tested using the Yr-2 profiles of the 'unseen' 60 children. Using only IgE epitopes 51/60 (85.4%) children were correctly classified, for a balanced accuracy of 85%, AUC=87.5%, Sensitivity=73.1%, Specificity=96.5%, PPV=95%, NPV=80%.

Conclusions: Evaluation of the peanut allergenic epitope repertoire at ~2 yrs of age is predictive of peanut allergy status at 5 years. If confirmed in other studies, this assay should enable physicians to identify infants with persistent peanut allergy for initiating early immunotherapeutic interventions.

0124 | Peanut, tree-nut and sesame seed allergies: do children allergic to a nut or sesame seed need to avoid all nuts?

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Introduction: There is a large cross-sensitivity by IgE testing among tree nuts, peanut and sesame seed; however, rate of challenge proven co-allergy has not been proven. The introduction of 'safe' selective nuts and seeds in children already allergic to one nut is gaining momentum, especially following studies showing that oral peanut exposure prevents peanut allergy.

Objectives: The Pronuts study recruited children up to 16 years of age with at least one confirmed nut or seed allergy. Based on sequential challenge to 11 nut/seeds, our objective was to determine the true rate of co-existent peanut, tree-nut and sesame seed allergy. Patients were then asked to introduce all 'safe' nuts/seeds regularly into the diet to determine the feasibility and safety of this approach.

Results: Ninety three children completed over 800 food challenges performed according to the PRACTALL guidelines. Up to three nuts/seeds were performed in a single challenge. Monoallergy was present in 45% of children and 2 nut allergies were present in 61%. The UK had a younger population and had a 52% rate of monoallergic children. In the UK peanut allergy was the most common index nut allergy followed by walnut and hazelnut; in Geneva it was cashew followed by peanut then pistachio. All 32 children with pistachio nut allergy had cashew nut allergy, but 6 cashew allergic children could tolerate pistachio. We noted clusters of other nut allergies, most notably between pecan, walnut and hazelnut allergy.

Conclusions: This is the first prospective study to establish the rate of challenge proven co-existent allergy among peanut, all tree-nuts and sesame seed allergy. The majority of participants were able to introduce up to 9 additional nuts and seeds into their diets. The differences between Geneva and London in the prevalence of monoallergy, specific nut allergies and clusters may be due to difference in age and/or dietary or environmental exposure to nuts.

0125 | Long-time evolution of oral immunotherapy in highly sensitized milk allergy children

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Introduction: Oral immunotherapy (OIT) with milk is an alternative treatment for children with persistent milk allergy. It could be a risky procedure, especially in highly sensitized patients. The aim of this study was to evaluate long-time evolution of milk allergic patients with milk/casein-specific IgE>100 KU/l who underwent OIT.

Objectives: Oral immunotherapy (OIT) with milk is an alternative treatment for children with persistent milk allergy. It could be a risky procedure, especially in highly sensitized patients. The aim of this study was to evaluate long-time evolution of milk allergic patients with milk/casein-specific IgE>100 KU/l who underwent OIT.

Results: Seventeen patients were included (53% male); of which 35.2% had atopic dermatitis and 88.2% asthma. Mean age of patients was 8.64 years±3.04 SD and mean casein-specific IgE was 404.17 KU/l±393.9 SD. Mean time duration of OIT was 28.57 weeks±16.1 SD.

Fifteen patients were able to complete treatment; 80% of them reached total tolerance (200 ml) and 20% partial tolerance (<200 ml). Only two patients leaved the treatment for repeated severe reactions. Although all patients had adverse reactions during OIT, most of them were mild to moderate. In the follow-up 12 and 24 months after OIT, all patients continue tolerating; however, most of them with adverse reactions at home: 12 months after OIT, twelve patients (80%) had a total of 71 adverse reactions (38.0% mild, 57.7% moderate, 4.3% severe). Between month 12 and 24 after OIT, eight patients (53.3%) had a total of 21 adverse reactions (33.4% mild, 57.1% moderated, 9.5% severe).

Of the total number of adverse reactions, 8.7% were caused by goat/sheep cheese contamination and 43.5% were associated with a cofactor, being exercise the most frequently involved.

At 24 months, milk/casein-specific IgE decreased in all patients. Twelve patients have been followed over 24 months, and only one patient had a severe adverse reaction, caused by goat/sheep cheese contamination ingestion.

Conclusions: OIT seems to be an effective treatment in high sensitized milk allergy children. Cofactors, specially exercise, are frequently involved in adverse reactions in our patients.

0126 | Improved quality of life and less diet restrictions if CRD is used: simulation analysis in a prospective food allergy study among school children

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Introduction: Food allergy is known to be associated with poorer Quality of Life (QoL) in children and in their caregivers. The overall QoL is significantly lower in those parents who diagnosed their kids' food allergy themselves, w.r.t. to the ones who received a proper medical diagnosis.

Objectives: In accordance to Finnish Allergy Program 2008–2018, a prospective study was initiated to investigate how to decrease food avoidance diets by 50% in school children with suspected food allergy, based on an algorithm in which patient history is sided by CRD (ImmunoCAP ISAC). The present analysis explores how diet changed after ISAC results, simulating also QoL variations in children and in their caregivers.

Results: Out of 2885 children attending school in the Härkätie region in Finland, 205 were on diet due to food allergy, the diet being reported on a school diet register and based on a medical certificate. 157 kids agreed to participate to the study, 90 were referred to laboratory tests for food sIgE and ISAC, and in 11 cases food challenges (FC) were performed. 7 children dropped out, leaving 83 children in this simulation analysis.

QoL average values were extracted from recent literature for both parents (WHOQoL-BREF scale, a 5-points Likert score, higher scores reflecting higher QoL) [Birdi 2016], and children (PedsQM™-generic questionnaire, 0–100 scale, higher scores reflect higher QoL); data from [Cummings 2010] for nuts allergic children and from [Valentine 2011] for other food allergies). QoL changes in children and in their parents were evaluated based on the diet register, and after ISAC+FC.

Results from the school register show that 31 children requested to avoid one (37.3%), 16 (19.3%) two, and 36 (43.4%) 3–8 foods. Among foods avoided were nuts (in 26 kids), staple foods (in 30) and fruits/vegetables (in 52). After ISAC+FC, only 14 children (16.9%)

had to keep their diet, while 69 (83.1%) resumed eating regular meals.

For parents, the average QoL based on the school register data was 3.45, and after ISAC+FC it increased to 4.74 (37.1% increase). In children, QoL moved from 81.8 to 88.4 respectively (almost healthy person).

Conclusions: This study shows that many school children are requested to stay on a food avoidance diet, but they could actually eat freely, making it possible to reduce avoidance diet by 50% when using ISAC. Results show also that correct diagnosis can improve importantly the QoL of children and of their caregivers.

TUESDAY, 20 JUNE 2017
OAS 29
ASTHMA IMMUNOPATHOLOGY/INFLAMMATION

0127 | Neutrophil extracellular traps are associated with asthma severity and neutrophilic phenotype

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Introduction: Neutrophil extracellular traps (NETs) and eosinophil extracellular traps (EETs) are web like structure formed during neutrophil and eosinophil activation respectively. These filaments are made of chromatin associated with different enzymes and antimicrobial proteins depending on their cellular origin. Beside the well documented anti infectious properties of both structures, several lines of evidence now suggest a major role for NETs in thrombosis, autoimmune diseases and cancer whereas EETs functions remain poorly documented.

Objectives: To investigate the presence of NETs and EETs in a large cohort of asthmatic patients and the relationship of both source of extracellular DNA with inflammatory phenotype and clinical features of asthma.

Results: Circulating NETs and EETs were evaluated in 190 asthmatic patients (N=100 atopic asthma, N=90 non atopic asthma) from the national cohort COBRA, using two complementary approaches: cell free DNA quantification by fluorimetry and DNA-enzyme complex quantification by ELISA: DNA-MPO (myeloperoxydase) for NET evaluation, and DNA-EMBP (eosinophil major basic protein) for EET evaluation. Local production of these extracellular traps (ETs) was assessed by evaluating these markers in 22 BAL supernatants (eosinophil count median, 2%; IQR, 0–5.5%; neutrophil count median 8%; IQR, 3.1–25.6%) and by imaging ETs in bronchial biopsies using immunofluorescence confocal microscopy.

We found higher circulating NET levels in severe asthma compared to moderate asthma whereas NET levels were similar between the atopic and the non atopic groups. In particular, NET levels correlated with some severity and poor control markers like persistence of symptoms between exacerbation, exertional dyspnea or wheezing. When analyzing local production of NETs, we evidence an association between NET levels in BAL and bronchial neutrophilia. Surprisingly, while detecting circulating DNA-EMBP complexes in some patients, we failed to detect them in any of the 22 BAL supernatants available. To date, the visualization of NETs and EETs in bronchial biopsies is under evaluation.

Conclusions: NETs are associated with the clinical severity, the poor control and the neutrophilic phenotype of asthma independently of atopy, whereas EETs seem to be less important in this setting.

0128 | Type 1 interferon alpha (IFN-a) regulation by respiratory viruses in pediatric asthma

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Introduction: Two third of the virus associated asthma exacerbations are caused by Rhinovirus (RV). The RV activated and regulated antiviral genes like Interferon type I and their signal transduction need to be better clarified. In this study, we analyzed the influence of RV infection in the nasopharyngeal fluid (NPF) on the Interferon type I, IFNa, responses in two cohorts of pre-school children with and without asthma and related them to their clinical outcome at baseline and during disease exacerbations. Interferons (IFNs) are large group of agents with antiviral activity. Type I IFNs are primarily responsible for antiviral activities in humans. Binding of the IFN alpha to its receptor activates Jak1 and Tyk2 and their principle targets for phosphorylation, signal transducer and activator of transcription 1 (STAT1) and 2 (STAT2). Together with IRF9, phosphorylated STAT1 and STAT2 form the so called Interferon-Stimulated Genes Factor 3 (ISGF3) complex. In asthma, type I IFNs are described to be deficiently induced upon respiratory viruses challenge.

Objectives: In our prospective European multicenter study PreDicta, we analyzed IFN-alpha levels in serum of children with and without asthma and sub-grouped the children in accordance to the presence (+RV) or absence (-RV) of Rhinovirus (RV) in their airways in vivo to analyze the influence of rhinovirus (RV) in nasopharyngeal fluid (NPF) on the interferon (IFN) type I responses like IFN-α and

their signal transduction, at baseline and during disease exacerbation in the blood of a cohorts of pre-school children with and without asthma.

Results: At baseline, in healthy children, RV detection in vivo was found associated with an increase in IFNA mRNA expression in their PBMCs, as compared to asthmatic children with a positive RV detection in their NPF. However, by looking at PBMCs from asthmatic children infected *in vitro* with RV1b we found an upregulation of *MYD88,IRF1*, *STAT1* and *STAT2* mRNA, whereas in control children *MYD88,IRF1*, *STAT1* and *IRF9* were predominantly induced. Moreover, during symptomatic visits because of disease exacerbation associated with RV detection in NPF, IFN-α production was found increased

Conclusions: In conclusion, during asthma exacerbations associated with RV, asthmatic children can induce IFN-α secretion indicating that infections that cause asthma exacerbations, can help us to better understand the regulation of antiviral immune responses in asthma.

0129 | Increase of systemic and local regulatory B cells coincides with a shift in T cell subpopulations in and out of grass pollen season

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Introduction: Regulatory immune cells play a pivotal role for restoring tolerogenicity in allergic disease entities. IL-10-producing regulatory B cells (Bregs) are known to maintain regulatory capacities of T cells while restricting Th1 and Th17 differentiation. Recently, it has been shown that regulatory T cells (Tregs) can not only differentiate into effector Th17 cells via an intermediate subset expressing FoxP3 and IL-17 simultaneously, but also, that Th17 cells carry the ability to transdifferentiate "back" into Tregs.

Objectives: In this study, we extracted sputum and blood cells from 40 allergic rhinitis patients with and without grass-pollen specific immunotherapy (AIT) as well as 25 healthy subjects in and out of season and analyzed different immune cell populations using flow cytometry.

Results: Local and systemic T- and B-cell subsets were compared during grass pollen season and out of the season in allergic rhinitis patients and healthy controls. We found a significant increase in IL-10-producing regulatory B cells out of the grass pollen season, while a positive influence of AIT on Breg populations became visible. On top, the IL-37$^+$ regulatory B cells were significantly increased in season, while no significant intra-seasonal group difference arose. The increased local regulatory B cell population in sputum coincides with a significant decrease of effector Th17 cells and, notably, with an extra-seasonal increase in the local IL-17-expressing CD4$^+$FoxP3^{+-} Treg population.

Conclusions: In summary, we could show that the seasonal immune response to specific immunotherapy involves the local induction of B cells carrying immunoregulatory functions. We postulate that this increase leads to a shift of Th17 cells towards a rather regulatory phenotype, as not only the Th17 population, but also the intermediate IL-17$^+$FoxP3$^+$ Treg subset was significantly increased out of the season. In conclusion, the frequency of Th17 cells and IL-17-producing regulatory T cells in the peripheral blood and induced sputum may represent early biomarkers of immunotherapy efficacy.

0130 | Tgf-beta regulation and regulated anti-rhinovirus (RV) immune responses in asthma

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Introduction: The majority of asthma exacerbations in children are caused by Rhinovirus (RV). TGF-β induces Rhinovirus replication and it is secreted in a latent inactive complex enclosing the inhibitor latency-associated protein, LAP.

Objectives: Here, we analyzed the role of TGF-β before and after RV challenge of PBMCs from preschool healthy and asthmatic children and in experimental asthma.

Results: In PBMCs from asthmatic children as well as in murine lung cells from asthmatic mice, after RV challenge ex vivo, TGF-beta is maintained and inhibited by LAP3 and IDO production in RV infected host cells leading to T-bet driven and CD8+IFN-gamma+ cell mediated anti-viral immune responses. However, administration of active exogenous mature TGF-beta to lung cells from asthmatic mice, after RV infection ex vivo, reduced an antiviral immune responses as shown by reduction of T cells producing IFN-gamma.

Conclusions: These data suggest that RV uses active TGF-β present in the environment to replicate and to inhibit effective antiviral immune responses. Moreover, during acute RV infections, endogenous TGF-beta is retained and inactivated in RV infected host cells, resulting in a CD4+T-bet+ and CD8+IFN-gamma+ T cell mediated acute immune response.

0131 | Dupilumab efficacy in severe asthma exacerbations by different baseline patient characteristics in patients with uncontrolled persistent asthma

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Introduction: Dupilumab (DPL), a fully human IL-4R-α monoclonal antibody, inhibits IL-4 and IL-13 signalling, key drivers of type 2-mediated inflammation. In a randomized pivotal phase 2b study (NCT01854047), DPL improved lung function (FEV_1), reduced severe asthma exacerbations, improved quality of life measures, and was generally well tolerated in adults with uncontrolled persistent asthma on medium-to-high-dose ICS+LABA.

Objectives: This post hoc analysis reports the effect of DPL on the rate and risk of severe asthma exacerbations in various subgroups. Severe exacerbations in the 24-week treatment period are reported for the intent-to-treat (ITT) population receiving DPL 200 and 300 mg every 2 weeks (q2w), doses currently being assessed in phase 3 (NCT02414854), and placebo (PBO). Subgroups are defined by: BMI, FEV_1, FEV_1 % predicted, ACQ-5 score, severe exacerbations in the year prior to study, ICS+LABA dose, and age at asthma onset at baseline.

Results: DPL q2w ($P < .001$ vs PBO) reduced severe exacerbations in the ITT population (−70.0 to −70.5%; $P < .001$ vs PBO) and in all subgroups examined (numerically/significantly; Table). A significant treatment-by-subgroup interaction was observed for the subgroup defined by baseline FEV_1 % predicted receiving DPL 300 mg q2w. Numerically better reductions in severe exacerbations were observed with DPL 300 mg q2w in patients with baseline FEV_1 predicted <60% vs those with FEV_1 predicted ≥60%.

Conclusions: Dupilumab q2w demonstrated a consistent effect in reducing severe asthma exacerbations across baseline characteristic subgroups in patients with uncontrolled persistent asthma.

	Subgroup, (n)	Adjusted annualized rate of severe exacerbations, estimate (95% CI)			Relative risk vs PBO (95% CI)		Reduction vs PBO, %		*P* value for interaction	
		PBO	200 mg q2w	300 mg q2w	200 mg q2w	300 mg q2w	200 mg q2w	300 mg q2w	200 mg q2w	300 mg q2w
Overall population		0.897 (0.619–1.300)	0.269 (0.157–0.461)	0.265 (0.157–0.445)	0.300 (0.159–0.565)	0.295 (0.159–0.546)	70.0	70.5		
BMI (kg/m^2)	< **30 (276)**	0.793 (0.474–1.328)	0.233 (0.109–0.501)	0.247 (0.120–0.507)	0.294 (0.118–0.731)	0.311 (0.130–0.745)	70.6	68.9	0.9955	0.8316
	≥ **30 (186)**	0.832 (0.406–1.706)	0.259 (0.108–0.624)	0.213 (0.087–0.520)	0.311 (0.128–0.758)	0.255 (0.107–0.608)	68.9	74.5		
FEV_1 (L)	≤ **1.75 (238)**	1.399 (0.881–2.220)	0.394 (0.206–0.756)	0.272 (0.132–0.560)	0.282 (0.129–0.616)	0.194 (0.083–0.455)	71.8	80.6	0.8847	0.0866
	> **1.75 (224)**	0.388 (0.205–0.737)	0.122 (0.045–0.333)	0.219 (0.103–0.466)	0.314 (0.102–0.968)	0.564 (0.227–1.400)	68.6	43.6		
FEV_1 (%) predicted	< **60 (201)**	1.359 (0.824–2.241)	0.464 (0.239–0.901)	0.211 (0.089–0.498)	0.342 (0.154–0.760)	0.155 (0.059–0.409)	65.8	84.5	0.6438	0.0365
	≥ **60 (261)**	0.498 (0.288–0.861)	0.106 (0.040–0.283)	0.263 (0.137–0.504)	0.213 (0.073–0.621)	0.527 (0.242–1.150)	78.7	47.3		
ACQ-5 score	≤ **2 (113)**	0.354 (0.129–0.973)	0.036 (0.004–0.319)	0.250 (0.078–0.800)	0.101 (0.011–0.967)	0.705 (0.184–2.697)	89.9	29.5	0.2630	0.1308
	> **2 (349)**	1.115 (0.748–1.661)	0.380 (0.216–0.669)	0.263 (0.146–0.471)	0.341 (0.175–0.667)	0.235 (0.119–0.467)	65.9	76.5		
No. severe asthma exacerbations in the year prior to study	**1 (236)**	0.766 (0.426–1.379)	0.199 (0.087–0.456)	0.182 (0.070–0.470)	0.260 (0.094–0.713)	0.237 (0.078–0.722)	74.0	76.3	0.6694	0.5555
	> **1 (226)**	0.938 (0.567–1.551)	0.321 (0.153–0.674)	0.320 (0.170–0.605)	0.342 (0.153–0.767)	0.342 (0.166–0.704)	65.8	65.8		
ICS+LABA dose	**Medium[a] (221)**	0.525	0.199	0.217	NE	NE	62.1	58.7	0.4247	0.3173
	High (229)	1.525 (0.972–2.392)	0.417 (0.212–0.819)	0.404 (0.215–0.761)	0.273 (0.123–0.605)	0.265 (0.124–0.568)	72.7	73.5		
Age at asthma onset, years	≤ **40 (334)**	0.726 (0.462–1.141)	0.312 (0.174–0.558)	0.192 (0.100–0.366)	0.430 (0.221–0.834)	0.264 (0.128–0.543)	57.0	73.6	NE	NE
	> **40[a] (125)**	1.081	0.000	0.449	NE	NE	100.0	58.5		

NE, Not estimable. aUnadjusted data are presented as the adjusted rates could not be estimated for these subgroups.

0132 | FoxP3+ regulatory B cells were higher in sputum and blood, but lower in bone marrow following whole lung allergen challenge in allergic asthmatics

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Introduction: Regulatory B cells (B_{regs}) modulate IgE-mediated inflammatory responses in allergic asthma through production of IgG_4 neutralizing antibodies, and immunomodulatory cytokines, including IL-10. Additionally, FoxP3 is a transcription factor that modulates the development and function of T_{regs}, but its role in B_{regs} is currently not known. We previously showed that peripheral blood (PB) and bone marrow (BM) levels of CD5+FoxP3+, CD1d+CD5+, CD27+CD24+, and CD24+CD38+ B_{reg} phenotypes were detectable in PB and BM following allergen challenge. This current study evaluated the kinetics of FoxP3+ B_{regs} in sputum, peripheral blood, and bone marrow of allergic asthmatics following whole lung allergen challenge.

Objectives: Fifteen subjects with allergic asthma were recruited for whole lung allergen challenges and spirometry was measured for 7HR after allergen inhalation. Diluent inhalation challenges were conducted as controls. Sputum, PB and BM aspirates were collected at baseline (BL) and up to 24HR post-challenge. Cells were isolated and stained with CD1d, CD5, CD24, CD27, CD38, CD19, CD45, IL-10 and FoxP3, acquired using a Becton Dickinson LSR Fortessa flow cytometer, and data were analyzed using FlowJo software. Lymphocytes were defined as $SSC^{low}CD45+$, and B cells were defined as $SSC^{low}CD45+CD19+$. GraphPad Prism was used to perform 2-way ANOVAs and Bonferroni post-hoc analyses. Data were expressed as median (range).

Results: FoxP3+ B_{reg} levels measured in sputum were 7 (3–13)% at BL, 17 (7–23)% 7HR post-allergen, and 21 (4–28)% 24HR post-allergen. Additionally, FoxP3+ B_{reg} levels measured in PB were 6 (3–17)% at BL, 9 (1–52)% 7HR post-allergen, and 10 (3–23)% 24HR post-allergen. Furthermore, FoxP3+ B_{reg} levels measured in BM were 10 (3–21)% at BL, and 5 (2–13)% 24HR post-allergen. Sputum and PB FoxP3+ B_{reg} levels at 24HR post-challenge were significantly higher compared to diluent ($P < .05$), while BM FoxP3+ B_{reg} levels at 24HR post-challenge were significantly lower compared to diluent ($P < .05$).

Conclusions: Allergen-induced changes in the kinetics of FoxP3+ B_{regs} appear to be related to their relative compartment or location in the body of allergic asthmatics. At 24HR post-challenge, we observed decreased FoxP3+ B_{regs} in BM, and increased FoxP3+ B_{regs} in PB and sputum. Taken together, these findings suggest that FoxP3 expression may play an important role in the mobilization of B_{regs} from BM, through circulation, and into the airways of allergic asthmatics after allergic stimulation.

TUESDAY, 20 JUNE 2017
OAS 30
DRUG ALLERGY: DIAGNOSIS

0139 | Eicosanoid mediators levels in patients with NSAIDs-induced acute urticaria/angioedema

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Introduction: Hypersensitivity reactions to non-steroidal anti-inflammatory drugs (NSAIDs) can induce a variety of phenotypes including NSAIDs-exacerbated respiratory disease (NERD) and NSAIDs-induced acute urticaria/angioedema (NIUA). Experimental data from NERD support that cycloxygenase-1 inhibition, which shunts the arachidonic acid pathway from the prostaglandin (PG) pathway towards the biosynthesis of cysteinyl leukotrienes (LTs), plays a central role in the development of clinical symptoms. However, despite being the most frequent entity induced by NSAIDs-hypersensitivity, the underlying mechanisms of NIUA remains still unknown.

Objectives: We analyzed urine eicosanoids levels in NIUA patients (n=10) with a positive ASA challenge. Urine samples were taken before ASA challenge (basal), and at three 3-h intervals after challenge: T1 (within the first three hours), T2 (within 4–6 h), and T3 (within 6–9 h). Control urine samples were obtained from healthy individuals (n=13) at the same time intervals who also took ASA. LTE4, 13,14-dihydro-15-keto-PGE2 (PGE2) and 9a,11b-PGF2 (PGF2) levels were measured by gas chromatography/mass spectrometry (GC-MS) and high performance liquid chromatography/tandem mass spectrometry (HPLC-MS).

Results: A total of 10 patients and 13 controls were included. LTE4 levels were statistically increased in patients at T1 compared to controls (P=.013), then subsequently decreasing at T2 and T3. Conversely, PGE2 levels were lower at T1 (P=.015) and tended to increase at T2 and T3. Although not statistically significant, PGF2 levels were slightly raised at T2. No statistically significant changes were observed in these metabolites in the control group for any time interval.

Conclusions: Our results show that inflammatory mediators from the arachidonic acid pathway could play a role in NIUA, with differential patterns shown by LTE4 and PGE2. These data help shed new light into the pathomechanisms of NIUA.

0140 | Diagnostic value of T cell analysis of CD69 up regulation and in vitro release of interleukins in patients with nonimmediate hypersensitivity to amoxicillin

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Introduction: Nonimmediate reactions to betalactam (BL) antibiotics are one of the most common drug hypersensitivity reactions. Diagnostic evaluation of nonimmediate reactions is hampered by low sensitivity of skin testing and lack of other diagnostic methods. Drug provocation tests, that are time consuming and possibly dangerous for the patients, are still needed. With this study we aimed to assess diagnostic value of quantitative T cells analysis of CD69 up regulation and *in vitro* release of IL-2, IL-5 and IL-13 in diagnosis of patients with nonimmediate reactions to amoxicillin.

Objectives: CD69 upregulation on CD3 + , CD3 + CD4 + and CD3 + CD8 + T cells was analysed by flow cytometry after stimulation and incubation for 4 hours with 25 μg/ml and 100 μg/ml amoxicillin in 13 patients with confirmed amoxicillin hypersensitivity. PBMC were also incubated for 48 hours with 25 μg/ml and 100 μg/ml amoxicillin. IL-2, IL-5, IL-13 and IFN-γ concentrations were measured in supernatants with multiplex flow cytometry CBA Flex Array. To evaluate predicted normal values the same measurements were done in 7 patients in whom hypersensitivity to amoxicillin was excluded and 5 healthy controls that were exposed to amoxicillin in the past and had no adverse events.

Results: In six patients hypersensitivity was confirmed with skin tests and in seven patients drug provocation test was necessary. For all different measurements results were considered positive if values measured in patients with confirmed hypersensitivity were higher than the range of values for all negative patients. Highest number (4) of positive patients were found for CD69 upregulation of CD3 + cells after stimulation with 100 μg/ml amoxicillin. In three of those patients, amoxicillin hypersensitivity was confirmed with drug provocation test, so the provocation test could be omitted. Additional provocation test could be omitted with positive values for CD3 + 25 μg/ml, CD3 + 4 + 25 μg/ml, CD3 + 8 + 100 μg/ml, IL2 100 μg/ml.

Conclusions: If all positive *in vitro* results would be used in diagnostic evaluation of patients with amoxicillin hypersensitivity, potentially dangerous and time consuming provocation test, could be omitted in all seven patients.

0141 | Surveillance of severe cutaneous adverse reactions in Korea based on a nationwide registry

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Introduction: Severe cutaneous adverse reactions (SCARs) are life-threatening delayed hypersensitivity induced by various drugs consisting of Stevens-Johnson syndrome (SJS), Toxic Epidermal Necrolysis (TEN), and Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS). Despite the seriousness of these conditions, epidemiologic and clinical information is still not sufficient due to their sporadic occurrence. Therefore, a nationwide surveillance system is required to gather epidemiologic data of SCARs.

Objectives: To investigate the nationwide occurrence and clinical outcomes of SCARs in Korea.

Results: We built a retrospective SCARs registry which comprised of 34 tertiary referral university hospitals in order to recruit SCAR cases occurred from 2010 to 2015. The demographic information, culprit agents, and clinical outcomes were assessed. A total of 745 SCAR cases were enrolled; 384 cases of SJS or TEN and 361 cases of DRESS. Median age was 55 years and the male proportion was 49%. Patients with SCARs were hospitalized for average 22 days; 51 patients (6.8%) were transferred to intensive care units. Overall mortality rate was 6.6% and long-term sequelae were reported in 8.2%. While 92.2% of patients with DRESS completely recovered, only 76.6% of patients with SJS/TEN recover their health. Aging was the most important risk factor of fatality. For 745 cases, 1,393 drugs were suspected as culprit drugs. Main causative agents of SCARs in Korea were allopurinol (13.8%), carbamazepine (9.3%), and vancomycin (4.7%). Outcomes of SCARs were varied by culprit drugs; recovery rate was 91.6% in carbamazepine-induced SCAR cases while it was only 76.9% in methazolamide-induced SCAR cases.

Conclusions: In Korea, allopurinol and carbamazepine were the most frequently reported causative agents of SCARs. Clinical outcomes of SCARs showed the difference by related culprit drugs.

0142 | Basophil activation using synthetic antigenic determinants for diagnosing immediate hypersensitivity to clavulanic acid

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Introduction: Consumption of amoxicillin and clavulanic acid (CLV) in combination has increased in recent decades. Reports of selective reactions to CLV have also increased, and now account for around 30% of allergic reactions to the amoxicillin-CLV administration. In general, the basophil activation test (BAT) is useful for diagnosing betalactam immediate hypersensitivity and the only available *in vitro* assay for diagnosing patients with immediate hypersensitivity to CLV, although its sensitivity is still not optimal. One of the possible reasons could be the use of the incorrect drug metabolite.

Objectives: The aim of this study was to improve BAT sensitivity in the diagnosis of immediate hypersensitivity to CLV using different synthetic CLV determinants. These determinants were designed

considering possible fragments of the CLV molecule bound to a protein carrier.

Results: Six synthetic determinants of CLV derived from two hypothesized antigenic determinants (AD-I and AD-II) were designed presenting different functionalities, respectively: aldehyde (Clav1, Clav2 and Clav3) or amine (Clav4, Clav5 and Clav6). These determinants were tested using BAT in 30 patients with selective immediate hypersensitivity reactions to CLV and 20 non-atopic controls that tolerated CLV. Positive BAT results in patients were found for CLV (40%) and two of the synthetic determinants, Clav2 (56.7%) and Clav3 (26.7%). Sensitivity increased to 66.6% when CLV and Clav2 results were combined. Specificity was 88%. Finally, to prove that the observed activation was IgE mediated, BAT was performed on cells previously incubated with wortmannin, a potent inhibitor of the IgE signalling pathway. In all cases wortmannin treatment significantly reduced the CD63 expression compared to BAT performed without inhibition ($P<.001$ for CLV; $P<.001$ for Clav2; $P=.007$ for Clav3), showing the involvement of an IgE-mediated mechanism.

Conclusions: Determinants derived from AD-I (Clav2 and Clav3) are able to activate basophils by a specific IgE mechanism in selective reactors to CLV. Current BAT sensitivity using the parent drug can be significantly improved by including Clav2. These results indicate that AD-I could be the determinant involved in CLV IgE recognition by the immune system in those patients with hypersensitivity to CLV. These findings may be important for the development of new *in vitro* tests.

0143 | Penicillin major and minor determinants: are they still relevant?

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Introduction: Penicillin allergy has been estimated to be responsible for up to 20% of drug related deaths in Europe. This is routinely diagnosed in adults with skin testing followed by challenge if skin tests are negative. It is estimated that the positive predictive value of skin test to penicillin is approximately 50%.

Objectives: We reviewed the notes of all patients who underwent penicillin allergy testing at Guy's and St Thomas' NHS Foundation Trust between July 2010 to December 2016. Patients underwent skin testing and intradermal testing with neat Benzylpenicilloyl octa-L-lysine (PPL), neat Sodium Benzylpenilloate (MD), 1:10 dilution Benzylpenicillin (BP), neat Amoxicillin and the index penicillin. Patients who had negative skin test were invited for an oral challenge with either BP, Amoxicillin or the index penicillin.

Results: 1093 patients had undergone penicillin allergy testing. 125 patients (11%) were diagnosed to have penicillin allergy. 83 (66%) patients were diagnosed to have a Type I IgE mediated allergy while 42 (34%) diagnosed to have a delayed type allergy. 4 patients who tested positive to only PPL and/or MD whose history was incongruent with an allergic reaction had a negative oral challenge with penicillin, they were removed from further analysis. 78% of patients were diagnosed by skin testing, 21% by challenge testing and 1% by specific IgE. Of the immediate positive skin test patients 44% were positive to only Amoxicillin, 14% to only BP and Amoxicillin, 14% to only index, with 92% of patients testing positive to either BP, Amoxicillin and/or Index. 789 patients (80% of skin test negative) had undergone oral challenges of which 9 (1%) had an immediate reaction and 17 (2%) had a delayed reaction. In our case series the negative predictive value of skin testing is 96.6%, and 95.8% if PPL and MDM are omitted from skin testing. Furthermore we estimate that for every 181.5 patients skin tested there would be a single patient who tested positive to PPL and/or MDM, which is approximately 3.5x the cost of one drug challenge.

Conclusions: Our case series is supportive of the requirement of challenge testing to diagnose penicillin allergy. It also shows that the negative predictive value of skin testing when omitting PPL and MD is reduced by less than 1%, and the cost of its use to detect one patient is significantly more than by challenge testing. We should reconsider the cost effectiveness of using PPL and MDM in penicillin allergy testing.

0144 | Drug provocation tests with beta-lactams in patients with nonimmediate reactions: comparison of two protocols

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Introduction: Beta-lactams are the most frequent cause of drug reactions mediated by specific immunological mechanisms. Drug provocation tests (DPT) are considered the gold standard in the diagnosis of drug allergy, though in nonimmediate allergic reactions (NIR) it is not consensual its optimal duration.

Objectives: To compare two distinct protocols of DPT in patients with history of NIR to beta-lactams.

Methods: A retrospective study of patients with history of NIR to beta-lactams (>1 hour after last administration) was conducted in our Department, between January 2014 and June 2016. Data was collected from patients' electronic medical records. The protocols consisted in: Protocol 1—administration of beta-lactam in gradually increasing doses, in hospital setting, until treatment dose was reached. Protocol 2 - administration of beta-lactam in gradually increasing doses, in hospital setting, until treatment dose was reached, followed by continuation of medication at home (minimum 3 days).

Results: During the referred period 111 DPT, with the characteristics described above, were performed. The majority of patients were female (53%). Median age at time of reaction was 4 years (p25-p75: 2-14.5 years). In terms of severity, 44% had history of mild reaction, 54% moderate and 3% of severe reaction. Mean day of occurrence of self-reported reaction was 3 days (p25-p75: day 3-day 5). The antibiotics associated with the reactions, and used in the DPT, were in 51% of cases amoxicillin-clavulanic acid, 46% amoxicillin and in 3%, cephalosporins. 57 patients followed protocol 1 and 54 protocol 2. From those 57 patients on protocol 1, four (7.5%) had a positive DPT and from those 54 following protocol 2, five DPT were positive (10.2%). There were no statistically significant differences between protocols in regards to DPT outcome (P=.931), severity of the reaction (P=.934) or age at the time of self-reported reaction (P=.734).

Conclusions: In our sample the frequency of positive DPT was low. There were no statistically significant differences in relation to positivity of DPT between protocols. These results suggest that both protocols may be valid options in the work-up of NIR, however more studies with a larger population are needed.

TUESDAY, 20 JUNE 2017
OAS 31
CELLULAR AND MOLECULAR DIAGNOSTIC TECHNIQUES

0145 | Generation of enriched mature basophil cultures from CD34+ haematopoietic stem cells highly responsive to allergen stimulation upon sensitization

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Introduction: Although various basophil purification/enrichment methods have been described, the generation of mature human basophil cultures remains a challenge. In previous work primary human basophils have been generated *in vitro* using $CD34^+$ cells (haematopoietic stem cell) from $CD34^+$ enriched blood sources, such as cord blood or leukapheresis products mobilized by GCSF/GMCSF. However, $CD34^+$ enriched blood sources are not readily available, which has significantly hampered its widespread use.

In this study, we demonstrated a new recipe for culturing primary basophils. This culture method allows rapid expansion of $CD34^+$ cells and is able to generate sufficient basophils using only PBMCs from the normal blood source.

Objectives: The main objective of this study is to generate mature and functional basophils from $CD34^+$ cells of normal PBMCs with high yield.

Results: Basophils were characterized as $2D7^+/Fc\varepsilon RI^+/CD117^-/HLADR^-$. The optimum maturation window was between day 16-21. This was further confirmed with BB1 expression (a marker of basophils) and activation tests using anti-IgE. Within this window, we usually get $1\text{-}4x10^7$ cells where 30-50% are basophils.

Activation tests performed on cells passively sensitized with peanut-allergic sera or grass-allergic sera showed significant association between the percentages of activated basophils (CD63) and sera Ara h2 IgE level (P=.018, r^2=.69) and whole timothy grass IgE level (P=.003, r^2=.84) respectively.

Levels of pERK1/2 peaked after 10 mins of anti-IgE stimulation, which broadly similar to the previously published results.

Conclusions: We generated primary basophil from hematopoietic blood progenitors with high yield. These cultures demonstrated to be functional as demonstrated by their degranulation activity and the phosphorylation of ERK in a passive basophil activation test. Thus the obtained basophil cultures promise to be a useful tool in clinical allergy and basic basophil biology research.

0146 | Recombinant basophil activation test and "fingerprint" modeling of allergenic activity

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Introduction: The aim of this work was to introduce a novel model of analysis of recombinant basophil activation test (rBAT), which allows displaying multi parameter features of allergenic activity of all major allergens of a specific allergen source on the level of individual patient.

Objectives: 27 house dust mite (HDM) allergic patients and 30 patients with anaphylactic reactions to honey bee venom (HBV) (n=24) or yellow jacket venom (YJV) (n=6) were included. We also tested 6 healthy controls. Some HBV allergic patients were also followed during immunotherapy. Allergenic activity of HBV or HDM allergens was evaluated with basophil CD63 testing on heparinized whole blood with serial dilutions of nDer p 1, rDer p 2, rDer p 5, rDer p 7, rDer p 10, rDer p 21 and rDer p 23 allergens (from 10^{-35}-100 ng/ml), or rApi m 1, nApi m 1, rApi m 2, rApi m 3, rApi m 5, rApi m 10, rApi m 11 allergens (from 0.001-10 μg/ml). IgE reactivity of the same recombinants was determined with dot blots or ELISA.

Results: For HDM allergens the protocol was performed in two steps. First, in sera of all HDM allergic patients IgE reactivity was determined and in next step, positive recombinants were tested with BAT and quantified with CDsens. Unfortunately, CDsens results showed extremely wide range and as such different allergens could not be combined and compared in individual patients. For HBV allergens, we measured IgE reactivity and BAT for all recombinants in all patients. Recombinant BAT was analyzed as area under the curve (AUC). On the level of individual patient, we developed a 3D-plot of AUCs to all tested HBV allergens, which represent multiparameter features of allergenic activity, including quantification for single allergens and summary for all tested allergens. This analysis represents a patient's fingerprint of allergenic activity of selected allergen source, and was successfully tested to evaluate clinical reactivity or follow up immunotherapy. Overall, both in HDM and in HBV models the IgE reactivity did not correlate with allergenic activity.

Conclusions: Fingerprint modeling of allergenic activity pointing out the actual allergens from the offending allergen source that eliciting the allergic response in individual patients. This approach offers

a new tool to address patient's individual clinical reactivity at the molecular level and to monitor allergen immunotherapy.

0147 | Dipeptidyl peptidase I is stored in secretory granules and released on mast cell activation

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Introduction: Activated mast cells orchestrate inflammatory events through the explosive release of diverse inflammatory mediators. Dipeptidyl peptidase I (DPPI) has been implicated in the activation of several proteases prior to their secretion from mast cells, but its subcellular location is not clear.

Objectives: Our aim has been to examine the subcellular location of DPPI and investigate if it can be released following mast cell degranulation.

Results: Localization of organelle protein by isotope tagging (LOPIT) was employed on cells of the LAD2 mast cell line to investigate subcellular compartments in which DPPI and other proteases are located. This involved fractionation of organelles using density gradient centrifugation which allowed proteins from the same organelle to co-fractionate. The protein distribution in the fractions was analysed by mass spectrometry. Immunocytochemistry was performed on LAD2 cells with specific monoclonal antibodies we have developed against DPPI, tryptase, carboxypeptidase A3 (CPA3) and chymase. LAD2 Cells were stimulated experimentally, and the presence of DPP1 along with the other mast cell products in cell supernatants were investigated by specific enzyme immunoassays; and alterations in gene expression studied by quantitative polymerase chain reaction.

LOPIT analysis indicated that DPPI, tryptase, CPA3 and chymase were clustered in a fraction distinct from nuclear and mitochondrial fractions. DPPI was also co-localised with these mast cell proteases in immunostained preparations, though relative amounts of each protease differed between cells. Stimulation of the cells resulted in the secretion of DPPI in parallel with β-hexosaminidase and other mast cell proteases. DPPI gene expression was increased after cell activation accompanied by a similar increase in that for other mast cell proteases.

Conclusions: The presence of DPPI in mast cell granules and its release from activated cells indicates the potential for DPPI to have an extracellular role and it deserves consideration as a new marker of mast cell degranulation.

0148 | Specific ige to alpha-Gal in cow's milk allergic children: prevalence, quantification and relationship with meat allergy

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Introduction: Specific IgE to animal-derived carbohydrate determinants Gal-alpha-1,3-Gal-bêta-1,4-GlcNAc (alpha-Gal, AG) has been associated to cetuximab and red meat-induced allergic reactions. Human glycoproteins lack AG. As a result, the most abundant (around 1%) specificity of naturally occurring IgG, IgM and IgA antibodies in humans is directed against AG epitopes. Isotype switch leading to production of IgE to AG has been reported only in association with tick bites. Thus, meat allergy is associated with specific IgE to AG, but may also occur in milk-allergic children.

Objectives: We sought to describe specific IgE to AG in a cow's milk-allergic pediatric population and its possible relationship with meat allergy and cow's milk main allergens.

Methods: Specific IgE to AG, cow's milk extract, beef extract, and cow's milk proteins casein, alpha-lactalbumin, beta-lactoglobulin, serumalbumin, and lactoferrin were measured in 50 cow's milk-allergic children (median age 2.7 years, range 15 days-18 years, 33 boys) among whom 14 reported clinical reactions to beef.

Results: 25 children (50%) displayed IgE to AG, but none before the age of 10 months (n=16). From 10 months to 18 ans, prevalence of IgE to AG was stable (mean 74%, 60-82% depending on age). The median level of detectable IgE to AG was 0.76 kUA/ (0.11-13 kUA/l) and represented on an average 21% of the level of IgE to cow's milk extract in the corresponding serum.

We found a strong correlation (0.97) between IgE levels to AG and to beef extract in children with a history of clinical reaction to beef. No such correlation was found between IgE levels to AG and to cow's milk extract or individual milk allergens.

Follow-up data over a period of one year or more were available in two patients : a 2-year old girl displayed increasing levels of IgE to AG despite a 50% decrease of IgE levels to beef, and a 5-year old boy whose IgE to AG were undetectable on two samples, despite novel sensitization to beef evidenced during the follow-up.

Conclusions: We report here that specific IgE to AG in cow's milk allergic children are highly prevalent but only after the age of

10 months. The level of IgE to AG represents on an average 21% of the level of IgE to cow's milk extract in a given patient. IgE to AG and to beef extract are strongly correlated, but an independent evolution seems possible. Pathophysiology of IgE sensitization to AG in cow's milk allergic children and its clinical significance need further work.

0149 | IgE against 2S albumin Ara h 7 has a diagnostic value comparable to Ara h 2 and 6

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Introduction: Little is known on the clinical relevance of Ara h 7, the third 2S albumin acting as an allergen in peanut allergy.

Objectives: To investigate the discriminative ability of Ara h 7 in diagnostics of peanut allergy and assess the role of cross-reactivity between Ara h 2, Ara h 6 and Ara h 7 isoforms.

Results: IgE binding to recombinant peanut storage proteins Ara h 1, 2, 3, 6 and 7 was assessed in sera from food challenged confirmed 40 peanut tolerant (40) and peanut allergic patients (40). The area under the receiver operating characteristic (ROC) curve (AUC) was determined to evaluate the ability of the tests to discriminate between allergy and tolerance. A dose-dependent ELISA inhibition experiment was performed with recombinant Ara h 2, 6 and Ara h 7.0101, 0201 and 0202 isoforms.

For Ara h 7.0201 an AUC value was found of 0,83, comparable to Ara h 2 (AUC 0,81) and Ara h 6 (AUC 0,85). Ara h 7 levels strongly correlated with those from Ara h 2 and 6 (both Spearman's r=.81). The majority sensitized to 2S albumins Ara h 2, 6 or 7 was co-sensitized to all three (n=24, 68%), although mono-sensitization to single 2S albumins was also observed in selected patients (Ara h 2: n=6, 17%; Ara h 6: n=2, 6%; Ara h 7: n=2, 6%). Binding to Ara h 7.0101 and Ara h 7.0202 could be potently inhibited by the other isoforms, but not for Ara h 7.0201. IgE binding to Ara h 2 and 6 was variably inhibited with Ara h 7.0201. These findings indicate the presence of both cross-reactive and unique epitopes on Ara h 7.0201.

Conclusions: Ara h 7.0201 is the third clinically relevant peanut 2S albumin, with a discriminative ability for peanut allergy comparable to Ara h 2 and 6. While co-sensitization to all three 2S albumins is most common, mono-sensitization to either Ara h 2, 6 or 7 occurs in selected patients, leading to a risk of misdiagnosis when testing for a single 2S albumin. The Ara h 7.0201 isoform possesses cross-reactive epitopes, but also unique IgE epitopes, not present on the other Ara h 7 isoforms as well as Ara h 2 and 6.

0150 | Introducing a multiplex IgE diagnostic test, a new nanobead-based tool for allergy diagnosis: reporting on IgE reactivity of single allergen preparations and reproducibility performances

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Introduction: Allergy diagnosis is currently based on the use of purified allergenic proteins and protein extract. Diagnosis can be performed in vivo using a limited number of extracts, whereas all allergenic preparations can be tested in vitro by measuring specific IgE. In addition to third generation singleplex lab tests, multiplex testing tools are available since 10 years, allowing to have many IgE results from a single sample by testing allergens as micro spots on a rigid surface. Recently a new generation of lab diagnostic device has been released, the FABER test, adopting modern nanotechnologies, namely nanobeads, for allergen immobilization.

Objectives: To report the general set up of a multiplex test and the evaluation of some of its performances.

Results: The test is based on 244 molecules (122) and extracts (122), coupled to nano-particles. Particles are arrayed to a solid phase matrix, to form a one-step comprehensive array-based testing solution, using 120 μl of serum per test. Each allergen particle population can be individually optimized to achieve the maximum testing performance. The proof of the IgE binding for the 244 allergen preparations has been obtained by using routine serum samples from the sera bank. The evaluation of the IgE binding was obtained by using a standard polyclonal commercial preparation obtained by pooling human sera. The test was used on 22 consecutive batches. 243 allergen preparations, even the rarest one in term of prevalence in the general population, gave positive IgE result using single serum samples. Negative results are recorded for HSA which is the negative control test. 174 out of 244 allergens gave positive IgE results. Average CV values were between 15 and 25%. Allergen specificities having values in the lower IgE range had a lower reproducibility rather than those in the higher range, anyhow never dropping to negative IgE detection. The multiplex test reproducibility performance was not related to the kind of allergen preparations, namely purified molecule or verified extracts.

Conclusions: This test is a new lab test for multiplex specific IgE detection using allergenic molecules and extracts at the same time showing good performances. All steps in assembling the test are verified and the present study reports that all allergens bind IgE. Evaluation of missing allergen specificities is performed with in-house made serum pools and performances could be further improved.

TUESDAY, 20 JUNE 2017
OAS 32
AIT IN CHILDREN AND ADULTS

0151 | Subcutaneous allergen immunotherapy evaluation in a pediatric population

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Introduction: Respiratory allergic disease (RAD) is a growing concern in our population. Allergen immunotherapy (AIT) is, currently, the only approach to IgE-mediated allergic disorders that can induce specific immune tolerance, and is well established, mostly in adults. New polymerized extracts, more immunogenic and less allergenic, are emerging. The safety and efficacy assessment of this new extracts in children is important.

Objectives: Evaluate safety and efficacy of the subcutaneous immunotherapy (SCIT) with polymerized extracts in children.

Material and methods: Children with RAD, to whom SCIT was proposed, were included and followed during 24 to 60 months ($\mu\pm$SD 39.28$\pm$8.23). Amount of pharmacotherapy needed to achieve RAD control, skin prick test (SPT), specific IgE (sIgE) and adverse reactions were analysed in 2 moments, initial (IE) and final (FE) evaluation, and compared between two groups, with SCIT (y-SCIT) and without SCIT (n-SCIT). For the statistical analysis was used SPSS V23.

Results: 36 patients with ages between 6 to 17 years were included; 25 y-SCIT, 11 n-SCIT, age groups ($\mu\pm$SD) 10.84$\pm$3.24 and 8.55$\pm$2.67, respectively. In the IE, there is a significant (Monte Carlo *p* .002) and very high (V^2 0.54) association between y-SCIT and a higher pharmacologic step to achieve asthma control. It was found a significant association (Monte Carlo *p* .001) and of high intensity (V^2 0.36) between SCIT and less rhinitis pharmacotherapy needs in FE. The final sIgE was lower in y-SCIT group (*t* test; *p* .038). No difference was found between the two groups concerning SPT dimensions. 3 patients had a local reaction; none have had systemic reactions.

Conclusions: SCIT is safe in children. The higher pharmacotherapy step to achieve asthma control in IE of y-SCIT group may have been the reason to patients choose SCIT. AIT is associated with lower sIgE, and less preventive pharmacologic treatment needed to control RAD (consistent with previous studies). Our goal is to continue following these patients and increase the sample to improve this work accuracy.

0152 | A grass SLIT-tablet is well tolerated in the paediatric population

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Introduction: Several clinical trials with the a standardized grass SLIT-tablet (75 000 SQ-T/2800 BAU) have been conducted in paediatric populations across Europe and North America. Safety data were pooled from 2 phase I and 5 phase III, randomised, DBPC, multi-centre clinical trials conducted in children (5-18 years) with grass pollen allergic rhinoconjunctivitis.

Objectives: Here, the pooled safety data from these trials are presented.

Results: A total of 1816 subjects were included; 922 were treated with the grass SLIT-tablet and 894 were treated with placebo.

The proportion of subjects who experienced adverse events (AEs) were similar in the active treatment group (71%) and the placebo group (70%). In the active group, the percentage of subjects who experienced treatment-related AEs (48%) was larger than in the placebo group (18%). However, most treatment-related AEs were mild or moderate of intensity and few were severe (grass SLIT-tablet: 79%, 19%, and 2%; placebo: 84%, 14%, and 2%, respectively). Further, most subjects who experienced at least one treatment-related AE continued the trial uninterruptedly and with no additional medication (grass SLIT-tablet: 89%; placebo: 87%) and recovered (both groups: 99%). Although the frequency was higher in the active group (7%) compared with placebo (<1%), few subjects discontinued the trial due to treatment-related AEs.

The most commonly reported treatment-related AEs in the active group were oral pruritus, throat irritation, tongue pruritus, and ear pruritus, occurring in 25%, 16%, 5%, and 5% of subjects treated with grass SLIT-tablet and in 2%, 1%, <1%, and <1% in subjects treated with placebo, respectively. The most commonly reported treatment-related AEs typically occurred within the first days or weeks of initial treatment with the grass SLIT-tablet and were recovered within days. Three treatment-related AEs in the active group involved use of adrenaline. Fewer than 1% of subjects treated with the grass SLIT-tablet experienced serious treatment-related AEs and none of these included constricted airways. All but one subject with immune thrombocytopenic purpura recovered. No anaphylactic shocks or deaths occurred in any of the trials.

Conclusions: The grass SLIT-tablet is well tolerated in the paediatric population. Most of the reported AEs were local, mild, and transient allergic reactions, consistent with the sublingual administration of grass allergens in sensitised subjects.

0154 | Health related quality of life among children and adolescents treated with allergen immunotherapy (AIT)

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Introduction: Treatment of respiratory allergies includes symptomatic medication, allergen avoidance and allergen immunotherapy (AIT). Influence of AIT on Health Related Quality of Life (HrQoL) is not fully explored. DISABKIDS (DCGM-37) is a generic instrument measuring HRQoL in children and adolescents with chronic health conditions.

Objectives: The objectives of this prospective study were to investigate how children and adolescents with allergic asthma and allergic rhino-conjunctivitis undergoing AIT estimated HrQoL and allergy related symptoms before and after one-year treatment. Besides the effects on allergen specific IgE, IgG and IgG4 antibody-levels were evaluated.

Results: 116 children /adolescents, 69 boys and 47 girls, median age 11 (6-17) years were included. A majority (n=86, 74%) were treated with birch pollen allergen extract, 21 (18%) were treated with grass pollen allergen extract, the rest were treated with cat (n=4), dog (n=3) or mite (n=2) allergen extract.

Changes between base-line and one-year follow-up were analyzed with Wilcoxon signed ranks test. General HrQoL, measured with DISABKIDS, were improved ($P<.001$) after one year of treatment. Besides, self-assessment of allergic symptoms showed improvements ($P<.001$), allergen specific IgE decreased and IgG and IgG4 increased ($P<.001$) between the start of treatment and one year follow-up.

Conclusions: The study shows that HrQoL in children and adolescents with respiratory allergies improves already after one year of AIT and is combined with reduction in allergic airway symptoms and in favorable changes in the immunoglobulin profile.

0155 | House dust mite SLIT-tablet is well tolerated in patients with house dust mite respiratory allergic disease

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Introduction: A house dust mite (HDM) SLIT-tablet addresses the underlying cause of HDM respiratory allergic disease, and clinical effect has been demonstrated for both allergic asthma and allergic rhinitis.

Objectives: Here, we present pooled safety data from an adult population with HDM respiratory allergy, with a particular focus on the impact of asthma on the safety profile of the HDM SLIT-tablet.

Results: Safety data from 2 randomised DBPC phase III trials (MT-04 and MT-06) were pooled.

MT-04: 834 adults with HDM allergic asthma not well-controlled by inhaled corticosteroids and with HDM allergic rhinitis (EudraCT no. 2010-018621-19)

MT-06: 992 adults with moderate-to-severe HDM allergic rhinitis despite use of allergy pharmacotherapy with or without asthma (EudraCT No. 2011-002277-38)

Subjects with $FEV_1<70\%$ at randomisation or a severe asthma exacerbation within the last 3 months prior to randomisation were not eligible.

The proportion of subjects experiencing adverse events (AEs) was greater in the active treatment group (73% of subjects) compared to placebo (53%). Most AEs were mild in both groups (70% in active; 66% in placebo), and the proportion of subjects reporting severe AEs was similar for active (5%) and placebo (4%). The most frequent treatment-related AEs were local allergic reactions, most of which occurred within the first few days of treatment and subsided with continued use. No systemic allergic reactions were reported.

Subgroup analysis by asthma status revealed no statistically significant difference in the risk of moderate or severe treatment-related AEs between subjects with asthma and subjects without asthma ($N_{asthma}=863$, $N_{no\ asthma}=352$; $P=.88$). Among subjects on active treatment, 12% with asthma and 11% without asthma reported events. Likewise, the proportion was 12% regardless of GINA treatment step at screening. Further, subjects with partly or uncontrolled asthma at treatment initiation were no more likely to experience moderate or severe treatment-related AEs than subjects with controlled asthma (11% and 14% of subjects on active treatment, respectively).

Conclusions: The HDM SLIT-tablet was well tolerated in adults with HDM respiratory allergic disease and most treatment-related

AEs subsided with continued treatment. The safety profile was comparable for subjects with HDM allergic rhinitis with and without asthma, and GINA treatment step and asthma control level had no impact on the safety profile of the HDM SLIT-tablet.

0156 | Intralymphatic immunotherapy (ilit) with both grass and birch allergen- a randomized double-blind placebo controlled trial

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Introduction: Allergen specific immunotherapy is an effective but time-consuming treatment for allergic rhinitis. In order to shorten treatment duration, the use of three intralymphatic injections (ILIT) have been studied with diverging results.

Objectives: This study assessed safety, clinical effects and immunological changes after ILIT with two allergens given simultaneously.

Method: 58 patients with moderate to severe birch and grass pollen allergy were randomized 1:1 to receive three ultrasound-guided intralymphatic injections with placebo or Birch and Grass 1000 SQ/U in each groin at one-month intervals. Patients were followed for one year and the study was conducted 2012-2015.

Results: Baseline characteristics were the same in both groups. 56 patients received all three injections. 52 patients fulfilled the follow up-visits. Side effects reported were mild or moderate.

The primary outcome measure was symptom score after nasal grass allergen, which decreased after active treatment. Skin prick test reactions were reduced and the Juniper Quality of Life Questionnaire scores improved in the active group during the birch pollen season, as compared to placebo. The consumption of nasal steroids and β-2-agonists increased during the birch pollen season in the placebo group, but was unchanged in active group.

Immunological investigations showed that levels of S-IgG4 Timothy increased after active treatment. Allergen specific S-IgE levels remained stable or increased in the active group whereas the levels in placebo group decreased outside the pollen season. Analysis of lymph node aspirates and peripheral blood revealed an increased proportion of CD4 + effector memory cells in the active group but not in the placebo group. CD4 memory Th1-cells increased in the active group in blood; this was not seen in the placebo group.

Conclusions: We show for the first time that ILIT with two allergens appears to be a safe procedure with only limited side-effects. Allergen challenge indicates that ILIT reduces rhinitis-symptoms. Several secondary outcomes also point favorably toward ILIT, including improved quality of life and reduced need for rescue medication. The immunological results show that local administration of allergen in lymph nodes generates systemic changes in lymphocytes, further supporting the rationale for this alternative administration route.

WEDNESDAY, 21 JUNE 2017
OAS 33
MANAGEMENT OF DRUG ALLERGY

0157 | NSAIDs hypersensitivity reactions in children

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Introduction: NSAIDs hypersensitivity reactions can be cross-intolerant (CI) or selective according to the response to non chemically related drugs. Sufficient studies in children have not been carried out. We evaluated in our service children who developed reactions to NSAIDs.

Objectives: Subjects between 2-14 y.o. were evaluated. An allergy history plus skin test with inhalant allergens plus total IgE quantified by immunoassay were made. Incremental doses of ASA were administered adapted to body age/weight. If negative, we challenged with the culprit. Independent T test and Chi X^2 was made.

Results: We studied 113 children who experienced symptoms indicative of NSAID hypersensitivity. The 59% were males and the 41% females. Mean age 7,6 (0.5-14 y o). Atopic status occurred in the 46%. Drugs involved were: ibuprofen (81% of the episodes), paracetamol (10%), ASA (4.8%) and metamizol (1%). Cross intolerant were the 27%, selective immediate responders 1,7% and 74% had good tolerance. The clinical entities were urticaria/angioedema in 32% and isolated angioedema in 32%. Anaphylaxis occurred in 11.8% and asthma accompanied with facial angioedema in 17.6%. Cases were older ($P<.0001$), and had more episodes ($P<.05$) than those tolerant. Total IgE and atopy did not show significant differences.

Conclusions: Urticaria and/or angioedema are the most frequent clinical entities in children under 14 with CI to NSAIDs with group. Asthma accompanied with facial angioedema appeared in the 17.6%. Selective responders are uncommon. This contrast with previous studies done in adults and adolescents as well as other populations under 14 years.

0158 | Demonstration of a specific sensitization in children with amoxicillin clavulanic acid-induced cutaneous adverse reactions within infectious mononucleosis

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Introduction: Children with infectious mononucleosis frequently develop skin eruptions while on beta-lactams. Maculopapular exanthemas are commonly reported, but even more severe cutaneous adverse drug reactions may occur. The underlying pathomechanisms are still largely unknown.

Objectives: To investigate whether amoxicillin- clavulanic acid (AMX-CLV) induced skin reactions in acute Epstein Barr Virus (EBV) infections is a mere disease-associated phenomenon or results from specific sensitization to the drug. Ten patients with AMX-CLV cutaneous reactions within EBV infection were investigated in vivo by prick, intradermal and in two cases by patch tests and in vitro by means of the lymphocyte transformation test (LTT) and the generation of haptens-specific short term T-cell lines (TCLs) employing Penicillin (PEN), Ampicillin (AMP), Amoxicillin (AMX) and AMX-CLV.

Results: Drug specific sensitization to AMX/CLV in the LTT was observed in five patients, two of whom showed also positive intradermal tests. Seven out of ten patients were positive to the TCLs. Moreover four out of ten patients were tested after a second AMX-CLV reaction following the first episode within EBV infection.

Conclusions: These data showed that a real sensitization to AMX-CLV may occur during infectious mononucleosis and in vitro investigations may help in the diagnostic work-up.

0159 | Vaccine allergy is extremely rare: a decade of experience from two large UK allergy centres

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Introduction: Vaccination is one of the most significant achievements of modern medicine. It is safe and allergic reactions are

extremely rare. The general public and some physicians are often excessively concerned about reactions to vaccination. This leads to unnecessary avoidance or delays which render patients vulnerable to infections and weaken herd immunity.

Objectives: We reviewed the clinical data of all patients with suspected vaccine allergy referred to our allergy departments between 2007 and 2016.

Results: Ninety-five patients underwent 103 supervised vaccinations or vaccination provocation testing. Median age was 25 years, with a male:female ratio of 1:1.2. Influenza (25%), tetanus (18%), hepatitis B (14%) and measles, mumps and rubella (MMR) vaccines (13%) were most commonly tested. Most patients were referred because of previous reactions to index vaccines - most commonly cutaneous reactions (31%), angioedema (14%) and vasovagal syncope (11%). Other reactions: respiratory (8%), gastrointestinal (5%) and fever (7%) were less common. Twenty patients with egg allergy were referred for influenza (11/20), yellow fever (5/20), MMR (4/20) and rabies (1/20) vaccination. Other reasons included past reactions to an unrelated vaccine (4), forgotten/unknown reasons (3) and latex allergy (2). Vaccine skin tests were performed routinely only in Cambridge. All referred patients received their required vaccines successfully. There were only 3 local reactions with localized pain, swelling and/or erythema and 4 vasovagal reactions. In 35% patients with suspected egg allergy this diagnosis was removed after a thorough allergy workup.

Conclusions: Vaccinations are safe and the risk of an allergic reaction remains negligible. The lack of awareness of the predictable and common side effects of vaccination (local oedema, erythema, dizziness etc.) often leads to an incorrect diagnosis of allergy. In our experience, most (if not all) patients suspected to be 'allergic' to vaccines can be vaccinated successfully. Moreover, most of them could be vaccinated in primary care following standard protocols. Despite clear UK guidelines, patients with suspected egg allergy are still inappropriately referred for MMR vaccination. Allergists play a crucial role in improving physicians' knowledge of vaccine side effects and safety as well as administering vaccinations where genuine concerns remain. This will help address a public health issue of a diminishing routine vaccine uptake.

0160 | Fixed drug eruptions: Report of 8 clinical cases

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Introduction: Hypersensitivity drug reactions are common and often manifest as a cutaneous eruption. Fixed drug eruption (FDE) is a rare cutaneous manifestation of drug allergic reactions. The diagnostic hallmark is its recurrence at previously affected sites.

Objectives: Demographic characterization of patients, description of culprit drugs and clinical manifestation. We performed a retrospective chart-review study of patients with FDE followed in our Immunoallergology outpatient department.

Results: 8 patients (pts) were included, 5 men and 3 women, average age 51±10 years. 4 had previous history of rhinitis, 1 of eczema, 1 of food allergy and 2 of primary immunodeficiency. Average age of the first manifestation of FDE was 44±15 years and the average age at the first drug allergy appointment 47±11 years. Diagnosis was made, on average, within 7,7±8 months. Culprit drugs were: amoxicillin in 3 pts (one of which had cross-reactive FDE with penicillin), ciprofloxacin in 1 pt (had cross-reactive FDE with levofloxacin), etoricoxib in 2 pts and nimesulide in 2 pts. Lesions were multiple in 6 pts and single lesion in 2. The location of the lesions was: Head 2, trunk 1, pelvis 4, arms 3, legs 3. In 3 cases, only one region of the body was affected. The time elapsed from drug ingestion until appearance of the lesion was <12 hours in 3 pts, 12-24 hours in 3 pts and 24-48 hours in 2 pts. No lesions appeared more than 48 hours after ingestion of the culprit drug. Diagnosis was established based on: oral provocation test in 7 pts and patch test in 2 pts. 3 pts had negative patch tests with the culprit drug on the lesion site, but had positive oral provocation test.

Conclusions: FDE are a rare presentation of drug allergy. FDE manifests in middle-aged adults and up until 48 hours of ingestion of the culprit drug. Antibiotics seem to be more often associated with cross-reactive FDE with antibiotics of the same family. Oral provocation tests are important for diagnosis as some patients have negative patch tests on the affected site.

0161 | Carbonic anhydrase inhibitors-induced severe cutaneous adverse reactions: analysis of Korean SCAR registry database 2010-2015

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Introduction: Severe Cutaneous Adverse Reactions (SCARs) including Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) by carbonic anhydrase inhibitors (CAIs) have been reported in patients of Asian descent.

Objectives: The aim of study was to describe clinical characteristics and outcomes of CAIs-induced SCARs.

Methods: SCAR cases between 2010 and 2015 were retrospectively analyzed using a web-based Korean SCAR Registry database. SCARs caused by CAIs were compared with other drug-induced SCARs.

Results: Of the 783 patients with SCARs, 15 (1.9%) cases were reported CAIs including methazolamide and acetazolamide as major culprit drugs. They all developed SJS or TEN, but no Drug Reaction with Eosinophilia and Systemic Symptoms. More than half the CAIs-induced SCAR cases developed TEN (n=8, 53.5%), followed by SJS/TEN overlap (n=4, 26.7%) and SJS (n=3, 20%). The period of hospitalization was 24.5±14.1 days, which was longer than SJS/TEN cases caused by other drugs. The mean duration of exposure and latent period was 9.1±8.0 days and 11.3±5.7 days. CAIs-induced SJS/TEN cases had higher than average rate of complications (20% vs. 8.4%).

Conclusions: SCAR cases related with CAIs showed the most severe manifestations and poor prognosis despite the relatively short period of drug exposure.

0162 | A rare case of injection site reaction induced by icatibant and the skin test positivity

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Case report: Introduction: Icatibant is a practical and effective drug which is used to treat the acute attacks in hereditary angioedema. In general it is well tolerated by the patients. Mild or moderate erythema and enduration can be observed on the injection site which resolve in a short time.

Case: A, 22-year old female patient was diagnosed as type II hereditary angioedema due to abdominal pain and angioedema episodes 4 years ago. Although the number of her attacks were decreased with the long term prophylactic treatment, she experienced abdominal pain at least once in a month. She used C1 inhibitor extract or icatibant in her attacks. An erythematosus enduration was developed at injection site after second usage of icatibant. The reaction became more disturbing during later injections. A few hours after injection approximately 20 cm itchy, painful, erythematous enduration started and resolved in 24 hours. Skin prick testing with the 1/10 dilution of the drug was found positive . To confirm the diagnosis patient was provocated with the drug and similar reaction was observed. Therefore icatibant was banned to the patient.

Conclusion: This is a rare case of injection site hypersensitivity reaction induced by icatibant and proved by the skin and provocation tests.

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0163 | European Academy of Allergy and Clinical Immunology (EAACI) Anaphylaxis Survey (AXIS) 2016: The reality of anaphylaxis management

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Introduction: Despite widespread dissemination of EAACI anaphylaxis management guidelines, anaphylaxis mis-diagnosis continues, adrenaline is under-used and most physicians are unaware of how to correctly administer it. Adrenaline auto-injector (AAI) prescriptions are not keeping pace with the increasing anaphylaxis hospitalization rate, and, even when prescribed, AAIs are frequently not used.

Objectives: In this environment, EAACI developed the AnaphylaXIs Survey (AXIS) 2016 as part of its Allergy Awareness initiative to better understand EAACI member AAI prescription practices. This survey was sent by email to 8720 EAACI members. 873 members from 89 countries responded. Data were anonymous and analysed descriptively.

Results: Of those aware of the anaphylaxis guidelines (n=711), most (96.8%; n=688) felt that they followed them. 775 respondents (88.8%) reported prescribing AAIs; 55.9% (n=433) for all absolute and relative indications and 44.2% (n=342) for absolute indications or only for certain patients. Of these 342 respondents, 28.1% (n=96) did not prescribe an AAI for all absolute indications, and 77.2% (n=264) did not prescribe an AAI for all relative indications. The most common absolute indication contravened was prescription for those with 'co-existing unstable or moderate/severe persistent asthma and a food allergy*' (15.5%; n=53). The most common relative indications contravened were AAI prescription for those with 'previous mild to moderate allergic reaction to traces of food (48.8%; n=167) or to peanut and/or tree nut' (43.3%; n=148) but also for 'teenagers/young adults with a food allergy*' (43.0%; n=147). Lack of patient understanding (37.1%; n=127), likelihood of non-compliance (31.9%; n=109) and considered unnecessary until after the first anaphylactic episode (26.6%; n=91) were cited as the most common reasons for not prescribing an AAI. Only 55.9% (n=433) of respondents reported prescribing ≥2 AAIs.

Conclusions: Anaphylaxis patients are not adequately protected. Results reflect latest findings that AAIs are under-prescribed in daily practice. Although adherence to guidelines is perceived to be high, compliance with AAI prescription recommendations is actually low. Continued training, further simplification and wider dissemination of guideline messages are required.

* excl. food allergy syndrome

0164 | Significant improvement in diagnosing anaphylaxis over time: A major factor compounding time-trend data?

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Introduction: Recent studies based on hospital admissions have reported a significant increase of anaphylaxis prevalence. These studies rely on accurate diagnosis and coding data from medical records, which has been identified as a potential shortcoming.

Objectives: The aim of this study was to investigate the extent of anaphylaxis miscoding, how this changed after intensified training programs and new anaphylaxis guidelines, and whether this contributed to apparent changes in prevalence over a 10-year period.

Results: We conducted a retrospective chart examination of all cases coded as anaphylaxis presenting to the main tertiary Pediatric Emergency Department (PED) in Perth, Australia. We compared data from 2003/2004 with data from 2012, after intensified training programs and new anaphylaxis guidelines were introduced. Information was collected on standardized forms.

All charts were reviewed by 2 Allergists.

136/83832 ED presentations in 2003/2004 and 177/71822 ED presentations in 2012 were coded as anaphylaxis (ICD-10) with significantly more coding errors in 2003/2004 (44/136;32%) than in 2012 (18/177;10%).

A diagnosis of anaphylaxis was verified in 92/136 (68%) cases in 2003/2004 and 159/177 (90%) cases in 2012, indicating a two-fold increase in presentations with anaphylaxis ($P<.001$). Sub-analysis suggested that infants between 12 to 24 months showed the largest (5-fold) increase (0.54/1000 in 2003 compared to 2.69/1000 in 2012).

An increase in food allergy was the main contributing factor ($P<.001$) largely from tree-nut allergies.

We also examined for potential underdiagnosis of anaphylaxis analyzing 400 randomly selected cases of ED presentations attributed to conditions with an associated diagnosis.

Based on the estimate of total anaphylaxis cases for both time periods we found an underdiagnosis rate of 37% (63% correctly diagnosed) in 2003/2004 and 20% (80% correctly diagnosed) in 2012.

Conclusions: To our knowledge this is the first study to examine changing patterns of emergency presentations of pediatric

anaphylaxis in the context of coding errors. Comparing the years 2003/2004 and 2012 the rate of accurate coding improved which is crucial for the evaluation of epidemiological trends, adequate education/guidance of primary care takers and reduction of costs in the health care system. This could be achieved by improving collaborations between Pediatric Allergists and Emergency Physicians, reinforcing guidelines and training programs.

0165 | Exploring the mast cell compartment in patients with idiopathic anaphylaxis

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Introduction: It has been suggested that patients with idiopathic anaphylaxis (IA) may have a hyper-responsive mast cell compartment as one component of their disease. To explore this possibility, we examined the mast cell compartment in the bone marrow of these patients for evidence of in vivo activation. We also examined the in vitro growth rate and degranulation of mast cells cultured from these patients in comparison to mast cells cultured from healthy volunteers (HV).

Objectives: We prospectively enrolled 57 patients with frequent (IA) after informed consent, >3 episodes/year and analyzed mast cells cultured from peripheral blood CD34+ cells for rate of growth and releasibility following FceRI aggregation. Patients also underwent a bone marrow procedure to rule out clonal disease and the bone marrow mast cell compartment was analyzed by multi-parameter flow cytometry after bone marrow aspirates were processed and stained with antibodies for CD2, CD25, CD45 and CD117. Activation markers were identified with additional antibodies for CD11c, CD35, CD59, CD63, CD69 and CD203c in 52 subjects.

Results: The flow cytometric analysis showed that immunophenotypic characteristics of bone marrow mast cells were abnormal in patients with indolent systemic mastocytosis (ISM) or monoclonal mast cell activation syndrome with expression of surface makers such as CD25 and evidence of mast cell activation in vivo, with up-regulation of CD63 and CD203c. The bone marrow mast cell compartment in patients with IA was no different from that of HV including the degree of in vivo activation as shown by expression of CD63 and CD203c. Thus, there was no evidence of in vivo activation of mast cells in the bone marrow of patients with IA. CD34 + cells from the peripheral blood of patients with ISM or IA when cultured produced more mast cells compared to those obtained from HV. When all groups were compared, there was no difference in mast cell responsiveness as examined by IgE-dependent beta-hexosaminidase release.

Conclusions: We found no evidence for a hyper-responsive mast cell phenotype in patients with IA, either in vitro or in vivo. The possibility remains, however, that mast cells in patients with IA are hyperresponsive to a yet to be identified degranulation signal or combination of signals; or cell compartments reacting to mediators released by mast cells are themselves hyperresponsive.

0166 | TWEAK/Fn14 axis mediates histamine and PAF-induced subcutaneous vascular leakage and anaphylactic shock

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Introduction: Anaphylaxis is the most aggressive and acute manifestation of allergic disorders. In the usual clinical practice, treatments used to prevent a fatal outcome in anaphylaxis are aimed at treating the vascular system as the main target organ. Mechanisms underlying anaphylaxis include the activation of the classic effector cells - mast cells, which by IgE-FcεR stimulation releasing different mediators and cytokines that elicits reactions. However, basic studies on research dedicated to analyze the molecular functioning of the vascular system in anaphylaxis have been scarcely unexplored. The axis TNF-like weak inducer of apoptosis (TWEAK) and its functional receptor the fibroblast growth factor-inducible molecule 14 (Fn14) are two proteins belong the TNF superfamily that participate in tissue repair after acute injury. Gain of loss-of function experiments have demonstrated that TWEAK/Fn14 axis is also involved in endothelial dysfunction and inflammation.

Objectives: To study the role of TWEAK/Fn14 during anaphylactic reactions.

Results: *In vitro* Fn14 expression was evaluated in: a) IgE-FcεR sensitized bone marrow-derived mast cells (BMMCs) and in b) Histamine (His) and PAF - stimulated mouse aortic endothelial cells (MAECs). *In vivo* experimental models of subcutaneous permeability in response to these mediators and mice models of passive (PSA) and active (ASA) systemic anaphylaxis were studied in WT, TWEAK and Fn14 knockout mice.

By *in vitro* studies we have observed that Fn14 is regulated in response to IgE-FcεR stimulation in BMMCs, which might be related to the action of His and PAF as they isolated increase Fn14 expression in MAECs. *In vivo* subcutaneous injection of His or PAF results in strongly reduced local effects in TWEAK and Fn14 knockout mice, indicating their role in vascular permeability. In addition, experimental mice models of PSA and ASA show that the absence of either TWEAK or Fn14 prevents anaphylaxis related symptoms. These

knockout mice show resistance to drop in corporal temperature, lower index of severity of reactions and less affectation to the decrease of activity after anaphylaxis challenges.

Conclusions: Our data identifies a functional role for TWEAK-Fn14 axis as a critical mediator of hypersensitivity reactions and highlight it as a potential new target in anaphylactic reactions.

0167 | Identification of an unknown mastocytosis in patients with severe anaphylaxis

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Introduction: Anaphylaxis is a severe, life-threatening, systemic hypersensitivity reaction. Mastocytosis can promote the onset and severity of allergic reactions. As not all patients who experienced an anaphylactic reaction undergo a comprehensive work-up for mastocytosis, this condition might be underdiagnosed. Recent studies suggested the detection of KIT D816V mutation in peripheral blood as a screening marker of systemic mastocytosis.

Objectives: We hypothesize that in patients with severe anaphylaxis regardless the elicitor an underlying mastocytosis should be considered.

Methods: Between January 2015 and January 2016, 50 subjects with a severity grade III or IV according to Ring&Messmer were subjected to the analysis of KIT D816V mutation in peripheral blood. All subjects were registered in the anaphylaxis registry (Network for Online Registration of Anaphylaxis - NORA). Written informed consent was obtained.

Results: In 47 of 50 subject the KIT D816V analysis was successfully performed. The subjects comprise 29 females and 18 males with a median age of 48 (17-76) years. The elicitors were insect venom (n=21, mainly yellow jacket), food (n=17, various food) or unknown (n=8). One anaphylaxis was triggered during specific immunotherapy to bee. In 26 subjects, the elicitor was known and in 13 reasonably suspected.

Four out of 47 subjects (9%) were positive for KIT D816V mutation. These 4 patients were females and experienced an anaphylactic reaction of grade III severity. The elicitors were yellow jacket (2x), shrimps (1x) and one reaction was idiopathic. In one case mastocytosis was already diagnosed and an elevated tryptase level was known prior the reaction. In the other 3 cases the mastocytosis was not considered beforehand.

Conclusions: Our data suggests that in adult patients who experienced a severe anaphylactic reaction a mastocytosis should be considered as an underlying disease. This seems to be important not only in venom induced anaphylaxis but in rather all cases, however more data is needed to better define the target population for the genetic analysis.

0168 | The body mass index is an indicator of advanced systemic mastocytosis: preliminary results from a registry project of the European Competence Network on Mastocytosis (ECNM) registry

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Introduction: Systemic mastocytosis (SM) is a myeloid neoplasm caused by infiltration of mast cells (MC) in various organs. In

advanced SM, MC infiltration leads to organ damage.. Changes in metabolic cascades may play an important role in patients with advanced SM. For example, malabsorption related to gastrointestinal involvement in SM may contribute to malnutrition observed in several cases of SM.

Objectives: The aim of the study was to analyze the body mass index (BMI) in mastocytosis patients collected in the registry of the European Competence Network on Mastocytosis (ECNM registry) and to correlate BMI values with clinical parameters and the type of SM.

Results: Methods: A total of 1513 patients enrolled in the ECNM registry were analyzed. In these patients, BMI, malnutrition, weight loss >10%, malabsorption, osteopenia/osteoporosis, and the variant of SM were analyzed.

Results: Malnutrition was observed in 1.4% of all subjects. In most patients the BMI was >=21. Weight loss >=10% was found in 6.7% of all patients, and . malabsorption was reported in 2.9% of all cases. Osteopenia/osteoporosis were found in 28.2% of the patients examined.

Weight loss, malnutrition, and malabsorption were most prevalent in patients with advanced forms of SM (ASM, AHNMD), while low BMI and osteopenia/osteoporosis were found in all forms of the disease.

Conclusions: Weight loss, malnutrition, and malabsorption are indicators of an advanced form of mastocytosis.