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ABSTRACTS

SUNDAY, 27 MAY 2018
OAS 01
MECHANISMS OF FOOD ALLERGY

0001 | Early consumption of peanut alters the epitope-specific humoral immune response in high-risk infants enrolled in the LEAP trial

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Background: Results of the LEAP trial showed (Du Toit et al 2016) that early introduction to peanut (PN) in high-risk infants significantly reduced the prevalence of peanut allergy at 5 years. However, the mechanisms by which this is achieved are unknown. Utilizing a subset of subjects who completed the LEAP trial, we monitored the development of IgE and IgG4 binding to sequential allergenic PN epitopes in infants enrolled at 4-11 months and followed to 5 years. We evaluated changes in the epitope repertoire and its association with oral PN exposure and development of allergy.

Method: A novel high-throughput luminex-based assay was used to quantitate IgE and IgG4 binding to 64 sequential epitopes found on Ara h1-3. Sera from 341 subjects were evaluated for epitope-specific antibodies at baseline, 1, 2.5 and 5 years. Overall, 50 subjects were non-allergic in each arm, 84 and 119 were IgE-sensitized but not reactive in the consumer and avoidance groups; and 38 in the avoidance group had OFC confirmed allergy by 5 years. Epitope-specific binding (EB) scores were obtained and linear mixed-effect models were used to evaluate changes over time.

Results: IgE PN epitope-specific antibodies developed in patients in the avoidance group, and were unique for those who developed PN allergy by 5 years. IgE antibodies were generated to epitopes predominantly in two regions: AraH1 031-066 and AraH2 005-030 and were evident at 2 years, increasing further over time. Minimal increases of IgE epitopes occurred after 1 yr in the PN-consumers, but not to the same epitopes bound by patients developing PN allergy, suggesting that the production of transient IgE to certain epitopes may be a natural developmental phenomenon. Interestingly, no major changes in IgE epitope-specific antibodies were seen in patients who were non-allergic or sensitized but non-reactive at 5 years, regardless of treatment. Development of IgG4 epitope-specific antibodies increased in most patients regardless of consumption or allergy status, suggesting that exposure occurs via non-oral routes in PN-avoiders. However, PN-consumers developed greater quantities of IgG4 earlier to allergenic epitopes recognized by PN-avoiders IgE antibodies.

Conclusion: Early PN consumption in infants at high risk of PN allergy appears to divert the immunologic response to a "protective" form of immunoglobulin, i.e. from IgE to IgG4 epitope-specific antibodies, while non-consumers generated epitope-specific IgE antibodies first and later developed IgG4 antibodies to the allergenic epitopes.

0002 | Unprocessed cow's milk suppresses allergic symptoms in a murine model for food allergy: A potential role for epigenetics

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Background: Epidemiological studies have shown an inverse relation between unprocessed cow's milk consumption and the development of asthma and allergies. This protective effect seemed to be abolished by milk processing. Previously, we confirmed the epidemiological findings on asthma by showing causality. In the present study, we investigated whether unprocessed cow's milk is also protective in a murine model for food allergy. Besides, we looked at possible changes in histone acetylation to investigate the involvement of epigenetic regulation.

Method: C3H/HeOuJ mice were sensitized intragastrically (i.g.) once a week for five weeks with ovalbumin (OVA) using cholera toxin (CT) as an adjuvant (d0, 7, 14, 21, 28). Prior to sensitization, mice were orally treated with unprocessed milk, processed milk or PBS (as control) for eight consecutive days (d-9 to -2). Five days after the last sensitization (d33), mice were challenged intradermally (i.d.) in the ear with OVA to determine acute allergic symptoms. On the same day, mice were challenged i.g. with OVA. Eighteen hours after the i.g. challenge mice were killed and organs were obtained for *ex vivo* analysis (d34). In addition, epigenetic modifications in Th1-, Th2- and regulatory T cell-related genes of splenocyte derived $CD4^+$ T cells were analyzed after milk treatment (d-1) as well as at the end of the study (d34).

Results: OVA sensitized mice receiving unprocessed milk showed decreased allergic symptoms compared to sensitized mice receiving PBS. The acute allergic skin response and anaphylactic shock symptoms were reduced and the body temperature remained high. OVA-specific IgE levels were also decreased. These protective effects were not observed when sensitized mice received processed milk. Looking

at epigenetic modifications, unprocessed milk exposure for eight days led to higher acetylation of Th1-, Th2- and regulatory T cell-related genes of $CD4^+$ T cells compared to processed milk (d-1). At the end of the study (d34) this general immunostimulation was resolved and acetylation of Th2 genes was lower compared to processed milk.

Conclusion: Unprocessed cow's milk reduces allergic symptoms in a murine model for food allergy. This protective effect was not observed after exposure to processed milk. The general immunostimulation in the spleen after exposure to unprocessed milk could be responsible for the observed tolerance induction, suggesting that epigenetic mechanisms contribute to the allergy protective effect of unprocessed cow's milk.

0003 | Deficient naïve CD4+ t-cell function and epigenetic remodelling of metabolic genes in childhood food allergy

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Background: Activation and differentiation of naïve CD4 + T-cells requires widespread transcriptional and epigenetic regulation of pathways regulating cell growth, metabolism, and proliferation. It remains unclear whether this process is dysregulated in IgE-mediated food allergy. Here we investigated the molecular regulation of naive CD4 + T-cell function in children with food allergy.

Method: Naïve CD4 + T-cells were purified from peripheral blood mononuclear cells of children with egg allergy (n = 44) and non-allergic controls (n = 21) at baseline (12-months of age) and follow-up (2 or 4 years of age). Naïve T-cells were activated using anti-CD3/anti-CD28 stimulation *in vitro* and proliferation, viability and cytokine production was measured. RNA-seq and genome-wide DNA methylation analysis was carried out both pre- and post- activation to study molecular determinants of the T-cell activation response.

Results: Naïve T-cells from infants with egg allergy exhibited a markedly attenuated capacity for proliferation in response to T-cell receptor (TCR) activation. This was associated with reduced expression of cell cycle related targets of the E2F and MYC transcription factor networks, and concomitant remodeling of DNA methylation at genes in the PIK3/AKT/mTOR metabolic pathway. In children who naturally developed tolerance to egg at follow-up, these differences were largely attenuated.

Conclusion: Food allergy is associated with inherent differences in the responsive capacity of naive T-cells from the resting state following TCR signaling. These pathways warrant further investigation as they are likely to influence T-cell fate decisions.

0004 | Does early exposure to cow's milk protein reduce the risk of cow's milk allergy?

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Background: Overall early exposure to allergenic foods in the infant's diet is a new strategy for preventing food allergy to that allergen, but the optimal timing of exposure for different allergens is not known. We aimed to examine the relationship between exposure to cow's milk protein in the first 3 months of life and risk of cow's milk allergy at age 12 months.

Method: HealthNuts is a longitudinal population-based food allergy study which recruited 5276 12-month old infants. Skin prick testing to cow's milk was conducted on the second half of the cohort (n = 2715) and sensitisation defined as a wheal $\geq$ 2 mm. Cow's milk allergy was defined as a parent reported reaction to cow's milk consistent with IgE-mediated allergy together with evidence of sensitisation. Early exposure to cow's milk protein was captured through parental questionnaire and defined as consumption of cow's milk-based infant formula during the first 3 months of life.

Results: Forty-two percent of infants were exposed to cow's milk in the first 3 months of life (n = 1977/4712) and 87% of these infants were also breastfed. Early exposure to cow's milk protein was associated with a reduced risk of cow's milk sensitisation (aOR 0.44, 95% CI 0.23-0.83), parent-reported reactions to cow's milk (aOR 0.44, 95% CI 0.29-0.67) and cow's milk allergy (aOR 0.31, 95% CI 0.10-0.91) at age 12-months. Age at exposure to cow's milk protein was not associated with the risk of other food allergies.

Conclusion: Exposure to cow's milk protein in the first 3 months of life was associated with a reduced risk of cow's milk allergy. Clinical trials are warranted to further assess this association.

0005 | Genome-wide association study identifies the SERPINB gene cluster as a susceptibility locus for food allergy

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Background: The genetic determinants underlying food allergy susceptibility remain largely unidentified to date. Due to the large spectrum of disease symptoms which may affect any organ system, a reliable diagnosis is often difficult to obtain.

Method: We performed a genome-wide association study on food allergy in patients in whom the diagnosis was based on oral food challenges. In total the discovery set comprised of 497 cases and 2387 controls. Another 1051 cases and 2510 controls were included in the replication.

Results: 5 loci reached the genome-wide significance threshold of $P < 5 \times 10^{-8}$, the clade B serpin (SERPINB) gene cluster at 18q21.3, the cytokine gene cluster at 5q31.1, the filaggrin gene, the *C11orf30/LRRC32* locus, and the human leukocyte antigen (HLA) region. The HLA locus was identified as a peanut-specific locus after stratification of the results for the most common food allergens peanut, hen's egg and cow's milk. The other identified loci were associated with any food allergy.

Publically available databases such as the Ensembl Variant Effect Predictor and the GTEx Consortium Database, that harbours information on gene expression data in various tissues, were used to evaluate whether there is evidence for functionally relevant single nucleotide polymorphisms in the identified loci. Variants in the SERPINB gene cluster were identified as expression quantitative trait loci for *SERPINB10* in leukocytes. GTEx expression data also showed high expression of SERPINB genes in the esophagus.

Conclusion: As the five associated loci play a role in the regulation of the immune response or in the formation of an intact epithelial barrier, our results highlight the importance of both mechanisms in food allergy development.

0006 | Metabolic characterization of severe allergic patients to profilin overexposed to grass pollen

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Background: Allergic diseases have presented a progressive rise in both, the incidence, which is increasing steadily worldwide, and in severity, with an increase of food allergy and asthma. In the case of food allergy, our previous results have shown that the allergen profilin may have an effective access to the body through the oral mucosa, specially in patients with a severe allergic phenotype overexposed to grass pollen. Oral provocation with profilin represents a unique diagnosis way to identify grass pollen severe allergic patients. To date, there is a huge lack of deep knowledge concerning the molecular mechanism involved in evolution to severity. 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.

Method: The aim of this study was to obtain the metabolic pattern of allergic patients and non-allergic subjects, 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 non-allergic subjects, mild, moderate and severe allergic patients to profilin, were analysed by high throughput liquid chromatography–mass spectrometry (LC-MS) technique.

Results: Results from the statistical models showed differences between the groups. Significant changes in amino acids, carnitines, fatty acids, sphingolipids, phospholipids and bile acids, which were found significantly correlated to the severity of the disease. In the case of carnitines; carnitine, acetylcarnitine and hexanoylcarnitine

were decreased on severe patients. Furthermore, lysophospholipids (LPC, LPE and LPS) were clearly increased on the severe group compared to the rest of allergic groups. Interestingly, sphingolipids such as sphingosine-1P and sphinganine-1P were increased on severe patients whereas xenobiotic C17-sphinganine was decreased.

Conclusion: These findings suggested that metabolite target analysis may be a guide of severity prediction and patient stratification in a near future for stratifying allergic patients in base to their clinical phenotype. This study represents a proof of concept of the potential of metabolomics in allergy diagnosis.

SUNDAY, 27 MAY 2018
OAS 02
PEDIATRIC ASTHMA AND RHINITIS

0017 | Cadherin-related family member 3 gene modulates asthma susceptibility in Chinese children

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Background: Asthma is the most common chronic lung disease in children. The gene encoding cadherin-related family member 3 (*CDHR3*) was recently identified as the receptor for human rhinovirus-C (HRV-C). Rs6967330 of this gene was a risk factor for severe asthma exacerbations in Caucasian preschoolers, but there was limited data on its association with childhood asthma. This cross-sectional study investigated the association between *CDHR3* and asthma diagnosis and subphenotypes in Chinese children.

Method: Ten tagging SNPs located within 5-kilobase both upstream and downstream from rs6967330 of *CDHR3* were selected by HaploView 5.0 based on 1000 Genomes database searched at pairwise $r^2 \geq 0.8$ for linkage disequilibrium (LD) for all SNPs with minor allele frequencies (MAFs) ≥ 0.01 in Southern Han Chinese (CHS). These tagging SNPs were genotyped by TaqMan assays on QuantStudio 12K Flex real-time PCR system. The genetic associations between these SNPs and categorical and quantitative variables were analysed by logistic and linear regression respectively. Haplotypes of *CDHR3* were assigned by Haploview 5.0, and their associations with asthma traits were analysed by haplo.stats 1.7.7 of R-package.

Results: 888 Chinese children with physician-diagnosed asthma and 1187 non-allergic controls were recruited, with their mean (SD) age in years being 11.0 (4.1) and 13.7 (4.5) respectively. Atopy was present in 75.2% of patients and 38.0% of controls ($P < 0.0001$). The overall genotyping efficiency was $\geq$ 95%. MAFs of tested *CDHR3* SNPs were comparable to those published for CHS, with rs448025 and rs543085868 being monomorphic. Asthma diagnosis was significantly associated with rs6967330 under addictive model (odds ratio [OR] 1.29 and 95% confidence interval [CI] 1.01-1.65; $P = 0.042$) and dominant model (OR 1.34 and 95% CI 1.02-1.76; $P = 0.036$). On the other hand, this SNP was not associated with atopy or spirometric indices. All other *CDHR3* SNPs were not associated with asthma subphenotypes. Asthma was also associated with GAC haplotype formed by rs4730125, rs6967330 and rs408223 (OR 1.32 and 95% CI 1.02-1.72; $P = 0.039$).

Conclusion: *CDHR3* is a candidate gene for childhood asthma susceptibility but not associated with other asthma subphenotypes. These results may reflect the importance of HRV-C infection for asthma. Our findings need to be replicated in large prospective studies.

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0018 | The TNFA, IL4, IL5 genes polymorphisms and asthma severity in Siberian children

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Background: Asthma is a multifactorial disease; its development is dependent on many environmental and genetic factors. Genetic risk factors can affect the clinical asthma phenotype and the level of therapeutic control over the disease. Cytokine genes are crucially important for ABA pathogenesis as they encode proteins participating in immune response and inflammation.

Aim: To evaluate the distributions of genotypes and allelic variants of immune response polymorphic genes *IL4* (*rs2070874*, *rs2243250*), *IL5* (*rs2069812*), and *TNFA* (*rs1800630*) in asthmatic children with different disease severity (Krasnoyarsk, Siberia, Russia).

Method: We analysed cytokines gene polymorphisms (*TNFA*, *IL4*, *IL5*) in asthmatic children with different disease severity (mild asthma (n = 72), moderate asthma (n = 67), and severe asthma (n = 71)) and population sample (n = 136). The analysis was performed on DNA isolated from peripheral blood by a standard method with a sorbent. Genotyping was carried out by PCR and RFLP-analysis with electrophoretic detection in an agarose gel. The comparison of the allele prevalence between the groups was carried out by Chi-square test using Gen Expert on-line calculator (http://gen-exp.ru/calculator_or.php).

Results: We observed statistically significant differences between moderate and severe asthma in comparison with control group for the prevalence of *TNFA* (*rs1800630*) polymorphism: CC genotype was more common in control group (75.7% compared to 59.2% in children with moderate asthma, $P = 0.01$ and 64.2% in severe asthma, $P = 0.001$; OR 2.37, CI 1.4-4.0). It was shown that the *TNFA**A allele and AA genotype of the *rs1800630* are associated with the increased risk of moderate and severe asthma.

Conclusion: Our data, obtained for the first time in children of European origin in Siberia, established that the studied cytokine genes are important for the asthma development in children. Our results may be especially interesting in terms of the accumulation of the data about the impact of *TNFA* production on asthma development and therapeutic control in children.

0019 | Development and applicability of lung function equations for Portuguese school age children

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Background: References values for spirometry should be derived from a population similar to the one that it is to be applied on. We aimed to estimate up-to-date reference values for Portuguese children and assess the external validity of the obtained reference values comparing them to the Global Lung Function Initiative (GLI).

Method: Anthropometry and spirometry (forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), forced expiratory flow between 25 and 75% of FVC ($FEF_{25/75\%}$)) were retrieved from 858 children attending primary schools in Porto, Portugal. Multiple linear regression models were calculated for each parameter with the stepwise forward selection method. Validity was assessed on a population of 2986 children, with matching age. Mean differences and Bland-Altman plot were used to compare the power of the two sources of reference values.

Results: Reference values were developed based on data from 481 children who correctly performed spirometry, aged 7 to 12 years, 267 (55.5%) boys. The strongest correlation was found for FVC with height (r = 0.609 for boys; 0.703 for girls), while the lowest correlation was observed in both sexes for $FEF_{25/75\%}$ with age. Height was the most significant predictor of FVC, FEV_1 and $FEF_{25/75\%}$. Final equations are presented in Table 1. The new derived references equations showed lower mean differences than GLI equations for measure values of FEV_1 [mean (SD) 0.0006 (0.23593) vs -0.0174 (0.2377) for boys; 0.0033 (0.22382) vs -0.0057 (0.21326) for girls], for $FEF_{25/75\%}$ [-0.0155 (0.46874) vs -0.1384 (0.46557)] and its predicted values. For FVC, GLI equations performed better for boys [-0.1331 (0.27828) vs -0.0727 (0.28112)] and our equations performed better for girls [(-0.0007 (0.25245) vs -0.0520 (0.24884)]. Bland-Altman plot visual inspections showed a similar fit for GLI equations and the new derived equations.

Conclusion: Our new derived equations performed slightly better when compared to GLI equations that tend to underestimate values. Moreover, they require less data and have simple calculation procedure, therefore facilitating diagnosis of respiratory conditions in children, by improving differentiation between health and disease.

Table 1

FVC equation boys	−3.042 + (0.038*H)+(0.004*W)
FVC equation girls	−2.253 + (0.029*H)+(0.034*age)+ (0.043*z-score BMI)
FEV_1 equation boys	−1.523 + (0.023*H)+(0.004*W)+(0.017*Age)
FEV_1 equation girls	−1.431 + (0.022H)+ (0.008W)+(−0.005ZscoreBMI)
$FEF_{25/75\%}$ boys and girls	−1.237 + (0.025*H)+(0.005W)+(0.039*z-score BMI)+(−0.01*Age)

0020 | Comparison of spirometry and impulse oscillometry to assess mannitol challenge test in children

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Background: The Impulse Oscillometry (IOS) is a noninvasive, no effort dependent validated technique that measures respiratory resistance (R) and reactance (X) at different oscillation frequencies. In this study we compare IOS and spirometry to assess mannitol challenge test in children.

Method: Mannitol tests was performed in 77 children with symptoms suggestive of asthma and in 21 without respiratory symptoms. The pulmonary function testing was performed baseline and after the administration of each mannitol doses. The following IOS values were analysed: Z5, R5, R20, X5, X15 and AX and FEV1 for spirometry. A fall of 15% in FEV1 from baseline after mannitol was considered as a positive challenge.

Results: A total of 98 patients, 50 females, with a mean age of 10.05 (±2.83) were enrolled. 27 had a ≥ 15 % fall in FEV1 after mannitol, in these patients the median variation of FEV1 was 17.9% and 18.7, 24.1 and 72.3% for R5, X5 and AX respectively. The changes in FEV1 correlated significantly with the IOS parameters with a Spearman coef. ≤0.40 ($P < 0.01$). The parameters with the greatest discriminative capacity according ROC curve analysis were: R5, X5 and AX (AUC >0.7). The most optimal cut-off points obtained for these parameters were: an increase ≥18 and 40% in R5 and AX and fall of 21% for X5. The median of PD15-FEV1 was 143.5 mg mannitol (41-417); however, when new cut-offs were used the mannitol concentration required to consider a positive test was lower, the median of PD18-R5, PC21-X5 and PC40-AX were 26.6, 14.3 and 23.1 mg respectively. This suggests that IOS could detect the bronchial obstruction before that the spirometry. The median increase over baseline of R5 was 18.8% (2.7-36.4) and 5.7% (−4-18) for R20 in patient with positive mannitol test, which was significant for R5 ($P < 0.01$) but not for R20 ($P = 0.05$). When the percent increase in R5 and R20 in positive vs negative mannitol subjects was rated, a significant difference was obtained only for R5. This difference indicates that mannitol has little effect on R20, consistent with no significant effect on central airways, on contrary to R5 measurement which include resistances of central and peripheral airways.

Conclusion: IOS can provide a reliable; shorter and patient-friendly technique for assessment of bronchial hyperresponsiveness in children. An increase in R5 with the lack of a significant change in R20 suggests that the airflow obstruction caused by mannitol is mainly due to an increase in peripheral airway resistance.

0021 | Comparison of bronchial and nasal allergen provocation in children and adolescents with bronchial asthma and house dust mite sensitization

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Background: Bronchial allergen provocation (BAP) is an established tool for the diagnosis of allergy in patients with asthma. However, BAP cannot be used in multi center trials due to possible side effects and lack of experienced investigators. Nasal allergen provocation (NAP) is an alternative method which is found to be safe without the need of sophisticated equipment. The aim of this prospective study was to evaluate the concordance of both methods in children and adolescents with suspected house dust mite (HDM) allergy.

Method: 157 patients with allergic asthma and positive prick test for HDM were screened and 112 patients underwent BAP with the following parameters being analyzed: PD20FEV1 allergen, exhaled NO, total-IgE, and specific-IgE to HDM. Within 12 weeks, NAP with HDM was performed in 74 of 112 patients. Results were evaluated using the Lebel score.

Results: 57 of 74 patients had an early asthmatic reaction (EAR) by BAP, of these 41 were identified by using the Lebel score. Lebel score had a sensitivity of 71.9% and a positive predictive value (PPV) of 89.1%, negative predictive values was 42.8%. In addition, an eNO $\geq$ 10 ppb (AUC 0.78) and a specific-IgE to HDM $\geq$ 25.5 kU/l (AUC 0.72) predicted an EAR; a correlation to Lebel score could not be determined.

Conclusion: The PPV of the Lebel score for an EAR was high. In contrast to PPV, low NPV could not exclude an asthmatic reaction sufficiently. In addition, specific- IgE as well as eNO could be identified as valid predictors of an EAR. According to our findings BAP cannot be substituted by NAP in patients with asthma and suspected HDM allergy.

0022 | The association between local and systemic immune response in seasonal allergic rhinitis children with or without pollen food syndrome

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Background: Seasonal allergic rhinitis (SAR) and pollen food syndrome (PFS) are both mediated by immunoglobulin E antibodies. However, the role of local and systemic immune response by these diseases in children is still unclear. The aim was to investigate the association between local and systemic immune response in seasonal allergic rhinitis children with or without pollen food syndrome.

Method: Eighty five children aged 7-12 years with SAR alone or SAR with PFS and 32 ages matched healthy control group were observed. The questionnaire on rhinitis and food symptoms, skin prick testing with commercial extracts and fresh fruits or vegetables were performed. Nasal and pharyngeal swabs were performed in all patients. Total and pollen specific IgE were assessed by ImmunoCAP, expression of IgE by immunocytochemistry.

Results: In children with SAR alone (63.5%) nasal and pharyngeal eosinophils count was remarkably higher compared with healthy control ($p<0.005$) and directly correlated with elevated peripheral blood eosinophils. All patients demonstrated increased expression of IgE in nasal and pharyngeal mucosa. In SAR with PFS children (36.5%) blood eosinophil count, nasal and pharyngeal eosinophilia as well as local and systemic production of IgE were more significant compared with SAR alone. Expression of IgE in pharyngeal mucosa directly correlated with elevated levels specific IgE to profillins in peripheral blood ($P < 0.05$).

Conclusion: SAR children have clear signs of allergic inflammation in nasal and pharyngeal mucosa, which is more expressed in children with PFS. The increased IgE expression and nasal and pharyngeal eosinophilia directly correlated with elevated levels of total and allergen–specific IgE in peripheral blood. These data support the concept of significant links between local and systemic immune response in allergic patients.

SUNDAY, 27 MAY 2018
OAS 03
PAN-OMICS IN RESPIRATORY AND SKIN DISORDERS

0012 | Where have all the flowers gone?

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Background: In the pre-GWAS era (1993-2007) numerous association studies have been published in renowned journals including The Lancet, New England Journal of Medicine, Nature, Nature Genetics, Nature Immunology, Science and Human Molecular Genetics. They all showed an association of allergy related traits while these results have not been systematically matched with results from current GWAS studies.

Method: We are now following up several prominent associations by comparing the previously published results with currently deposited data at the NHGRI-EBI Catalog of published genome-wide association studies http://www.ebi.ac.uk/gwas NHGRI-EBI listed phenotypes were only selected if they are not suffering themselves from serious problems like unstandardized outcomes. Also the SNP marker set should have a good coverage of the region of interest.

Results: In total 26 allergy associated genes could be reanalyzed. The initial association could not be confirmed for CD14, ADRB2, TNF, MS4A2, ADAM33, GSTM1, IL10, CTLA4, SPINK5, LTC4S, LTA, NPSR1, NOD1, SCGB1A1, GSTP1, NOS1, CCL5, TBXA2R, and TGFB1. Some genes showed borderline significant results like IL4 and IL4R while only IL13, HLA-DRB1, HLA-DQB1, IL1 cluster and STAT6 were clearly associated also in recent GWAS studies.

Conclusion: Most of the early SNP association studies could not be replicated which has also been described in other disease areas ("non-replication crisis"). Assumed reasons range from insufficient editorial oversight, poor review, phenotyping or genotyping errors, selective reporting or intentional fraud. In addition there are numerous study inherent problems like population stratification or wrong significance thresholds that may have led to largely irreproducible results.

0013 | Differential network and pathway analysis of transcriptome molecular markers associated with an adjuvanted grass allergoid immunotherapy

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Background: Specific Immunotherapy (SIT) with a 13 Grass MATA MPL vaccine, which combines broad spectrum grass modified allergens adsorbed on the depot adjuvant microcrystalline tyrosine (MCT) in combination with Monophosphoryl Lipid A (MPL), is a short course form of treatment for IgE-mediated allergic rhinoconjunctivitis. The aim of this analysis was to elucidate grass SIT-induced molecular mechanisms and identify signature genes associated with immunological pathways and cellular functions after treatment.

Method: This was a FDA approved phase I tolerability study with 30 subjects aged between 18 and 50 years with seasonal allergic rhinoconjunctivitis. Subjects were randomly assigned on a 1:1 ratio to receive either six injections of cumulative dose regimen 35600 SU of subcutaneous complex grass allergoid vaccine or placebo, which included MCT and all constituents of the active formulation except allergoid and MPL. RT^2 Profiler PCR Array gene expression analysis (178 genes) of peripheral blood mononuclear cells were analysed from both study groups after one week following the end of treatment. The raw PCR Array data was processed using GeneGlobe data analysis tool to calculate fold change values based on delta delta C_t method and P values based on a Student's t-test. Statistically significant output data ($P < 0.05$) was further analysed using differential network and pathway-based analysis system Ingenuity Pathway Analysis software.

Results: A cluster of differentially expressed genes related to the treatment group were identified with significant fold-changes in expression levels. Grass SIT-associated signature genes included T helper cell differentiation and proliferation markers (CD4, CD80, CD70) and Th1/Th2 cytokines, antigen presentation and transmembrane receptors (CD1a, HLA-DMA), transcription and signal transduction factors (FOXP3, GATA4, RUNX1, STAT5A), and a number of chemokines of cells adhesion and diapedesis. The top enriched categories of canonical pathways with P-value less than 10^{-20} were related to cytokine signalling between immune cells and the differentiation and activation of T-helper cells.

Conclusion: A distinct profile of SIT-induced responses from grass-allergic subjects revealed clusters of related genes associated with innate and adaptive immune responses one week after treatment compared with placebo. A number of genetic markers identified may be linked to early time-points during successful SIT with 13 Grass MATA MPL.

0014 | Risk variants in the CTLA4 gene locus associate with asthma susceptibility and regulatory T cell function

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Background: Genome wide linkage studies in diverse ethnicities have highlighted CTLA4 on chromosome 2q as an asthma candidate gene. Follow up studies however have not yielded conclusive results. Given the crucial role of CTLA4 in immune regulation and allergic pathophysiology, we wanted to evaluate the association in our Singapore population and other validation cohorts. To evaluate the role of *CTLA4* SNPs towards risk for asthma through genetic association cohorts and evaluate its functional relevance in immune cells.

Method: We used case-control populations collected from different ethnicities to determine association to asthma. We then used immune profiling using flow cytometry and serology analysis to association the disease variants of *CTLA4* to T cell immune subsets in a functional cohort.

Results: Genetic association of *CTLA4*SNPs to asthma in the Singapore population of 2880 individuals revealed a strong association of rs3087243 to asthma risk with a $P = 5.9 \times 10^{-06}$ and a strong odds ratio of OR = 1.52. This association was validated in a large meta-analysis of 10317 samples using independent cohorts from Netherlands, Australia and United States. Subsequently we showed in a functional cohort of 300 individuals that these associated disease variants influenced the CTLA4 protein expression in T cells but not the soluble levels (sCTLA4) in blood plasma. Interestingly we observed strongest effect of variants on CTLA4 expression in the naive regulatory T cell subset (Treg).

Conclusion: We have shown association of a *CTLA4* genetic variant with asthma risk in multiple independent population. We then showed association of this associated variant to expression in immune T cell subsets and especially the regulatory subset. This highlights possible strategies for intervention to attenuate local CTLA4 expression which could be crucial in identifying potential therapeutic targets.

CHR	SNP	BP	A1	F_A	F_U	A2	CHISQ	P_{trend}	OR	SE	L95	U95
2	rs56102377	204445880	A	0.054	0.061	G	0.556	0.46	0.884	0.166	0.638	1.223
2	rs11571319	204447183	A	0.101	0.123	G	3.261	0.071	0.801	0.123	0.63	1.019
2	**rs733618**	**204730944**	**G**	**0.382**	**0.439**	**A**	**9.002**	**0.0027***	**0.79**	**0.079**	**0.678**	**0.922**
2	rs4553808	204731005	G	0.1	0.121	A	2.909	0.088	0.810	0.124	0.636	1.032
2	rs16840252	204731519	T	0.105	0.128	C	3.468	0.063	0.798	0.121	0.63	1.012
2	**rs231775**	**204732714**	**A**	**0.362**	**0.3147**	**G**	**6.488**	**0.011***	**1.233**	**0.082**	**1.049**	**1.449**
2	**rs3087243**	**204738919**	**A**	**0.263**	**0.191**	**G**	**20.51**	**5.9×10^{-06}***	**1.52**	**0.093**	**1.267**	**1.822**

SNP, Single nucleotide polymorphism; CHR, Chromosome on which the SNP is present; BP, Position in base pair for the SNP location on the chromosome; AF (Allele frequency) and Odds Ratio (OR) is in reference to Risk allele; Case frequency and Control frequency is the frequency of the risk allele in affected (Cases) and unaffected (controls); Other allele – Allele 2 for the SNP considered for the association test. CHISQ, Chi square statistic for the SNP association test; SE, Standard error for the CAT (Cochran Armitage trend) test. P_{trend} is calculated by the Cochran Armitage trend test using the PLINK v 1.06 software. L95, U95 – lower and upper limits of the 95% Confidence Interval (CI) of odds ratio (OR).

*$P_{trend} < 0.05$ for the association test for AR is shown in bold.

0015 | Infants hospitalised for severe viral bronchiolitis manifest dysregulated interferon (particularly types 1 & 3) responses

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Background: A subset of infants are hyper-susceptible to respiratory viral insults, and resultant severe acute viral bronchiolitis (AVB) leads to hospitalisation more frequently relative to older children, for reasons unknown.

Aim: To characterise the cellular and molecular mechanisms underlying severe infant AVB in circulating cells and local airways tissues.

Method: Subjects were recruited at the time of hospitalisation for AVB and stratified by age: 0-18 months (infants) and 1.5-5 years (children). PBMC, nasal mucosal scrapings and saline washes were obtained at presentation with AVB and post-convalescence. Viral infection was confirmed by RT-qPCR in nasal saline washes. Immune response patterns were profiled by multiplex analysis of plasma cytokines, 9 colour flow cytometry, and transcriptomics. RNA-Seq transcriptomic data was analysed employing coexpression network analysis and personalised N-of-1-pathways analysis.

Results: Pathogen profiles were dominated by RSV in infants (50%) and RV (44%) in children. Cellular and molecular profiles in PBMC differed markedly: AVB infant responses were dominated by hyper-upregulated type I interferon signalling and pro-inflammatory pathways (TNF, IL6, TREM1, IL1B) independent of virus type, vs a combination of inflammation (PTGER2, IL6) plus growth/repair/remodelling pathways (ERBB2, TGFB1, AREG, HGF), IL4, and steroid signalling in some of the children. Overall age-related differences were not attributable to differential steroid usage. Cellular data in infants showed increased monocytes at AVB relative to recovery, and adjustment for proportions of myeloid cells suggests that the exaggerated infant responses are monocyte-associated. Network analysis combined with personalised immune response profiling confirmed differential upregulation of innate immunity in infants, and upregulation of NK cell networks in the children. Local airway molecular responses were comparable qualitatively in infants and children, and dominated in both by interferons type I-III, but the magnitude of upregulation of relevant genes was higher in infants (range 6-48 fold) than children (5-17 fold). Estimation of the cellular composition of airway samples revealed increased M1 macrophages at AVB in infants and increased NK cells at AVB in children.

Conclusion: Infants and children mount distinct immunoinflammatory responses to AVB. Myeloid innate antiviral immunity appears dysregulated/exaggerated in a subset of infants.

0016 | Proteomic analysis of the acid-insoluble fraction of human whole saliva from patients affected by non histaminergic-angioedema

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Background: Recurrent angioedema (AE) is characterized by recurrent bouts of swelling without urticaria, involving the subcutaneous or submucosal tissues, lasting 2–5 days and involving the skin, tongue, upper airways and gastrointestinal tract. Patients do not respond to treatment with corticosteroids and anti-histamines. Hereditary forms of angioedema include the one with a deficiency of complement component 1 esterase inhibitor (C1-INH-HAE), that with coagulation factor XII mutations (FXII-HAE), and that without an identified cause (U-HAE); acquired forms of angioedema comprise the idiopathic histaminergic (IH-AAE), the idiopathic non histaminergic (InH-AAE).

Method: In this study, we present the data from bidimensional electrophoresis coupled to high resolution mass spectrometry analysis on the acid-insoluble fraction of saliva from three classes of non-histaminergic angioedema patients and healthy controls aiming to highlight significant variations in normalised spot volumes. The peptide mixtures from the differentially-expressed spots were analysed by high resolution HPLC—ESI—MS/MS for protein identification.

Results: By this strategy, 16 differentially-expressed proteins among two or more groups were identified. Among other proteins implicated in immune response, we found an overexpression of proteins involved in immune response (interleukin-1 receptor antagonist and annexin A1, and of glyceraldehyde-3-phosphate dehydrogenase, α-enolase, and annexin A2 (proteins known to act also as plasminogen receptors) in patients affected by the idiopathic non-histaminergic or U-HAE with respect to healthy controls.

Conclusion: The involvement of plasmin has been previously proved in the pathogenesis of some subsets of HAE with normal C1-INH. Plasmin importantly interacts with the contact system, cleaving FXII. This data provide new insights on the molecular basis of these less characterised types of angioedema.

SUNDAY, 27 MAY 2018
OAS 04
DRUG HYPERSENSITIVITY RISK FACTORS

0007 | Low prevalence of aspirin hypersensitivity in mastocytosis: The results of a double-blind, placebo-controlled crossover study

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Background: Patients with mastocytosis are at increased risk of anaphylaxis and the use of several drugs, including nonsteroidal anti-inflammatory drugs (NSAIDs), is discouraged because of this reason. However, the prevalence and severity of NSAID-related hypersensitivity among patients with mastocytosis might be overestimated.

Method: We performed a double-blind, placebo-controlled, cross-over trial using a challenge with acetylsalicylic acid (ASA) up to a cumulative dose of 520 mg among adult patients with mastocytosis. In addition, we performed a retrospective search of our entire outpatient cohort for data on NSAID hypersensitivity.

Results: Fifty patients were included: 70% had indolent systemic mastocytosis, 18% mastocytosis in the skin, 12% advanced mastocytosis. The ASA challenge was positive in 1 patient, consisting of urticaria. No severe reactions were seen. Furthermore, 8 out of 191 patients in the total outpatient cohort had a history of NSAID-related hypersensitivity reaction(s), of whom 3 reported severe systemic reactions. All 8 patients had NSAID-related hypersensitivity reactions before they received the diagnosis of mastocytosis. The

Table 1 - Comparison of clinical characteristics of patients with and without NSAID hypersensitivity of mastocytosis cohort EMC, as proven by drug challenges.

	No NSAID hypersensitivity (n = 64)	NSAID hypersensitivity (n = 9)	*P*-value
Age, median (IQR)	55 (10)	51 (9)	NS
Male sex, n (%)	22 (34.4)	3 (33.3)	NS
Subtype, n (%)			NS
MIS	14 (21.9)[a]	1 (11.1)[c]	
ISM	45 (70.3)	7 (77.8)	
SSM	2 (3.1)	1 (11.1)	
SM-AHN	2 (3.1)	0	
ASM	1 (1.6)	0	
Presence of skin mastocytosis, n (%)	50 (78.1)	6 (66.6)	NS
Serum tryptase at diagnosis, median (IQR)	25 (7.8)	31.4 (11.4)	NS
History of anaphylaxis, n (%)	27 (42.2)	5 (55.6)	NS
Pruritus, n (%)[b]	50 (78.1)	1 (11.1)	NS
Flushing, n (%)[b]	27 (42.2)	2 (22.2)	NS
Dyspepsia, n (%)[b]	10 (15.6)	2 (22.2)	NS
Diarrhea, n (%)[b]	15 (23.4)	0	NS
Fatigue, n (%)[b]	37 (57.8)	2 (22.2)	0.04
Subjective cognitive problems, n (%)	22 (34.4)	0	0.045
Osteoporosis, n (%)	8 (12.5)	3 (33.3)	NS
Eosinophilia, n (%)	4 (6.3)	3 (33.3)	0.01
Atopy, n (%)	17 (26.6)	3 (33.3)	NS
History of hypersensitivity reaction to other drugs, n (%)[d]	5 (7.8)	4 (44.4)	0.002
Alcohol intolerance, n (%)[e]	29/50 (58)	4/5 (80)	NS
MC mediator related reaction to physical triggers, n (%)	48 (75)	5/6 (83.3)	NS

[a]Bone marrow investigation negative in 2 patients, other 12 never underwent bone marrow punction.
[b]Symptom present $\geq$3 days per week.
[c]Bone marrow investigation negative for bone marrow mastocytosis.
[d]See text for further explanation.
[e]Not known for all patients because some patients never consume alcohol.
[f]Physical triggers: heat, cold, stress, exercise, alcohol consumption.

major risk factor for NSAID hypersensitivity was a history of hypersensitivity reactions to other drugs (RR 5.7, 95% CI 1.9-11.3).

Conclusion: The prevalence of NSAID hypersensitivity in our cohort of adult patients with mastocytosis is 2 - 4.1%, which is only slightly higher than the general population. NSAIDs can be safely administered to most patients with mastocytosis; extra caution should be taken in patients who have a history of hypersensitivity reactions to other drugs.

0008 | Recording drug allergies and adverse drug reactions: Results of an audit at a district general hospital

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Background: Recording of adverse drug reactions (ADRs) is a cornerstone of safe prescribing. Where patient's carry a drug allergy label, particularly to antibiotics, the accuracy of this data is essential for effective risk stratification and clinical decision making. In line with UK National Institute for Clinical Excellence (NICE) Guideline 183 (Drug Allergy; diagnosis and management), the audit standard was for all ADR records to detail the drug name, the signs, symptoms and severity of the reaction experienced and the date when it occurred.

Method: This audit was undertaken in two arms in a hospital where the Cerner® health records system is utilised for inpatient records and prescribing. In the 'online' arm a dataset of 334 individual ADR records, generated from 521 current inpatients, was analysed. The 'ward-based' arm assessed 71 general medical inpatient's knowledge of their ADR history in comparison to their contemporaneous hospital records (29 ADRs identified).

Results: 'Online' audit arm: 31% of inpatients analysed were found to have one or more ADRs associated with their record. The percentage of inpatients with an ADR to a penicillin-based antibiotic was comparable to published literature at 12.5%. When assessed against the audit standard, 81% of ADRs had no associated severity and 42% had no detail of the reaction experienced. 'Type' of reaction was recorded for 96% of records, however, a very high preponderance for 'allergy' to be selected (73% off all ADRs) was noted.

'Ward based' audit arm: None of the 29 ADR records identified were compliant with the audit standard. In 9 cases, the patient was able to provide more detail, resulting in 6 records becoming complete. There were 4 ADRs reported by patients that were not previously documented, whilst 15 ADRs were documented but unknown to the patient.

Conclusion: This audit demonstrated poor compliance with the UK national standard for ADR records with important implications for both safe prescribing and appropriate risk stratification in patients carrying a drug allergy label. Importantly, whilst it was demonstrated that data quality could be improved in a significant proportion of cases by careful history taking, as a single intervention this would not be sufficient to complete all records. These results add to growing body of international evidence, highlighting the need for further investigation and quality improvement in adverse drug reaction recording.

0009 | Skin testing for drugs used in general anesthesia and factors associated with their positivity

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Background: Muscle relaxants, antibiotics, hypnotics, opioids, blood products and latex are the most frequent agents that lead to hypersensitivity reactions during general anesthesia. The positivity of skin tests is important to confirm the diagnosis of drug allergy, but few are known about the factors associated with positivity.

Aim: To examine the results of skin tests with aforementioned agents and identify the factors associated with skin test positivity.

Method: Records of 121 patients who underwent skin testing between 2010 and 2016 for agents used in general anesthesia were reviewed retrospectively. The demographic data, details of the previous perioperative reaction (if present) and detailed past medical history including allergic diseases were recorded.

Results: A total of 121 patients (97F/24M, mean age: 48.5 ± 12.8 years) were included. The majority (59.2%) was referred by their surgeon. The most frequent reason for referral was allergic drug reactions other than general anesthetics (64.5%, n = 78). The percentage of patients who had at least one previous general anesthesia was 53.7% (n = 65) and 60% (n = 39) of previously operated patients had a perioperative reaction. Majority of patients (84.3%) suffered from drug hypersensitivity suffered from NSAID and penicillin allergy respectively [(59.8%, n = 61) and (47.1%, n = 57)]. More than half of the patients (58.3%) suffered from at least one allergic disease (37.5% and 22.5% had asthma and allergic rhinitis respectively). In 30.6% (37/121) of patients, skin tests were positive with at least one agent. The most frequently performed skin test was for rocuronium (n = 88), followed by propofol (n = 85). Amongst all agents, most frequent skin test positivity was observed with vecuronium, rocuronium and atracurium, respectively. Factors that affect skin test positivity were having a history of perioperative reaction (P = 0.032, OR: 2.395, CI: 1.067-5.380) and a shorter interval between the last general anesthesia-requiring procedure and timing of skin testing (P = 0.006). Past medical history of non-general anesthetic drug allergy itself was not found significant, but in subgroup analysis, antibiotic allergy seemed to have an impact on skin test positivity (P = 0.026, OR: 2.934, CI: 1.110-7.760).

	Skin Test Positive (n:37)—%		Skin Test Negative (n:84) —%		*P*-value
Age (mean ±SD)	46.62 ± 14.54		49.33 ± 11.93		0.323
Sex (n %)					
Female	33	89.2	64	76.2	0.099
Male	4	10.8	20	23.8	
Number of Previous General Anesthesia (n %)					
None	15	40.5	41	49.4	0.304
Only once	8	21.6	22	26.5	
More than once	14	37.8	20	24.1	
Past Medical History of Perioperative Reaction (n %)					
Yes	17	45.9	22	26.2	0.032
No	20	54.1	62	73.8	
NSAID Allergy					
Yes	14	51.9	47	62.7	0.326
No	13	48.1	28	37.3	
Antibiotic Allergy					
Yes	20	74.1	37	49.3	0.026
No	7	25.9	38	50.7	
Local Anesthetic Allergy					
Yes	5	18.5	5	6.7	0.124
No	22	81.5	70	93.3	
Allergy to Any Other Drug Groups					
Yes	8	29.6	14	18.9	0.248
No	19	70.4	60	81.1	
Past Medical History of Allergic Diseases					
Yes	23	62.2	47	56.6	0.570
No	14	37.8	36	43.4	
Atopy Confirmed with Skin Prick Tests					
Atopic	7	25.9	15	30.6	0.666
Non-atopic	20	74.1	34	69.4	

Conclusion: History of perioperative reaction, antibiotic allergy and performing the skin tests within a shorter period of time after an anesthesia were found significant factors associated with skin test positivity for general anesthetics.

0010 | Evaluation of the potential risk factors in drug induced anaphylaxis

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Background Aim: To investigate the potential risk factors in the patients experienced anaphylaxis with drug.

Method: The study included the patients who were older than 17 years old and experienced immediate type of hypersensitivity reactions with a drug. The patients were grouped as anaphylaxis and non-anaphylaxis. Anaphylaxis was diagnosed according to the WAO criteria. Skin testing with culprit drugs were performed. In non-anaphylaxis group, drug provocation test (DPT) with culprit drug as well as in NSAID hypersensitive patients DPT with aspirin or diclofenac were performed. Atopy was determined by the skin prick test with the most common inhalant allergens. Demographic and clinical features of the patients, baseline tryptase and total IgE levels were compared.

Results: Among 281 patients, the median age was 40 (min-max: 16-90) and 76.5% of them were female. The median duration between the last reaction in the history and the evaluation was 7 months (min-max: 1-120). In 52.3% of the patients the reactions were defined as anaphylaxis. The most common culprit drugs were NSAID (56.9%) and beta-lactams (34.7%). The culprit drugs were used parenterally in 13.2% of the patients. In 34.9% of the patients had comorbid diseases and 24.6% of the patients were using additional drugs being the most common one antihypertensive (10%). Atopy was determined in 28.8% and 28.1% of the patients were smoker. Median serum levels of baseline tryptase and total IgE were 3.5 mg/L and 77 kU/L respectively. In 46.3% of the patients skin tests with culprit drugs were positive and the positivity ratio was higher in anaphylaxis group ($P = 0.002$). In univariate analysis, in the patients who were hypertensive and atopic and were using angiotensinogen converting enzyme inhibitors/angiotensinogen receptor blocker and used the culprit drug parenterally experienced anaphylaxis more commonly ($P = 0.034$; $P = 0.04$; $P = 0.03$; $P = 0.035$; $P = 0.013$; $P < 0.001$). In multivariate analysis, it was observed that the parenteral usage of drug and the presence of atopy were significantly higher in anaphylaxis group [$P < 0.001$, OR (CI):20.05 (4.75-88.64); $P = 0.012$, OR (CI):2.1 (1.17-3.74)]. Age, being smoker, family history, serum level of baseline tryptase and total IgE levels were not different between groups.

Conclusion: The parenteral route and atopy increase the risk of drug induced anaphylaxis.

0011 | Liver involvement complicating SJS/TEN in an HIV endemic setting

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Background: In HIV endemic settings, common culprit drugs causing SJS/TEN include nevirapine, cotrimoxazole and first-line anti-tuberculosis drugs. Hepatitis is an uncommon complication of SJS/TEN and may increase mortality. In our clinic, where ~80% of SJS/TEN cases occur in persons living with HIV, a significant proportion have hepatitis. The aim of this study was to determine the incidence of hepatitis and impact on outcome, as well as characterize the clinical features and patterns of liver injury.

Method: We conducted a retrospective clinical record review of all patients admitted to the tertiary dermatology service at Groote Schuur Hospital, Cape Town, South Africa, over a 10-year period (2005-2015). All available clinical and laboratory variables were collected, to where possible allow Naranjo and ALDEN drug causality assessment. Classification and severity of liver injury used criteria from ICM-CADALI and the CTCAE of the National Cancer Institute for adverse events version 4.03.

Results: Of the 184 SJS/TEN patients admitted, 77.2% (142/184) were HIV infected, with a median (IQR) CD4 count of 185 (97-264) cells/mm^3. SJS was the most frequent phenotype (56%, 103/184). The leading causative drugs were: Nevirapine 80/184 (43.5%), cotrimoxazole 35/184 (19.0%), and anti-TB drugs 12/184 (6.5%). 21.2% (37/184) had liver injury, with 28/37 (75.7%), 4/37 (10.8%) and 5/37 (13.5%) having a hepatocellular, cholestatic and mixed picture respectively. Of the 37 patients with liver injury, there was only one with pre-existing hepatitis B or other pre-existing chronic liver disease. CTCAE severity grades 1, 2, 3 and 4 occurred in 46.0%, 21.6%, 27.0% and 5.4% having life threatening liver injury.

Conclusion: Hepatitis amongst SJS/TEN in HIV endemic settings is common, occurring in more than one in five patients. The majority of hepatitis is of mild to moderate severity and death from liver failure did not occur.

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OAS 05
IMMUNE SENSORS IN ALLERGY

0023 | The aryl hydrocarbon receptor regulates allergic airway inflammation via non-hematopoietic expression of cytochrome P450 member CYP1B1

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Background: The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that is able to recognize xenobiotics as well as natural ligands such as tryptophan metabolites, dietary components and microbiota-derived factors. Functioning as an environmental sensor and transcription factor the AhR is important in the maintenance of homeostasis at mucosal surfaces. AhR activation induces the expression of cytochrome P450 1 (CYP1) enzymes which are able to metabolize various compounds including potential AhR ligands. Here we investigate the role of AhR and CYP1 family members in allergic airway inflammation.

Method: In this study we used AhR and CYP1-deficient mice to examine their role in pollen- and house dust mite-induced allergic airway inflammation. Repetitive exposure with ragweed extract or house dust mite (HDM) via intranasal instillation was followed by assessment of bronchoalveolar cell counts, histology, cytokine release and specific antibody production. Bone marrow chimeras were generated to determine the contribution of hematopoietic vs non-hematopoietic cells. Additionally, cell type-specific expression of CYP1 enzymes was determined by quantitative RT-PCR and immunofluorescence microscopy.

Results: Exposure to ragweed extract or HDM resulted in notable enhancement of total IgE and a heightened cellular infiltration of white blood cells, namely eosinophils, lymphocytes and macrophages, in the bronchoalveolar lavage of AhR- and CYP1B1-deficient mice. Interestingly, CYP1B1-deficient mice reconstituted with bone marrow of C57BL/6 mice phenocopied these results indicating a prominent role for CYP1B1 expressing non-hematopoietic cells. In line with these results RT-PCR and microscopy analysis of lung tissue showed a much higher CYP1B1 expression by non-hematopoietic cells.

Conclusion: AhR-dependent CYP1B1 expression by non-hematopoietic cells - presumably epithelial cells - is necessary to prevent exaggerated allergic airway inflammation. Further studies will aim to find underlying mechanisms of CYP1B1-mediated regulation of allergic airway inflammation.

0024 | The major milk allergen Bos d 5 prevents allergic sensitization in the mouse model only when loaded with quercetin-iron: Role for the aryl hydrocarbon receptor pathway

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Background: The major milk allergen Bos d 5 is a lipocalin and can be loaded with quercetin, a flavonoid with a great affinity to iron. We previously demonstrated in vitro that the loading of Bos d 5 with such a ligand is decisive for induction of immune tolerance. Here, we addressed the in vivo relevance of this observation by nasal allergic sensitization of BALB/c mice using unloaded (apo-) or quercetin-iron-loaded (holo-) Bos d 5, and further elucidated a potential mechanistic role of the aryl hydrocarbon receptor (AhR) pathway.

Method: BALB/c mice were sensitized nasally 6 times in biweekly intervals with apo-Bos d 5 generated by dialysis against deferoxamine, or holo-Bos d 5 generated by incubation of the apo-form with quercetin and iron. Quercetin-iron complex formation was assessed fluorometrically. Control groups were sham-treated with water or iron-quercetin complexes alone. Subsequently, allergic sensitization was assessed by recording body temperature drop after intraperitoneal challenge with Bos d 5. Specific serum antibodies and cytokines from Bos d 5-stimulated splenocytes were analyzed by ELISA. AhR activation was evaluated by stimulation of the reporter cell line AZ-AhR with Bos d 5.

Results: Application of apo-Bos d 5 resulted in significantly higher Bos d 5–specific antibodies (IgG1, IgG2a, IgA and IgE) and cytokine levels (IL5, IL13 and IL10) than in the group exposed to holo-Bos d 5 or controls. Sensitization with apo-, but not holo-Bos d 5 resulted in a significant body temperature drop upon allergen challenge. Quercetin-iron formation was confirmed by quenching of autofluorescence. Quercetin activated AhR in a concentration-dependent manner.

Conclusion: In the in vivo mouse model, the major milk protein Bos d 5 prevents allergic sensitization when properly loaded. In the holo-form, Bos d 5 transports ligands like quercetin-iron, which initiate immunosuppression by activating the anti-inflammatory AhR-pathway.

0025 | Free amino acid levels in human milk throughout lactation and their in vitro immunomodulatory potential

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Background: Human milk not only provides newborns with an optimal nutrient composition for development, but it also contains bioactive compounds that have an influence on the immune system and thus, potentially, on immune-related diseases like allergies. Free amino acids (FAAs), which make up 3-5% of the total amino acids present human milk, have gained considerable interest over the last decade. With the demonstrated immunomodulatory capacities of individual FAAs like glutamine, it is discussed that FAAs might have functional roles in the developing infant. To contribute to this discussion, the present study assessed the time-specific occurrence of FAAs in human milk and tested immunomodulatory effects of individual FAAs in *in vitro* immune/allergy models.

Method: A total of 135 human milk samples donated by healthy Dutch mothers participating in the PreventCD study (ISRCTN74582487) on coeliac disease were analyzed. It concerned 25 series of consecutively monthly samples up to 6 months of lactation. FAA content was analyzed by ultra-fast liquid chromatography. Immunomodulatory effects of individual FAAs were tested *in vitro* in monocyte-derived dendritic cells and in murine bone-marrow derived mast cells, which were activated for 24 hour with LPS and IL33, respectively. As readout, the T_H1/T_H2-cytokine profile was measured in absence and presence of FAAs during cell activation.

Results: At a concentration of 1295-1825 μM, glutamate was by far the most abundant FAA in milk at each stage of lactation, followed by glutamine. Whereas most of the FAAs remained stable levels throughout lactation, average levels of free glutamate, glutamine, aspartate, glycine, and serine significantly increased in the first three months of lactation. Of these, relative increases were highest for glutamate (1.5-fold) and glutamine (3.5-fold). Analyses of immunomodulatory effects of free glutamine and glutamate *in vitro* showed that glutamate significantly increased both the IL-12 production and the IL-12/IL10 ratio of LPS-stimulated dendritic cells in a dose-responsive manner, whereas glutamine decreased IL-13 production by IgE-stimulated mast cells.

Conclusion: FAAs in human milk display a time-specific occurrence throughout lactation. Free glutamine and glutamate, of which levels in human milk increased in the first 3 months of lactation, were demonstrated to exhibit T_H2-inhibiting and/or T_H1-skewing capabilities in *in vitro* allergy models. Additional research is required to study the physiological benefit of this finding.

0026 | Identification and quantification of functional cannabinoid receptor 1 (CB1) in human blood and tonsil immune system cells

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Background: The endocannabinoid system (ECS) is a complex signalling network involved in a large number of physiological processes. The ECS consists of the endocannabinoid ligands, the enzymes related to the synthesis and degradation and the cannabinoid receptors (CBRs). Immune system cells express CB1 but its functional role remains poorly understood due to the lack of suitable tools. In humans, the mRNA expression levels of CB1 are upregulated in tonsils and peripheral blood of allergic patients. The aim of this work is the visualization and quantification of CB1 at the protein level in human peripheral blood and tonsils and the study of the functional properties of this receptor in immune system cells.

Method: CB1-specific fluorescent chemical probe was used to visualize and quantify CB1 expression on B cells, T helper cells, cytotoxic T cells, monocytes, and total DCs from peripheral blood and tonsils by flow cytometry and confocal microscopy. Proliferative responses were measured by adding ^{3}H-thymidine and cytokine production was analysed by ELISA. Allogeneic co-cultures of human DCs and naïve $CD4^+$ T cells were performed and FOXP3 expression was analysed by flow cytometry.

Results: We developed and validated a novel chemical probe specific for CB1 to identify CB1-expressing immune system cells from blood by flow cytometry and in tonsils tissues by confocal microscopy. We demonstrated that $CD19^+$ B cells, $CD3^+CD4^+$ T helper cells, $CD3^+CD8^+$cytotoxic T cells, monocytes and total dendritic cells (DCs) from PBMC and tonsils express CB1. The CBR agonist HU210 but not the CB2 agonist HU308 increased the proliferation of activated tonsil T cells and reduced the production of Th2 cytokines IL-5 and IL-13 as well as IL-10. In addition, our data showed that the CBR agonist WIN55212-2 reduced the production of the pro-inflammatory cytokines TNF-α, IL-6 and IL-1β in LPS-activated monocytes, human monocyte-derived DCs and total DCs from peripheral blood. Interestingly, WIN55212-2 induced tolerogenic DCs with the capacity to generate functional $CD4^+CD25^+CD127^-FOXP3^+$ Treg cells.

Conclusion: A novel fluorescent chemical probe can be used to visualize and quantify the expression of functional CB1, which displays immunomodulatory effects in tonsil cells and blood immune system cells.

0027 | The expression of ST2+ mDCs and the effects of mDCs on human group 2 innate lymphoid cells in allergic rhinitis

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Background: It has reported that allergen inhalation increases the release of epithelial derived cytokine IL-33, which effects have been implicated in the pathogenesis of allergic disease such as allergic rhinitis (AR). Group 2 innate lymphoid cells (ILC2s) recently identified play significant roles in the pathogenesis of AR. However, the role of myeloid dendritic cells (mDCs), the professional antigen-presenting cells, on the ILC2s in AR is still unknown. Therefore, this study is going to investigate the IL-33 receptor (ST2) expression profiles on mDCs, the effects of inhaled allergens on the levels of $ST2^+$ mDCs, and the role of ($ST2^+$) mDCs on ILC2s in the pathogenesis of AR will be identified.

Method: AR patients who were positive to house dust mite were challenged with inhaled Der P1. Peripheral blood was collected, and $ST2^+$ mDCs were determined using flow cytometry at 0 h, 0.5 h, 2 and 4 hour after the allergen challenge. mDCs were prepared after stimulating CD14 + monocytes with granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-4. mDCs were co-cultured with both peripheral blood monocytes (PBMCs) and lineage negative cells isolated from AR patients. The effects of mDCs on human ILC2 intracellular cytokines and transcription factor expressions were assessed by flow cytometry. The secreted soluble cytokines were measured using ELISA kits.

Results: Inhaled Derp1 significantly increased $ST2^+$ mDC levels in the PBMCs of AR patients at 0.5 and 2 hour after challenge, and the $ST2^+$ mDC reached the peak at the time point of 0.5 h. Furthermore, mDCs up-regulated the production of type 2 cytokines, IL-5 and IL-13, after co-cultured with lineage negative cells. They also increased the levels of $IL\text{-}13^+$ ILC2s from PBMCs isolated from AR patients. Flow cytometry analysis revealed that p-STAT3 and p-STAT5 pathways were upregulated in ILC2s when co-cultured with mDCs.

Conclusion: The ST2 expression is up-regulated on blood mDCs after allergen inhalation in AR patients. mDCs activate human ILC2 function via p-STAT3 and p-STAT5, indicating that mDC can act as a bridge between innate and adaptive immunity in the pathogenesis of AR. Our findings reveal a novel regulatory pathway in ILC2 mediated allergic diseases.

0028 | The carbohydrate A10 (Ca10) from Ehrlich tumor cells promote tolerance by acting on dendritic cells

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Background: Dendritic cells (DCs) play a key role in linking innate and adaptive immune responses. Dysregulation of these mechanisms leads to immune tolerance-related diseases such as cancer. During cancer, alterations in glycosylation pattern of cell surface condition the immune response against the tumor. The carbohydrate A10 (Ca10) is located in the membrane of Ehrlich tumor (ET) cells and in certain human adenocarcinomas, but its potential immunomodulatory role remains fully elusive. We study the capacity of Ca10 to influence the differentiation of human monocytes to DCs (hmoDCs) and the capacity of this carbohydrate to inmunomodulate DCs function.

Method: Flow cytometry, ELISA, real-time quantitative PCR assays were performed to assess the effect of Ca10 in the generation of hmoDCs as well as the phenotype and function of Ca10-activated hmoDCs. Allogeneic co-cultures of hmoDCs and naïve $CD4^+$ T cells were performed and the *in vitro* generation of functional $FOXP3^+$ regulatory T (Treg) cells monitored by flow cytometry and suppression assays. C57BL/6J mice were immunized with ET cells. Tumor growth was monitored and the amount of Ca10 quantified in serum by ELISA. The *in vivo* generation of $FOXP3^+$ Treg cells was analysed in spleen by flow cytometry.

Results: HmoDCs generated in the presence of Ca10 express higher levels of PD-L1, ICOSL and CD14 than hmoDCs generated in absence of Ca10. After LPS stimulation, hmoDCs differentiated with Ca10 produce lower levels of pro-inflammatory cytokines and IL-10 than conventional hmoDCs. Conventional hmoDCs activated with Ca10 express PD-L1 at the protein level and produce IL-10. The expression of different tolerogenic molecules, such as PD-L1, ICOSL, IDO, SOCS1 and SOCS3, in Ca10-stimulated hmoDCs was analysed at the mRNA level. HmoDCs activated with Ca10 induce functional $CD4^+CD25^{high}CD127^-FOXP3^+$ Treg. In mice bearing ET, the levels of Ca10 in serum significantly positively correlate with tumor growth. The percentage of $CD4^+CD25^{high}FOXP3^+$ Treg cells increases in spleen from mice with ET.

Conclusion: Our results suggest that Ca10 might well favour the induction of tumor tolerance by generating Treg cells. These findings might contribute to develop novel cancer immunotherapy strategies in future.

SUNDAY, 27 MAY 2018

OAS 06

INNATE IMMUNE RESPONSE IN ASTHMA

0029 | Asthma and unified airways disease through epithelial cells

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Background: Emerging evidence suggests that the upper and lower airway are unified and that under disease settings, a pathological process in one region of the airway would affect the function of the entire airway. However, direct evidence supporting this is extremely limited and only inferred by the fact that the respiratory tract is continuous lined with epithelial cells and clinical observations of improved outcomes for lower airway disease following management of upper airway disease.

Method: Here, we directly tested this hypothesis by performing RNA-sequencing on predominantly matched nasal and bronchial epithelial brushings from 63 children with or without atopy or asthma. We then used a combination of differential gene expression, and gene co-expression analyses to determine similarity of the transcriptional landscape between the upper and lower airway.

Results: Overall, we report ~50% homology and ~50% divergence between the two sites, independent of disease phenotype and atopy. We identified sixteen modules of co-expressed genes (enriched for specific biological functions) to be conserved across nasal and bronchial epithelium. However, almost half of these were differentially expressed between the two regions.

Conclusion: Our findings suggest that in part the upper and lower airway do share a similar transcriptional composition, but also exhibit significant differences that is reflective of their region-specific functions. With significant interest in biomarker development, our data suggests that in certain settings nasal epithelial cells, may inform on lower airway disease and thus has considerable clinical implications.

0030 | Impaired conventional dendritic cell migration in absence of IL-17 in a model of experimental allergic asthma correlates with attenuated airway hyperresponsiveness and airway inflammation

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Background: Several studies suggest an involvement of Th17 cells and related cytokines in the pathology of allergic asthma. However, cellular mechanisms involved in induction, maintenance and pathophysiology of asthma are multifaceted and the exact manner in which IL-17 plays a role is still open.

Objective: Our aim was to decipher the cellular mechanisms involved in IL-17 dependent modulation of allergic asthma.

Method: WT, IL-17 $AF^{-/-}$ and IL-$17F^{-/-}$ mice were subjected to a murine experimental asthma model in which Ovalbumin was systemically and intranasally applied. Airway hyperresponsiveness, lung inflammation, antigen-specific IgG/IgE levels, cytokine levels, and dendritic cell (DC) trafficking was assessed. In Wt mice, anti-IL-17A specific monoclonal antibodies (mAbs) were used to neutralize IL-17A.

Results: In a model of experimental allergic asthma, we show that, influx of dendritic cells into the lungs and lung draining lymph nodes is reduced in IL-17A and IL-17F double knockout animals. These reductions correlated not only with impaired antigen specific responses in draining lymph nodes but also, with attenuated airway hyperresponsiveness, eosinophilic airway inflammation, mucus hypersecretion, reduction in serum immunoglobulin E levels as well as T helper 2 cells associated cytokine secretion by lung draining lymph nodes cells on re-stimulation in absence of IL-17. In-depth analysis of transcriptomic patterns of dendritic cells from lung draining lymph nodes revealed that absence of IL-17 led to attenuation of migration driving genes, expression of chemokines and cytokines involved in migration as well as antigen presentation and co-stimulation.

Conclusion: Thus, we hereby report that IL-17 influences migratory, co-stimulatory and antigen presentation capabilities of dendritic cells in lung draining lymph nodes and thereby, influences induction of murine experimental allergic asthma.

0031 | Analyzing miR-155 and receptor expression in human ILC2s and Th2 cells using a novel method: PrimeFlow miRNA

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Background: Type 2 innate lymphoid cells (ILC2) and T helper cells (Th2) have been implicated in chronic inflammation in asthma. Two important mediators in this disease are IL-33 and prostaglandin D2 (PGD2) which act as ligands for the receptors ST2 and CRTH2, respectively. Furthermore, both ST2 and CRTH2 can be expressed by ILC2s and Th2 cells. In addition, short non-coding microRNAs, such as miR-155, have been shown to be potent immune regulators through post-transcriptional gene regulation.

Aim: Our aim was to determine if miR-155 co-localized with the expression of ST2 and CRTH2 in blood derived ILC2s and Th2 cells obtained from asthmatics with or without recurrent viral exacerbations using a novel method, PrimeFlow miRNA analysis.

Method: Participants selected from an epidemiological cohort (West Sweden Asthma Study) were divided into three groups: Healthy volunteers (HV), asthmatics reporting recurrent viral exacerbation (VA) and asthmatics not reporting any recurrent viral exacerbations (NVA). Peripheral blood mononuclear cells (PBMCs) were subjected to five conditions: no stimulation, IL-33, PGD2, IL-33 + PGD2 and poly (I:C), a virus mimic. miR-155, ST2 and CRTH2 expression in ILCs and Th cells were analyzed by flow cytometry. Additionally, miR-155 was analysed in total PBMCs using qPCR.

Results: Analyzing miR-155 expression in PBMCs by qPCR, poly (I:C) demonstrated the highest increase of all conditions. Using Primeflow miRNA, poly (I:C) induced a clear upregulation of miR-155$^+$Th cells and miR-155$^+$ILCs in the VA group. This group also showed increased numbers of CRTH2$^+$miR-155$^+$Th cells when exposed to PGD2. ILCs from HV and VA groups responded with increased numbers of ST2$^+$miR-155$^+$ cells upon IL-33 + PGD2 stimulation in contrast to NVA group that demonstrated decreased numbers. Additionally, IL-33 and PGD2 respectively, also increased ST2$^+$miR-155$^+$ILCs in the VA group, but not to the same degree.

Conclusion: The PrimeFlow miRNA method enables analysis of miRNA in rare cell populations without prior cell sorting. Our results indicate an enhanced response in Th cells and especially ILCs to IL-33, PGD2, the combination of IL-33 + PGD2 as well as the virus mimic poly (I:C) in the VA subjects compared to both NVA and HV subjects. This data may suggest that circulating Th2 cells and ILC2s are more potent effector cells in asthmatics with recurrent viral exacerbation compared to healthy subjects or those asthmatics that report non-virus induced exacerbations.

0032 | The link between endoplasmic reticulum and mitochondria contributes to inflammasome-associated neutrophilic severe asthma in mice

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Background: Each asthma phenotype may have distinct observable molecular, cellular, morphological, functional, and clinical features, all of which can be possibly integrated into specific biological mechanisms, called as endotypes. Recently, one of new molecular phenotype of severe asthma has been reported as inflammasome-associated severe asthma.

Method: In this study, we investigated which mechanisms contribute to NLRP3 inflammasome activation in neutrophilic severe asthma focusing on the mechanical and functional link between endoplasmic reticulum (ER) and mitochondria using the neutrophilic dominant asthma murine model sensitized with OVA and LPS and then challenged with OVA (OVA_{LPS}-OVA mice).

Results: The OVA_{LPS}-OVA mice showed the typical features of neutrophilic asthma. Interestingly, confocal analysis and electron-microscopic findings revealed that in lung cells from OVA_{LPS}-OVA mice, the ER and mitochondria get closed each other even seemed to be united one compared to the finding of cells from control mice. An ER stress inhibitor, mitochondrial ROS inhibitor, or MCC950 significantly reduced the increases in inflammatory cytokines, mitochondrial ROS generation, NLRP3 inflammasome activation, airway inflammation, and bronchial hyper-responsiveness. Interestingly, the treatment restored the physical changes and distances of ER and mitochondria near normally.

Conclusion: These findings indicate that the development of ER-mitochondria complex in airway inflammatory cells may be implicated in the pathogenesis of neutrophilic bronchial asthma through NLRP3 inflammasome activation, providing the novel therapeutic target for bronchial asthma.

0033 | Dermatophagoides farinae extract reprograms the eicosanoid profile of human macrophages in vitro

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Background: Asthma is a complex disease influenced by genetic predisposition and environmental challenges. The ubiquitous indoor allergen Dermatophagoides (house dust mite, HDM) increases the risk for asthma development when sensitization occurs in the first three years of life. Alveolar macrophages persist in the lung for lengthy periods and sense inhalable particulate matter via pattern recognition receptors. HDM has been shown to elicit the production of proinflammatory cytokines TNF and IL6 in murine alveolar macrophages thus contributing to the pulmonary inflammatory response. Eicosanoids derived from arachidonic acid via 5-lipoxygenase (5LO) and cyclooxygenase-2 (COX2) are potent mediators and can be markedly dysregulated in asthma, especially in severe endotypes. Whether HDM contributes to the dysregulation of the eicosanoid profile of human macrophages remains unexplored.

Method: $CD14^+$ cells sorted from PBMC of healthy human donors were differentiated to alveolar-like macrophages for 6 days and stimulated with HDM extract for 24 h. Supernatants were analyzed via liquid chromatography-tandem mass spectrometry (LC-MS/MS) and cytokine multiplex assay. Cell lysates were subjected to western blot or RNA extraction and quantitative real-time PCR.

Results: 24 hour HDM exposure diminished macrophage 5LO expression and leukotriene (LT) B_4 and cysteinyl leukotriene secretion while inducing COX2 expression and prostaglandin (PG) E_2, D_2, $F_2\alpha$ and thromboxane (TX) B_2 (TXA_2 metabolite) output. Production of inflammatory cytokines IL6, IL8 and TNF was increased. P38 MAPK was phosphorylated in macrophages stimulated with HDM while inhibition of Dectin-2 with a blocking antibody did not affect transcript levels of COX2, 5LO, TNF and IL6.

Conclusion: Human macrophages responded to HDM *in vitro* with p38 activation and increased secretion of pro-inflammatory cytokines as previously shown for murine macrophages. The eicosanoid profile shifted from 5LO to COX2 resulting in enhanced prostanoid levels. In the lung, the marked increase of PGE_2 may have an anti-inflammatory and immunosuppressive effect as PGE_2 has been shown to suppress granulocyte activation and macrophage effector functions, resulting e.g. in impaired phagocytosis. On the other hand the induction of $PGF_2\alpha$, PGD_2 and TXB_2 contributes to airway inflammation and bronchoconstriction. Thus, exposure to HDM can profoundly reprogram the mediator profile in human macrophages, which may contribute to chronic airway inflammation.

0034 | Airway epithelial phosphoinositide 3-kinase delta regulates fungi-induced innate immune response

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Background: Respiratory fungal exposure is known to be associated with severe allergic lung inflammation. Airway epithelium is an essential controller of allergic inflammatory process. An innate immune recognition receptor, NLRP3 inflammasome, and phosphoinositide 3-kinase (PI3K)-δ in airway epithelium are critically involved in various inflammatory processes in lung. We investigated the role of NLRP3 inflammasome in fungi-induced allergic lung inflammation and examined the regulatory mechanism of NLRP3 inflammasome assembly/activation focusing on PI3K- δ isoform in airway epithelium.

Method: We utilized two *in vivo* models including PI3K-d knockout mice induced by exposure to *Aspergillus fumigatus* (*Af*) and *Alternaria alternata* (*Aa*) as well as *Af*-exposed *in vitro* experimental system. We also checked expression of NLRP3 protein in lung tissues from allergic bronchopulmonary aspergillosis (ABPA) patients.

Results: Assembly/activation of NLRP3 inflammasome was remarkably increased in the lung of *Af*-sensitized/challenged mice. Elevation of NLRP3 inflammasome assembly/activation was also observed in *Af*-stimulated epithelial cells. Similarly, pulmonary expression of NLRP3 in patients with ABPA was increased compared to that in healthy subjects. Importantly, neutralization of NLRP3 inflammasome-derived IL-1β alleviated various pathophysiologic features of *Af*-induced allergic inflammation. Furthermore, blockade of PI3K-δ improved *Af*-induced allergic inflammation through modulation of NLRP3 inflammasome assembly/activation, especially in epithelial cells. NLRP3 inflammasome was also implicated in *Aa*-induced eosinophilic allergic inflammation, which was improved by blockade of PI3K-δ.

Conclusion: These findings demonstrate that fungi-induced assembly/activation of NLRP3 inflammasome in airway epithelium may be modulated by PI3K-δ isoform. Inhibition of PI3K-δ may have potential for treating fungi-induced severe allergic lung inflammation in humans.

SUNDAY, 27 MAY 2018
OAS 07
UPDATES IN URTICARIA

0035 | Rationale of autologous serum skin test in acute vs chronic urticaria

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Background: Autologous serum skin test (ASST) is a rapid, in-vivo clinical test to detect functional autoantibodies in patients with chronic idiopathic urticaria (CIU), but its rationale in acute urticaria (AU) is unknown. This study was conducted to evaluate the efficacy of ASST among acute and chronic urticaria patients.

Method: Adult (age≥18 years) patients with a diagnosis of AU (<6 weeks' duration) and CIU were enrolled prospectively in a cross-sectional study. Healthy age- and sex-matched subjects served as controls. Urticaria patients were treatment-naive, thus detailed history and physical examination were recorded for all. ASST, besides B12, total immunoglobulin E (IgE), freeT3 (fT3), freeT4 (fT4), anti-thyroglobulin, and anti-TPO levels in serum were assessed in all subjects.

Results: Of 101 subjects, mean age was 34.35 ± 12.68 years with 58.4% female with no difference between AU (n: 27), CIU (n: 46), and control groups (n: 28). Ratio of positivity in ASST was higher in AU (25.9%), and CIU groups (21.7%) than in control (10.7%, $P = 0.33$ for all). Frequency of B12 deficiency (< 200 pg/mL) was significantly higher in CIU (39.1%) than AU (7.4%) and control (3.6%) ($P = 0.003$ and $P < 0.001$). Ratio of patients with high total IgE levels (>100) in AU (85.2%) and CIU (65.2%) were similar with each other ($P = 0.06$), but significantly higher than control group (10.7%) ($P < 0.001$ and $P < 0.001$). CIU group had significantly higher abnormal thyroid test results (45.7%) than AU (14.8%) and control groups (3.6%) ($P = 0.01$ and $P < 0.001$), whereas patients with clinically diagnosed thyroiditis were only in CIU group (6.5%). Among the possible risk factors for ASST positivity were age, gender, B12 deficiency, high total IgE, abnormal thyroid tests, and thyroiditis were assessed in logistic regression analysis, however there were no relation even analysed separately as AU, CIU and control.

Conclusion: Even tough thyroid tests and serum B12 was found to be related with CIU, and total IgE was associated with acute and chronic urticarial, this study did not show any significant difference between patients with AU and CIU regarding ASST.

0036 | Angioedema with and without wheals in patients with chronic spontaneous urticaria: Findings from the worldwide prospective observational aware study

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Background: Chronic spontaneous urticaria (CSU) patients frequently experience angioedema, with or without wheals. The real-world rate of these subtypes of CSU among patients and the clinical characteristics of these two populations is currently unclear.

Method: Here, we analysed CSU patients with angioedema, with (A^+/W^+) or without wheals (A^+/W^-), from Europe and Central/South America enrolled in the observational AWARE study. CSU patients were aged 18 years or older and refractory to at least one course of H1-antihistamine treatment. The two patient populations were compared on demographics, disease characteristics, disease control (Urticaria Control Test [UCT]; scores below 12 indicate poor control), quality of life impairment (Dermatology Life Quality Index [DLQI] and the Angioedema Quality of Life Questionnaire [AE-QoL]), and their use of health care resources.

Results: Of the 4174 AWARE patients assessed, 1971 (47.2%) had CSU with angioedema, of which 95.9% had A^+/W^+ and 4.1% had A^+/W^-. The two groups were demographically similar, but a slightly larger proportion of A^+/W^- patients were 65 years or older compared to A^+/W^+ patients (19% vs 11%). A^+/W^-patients had somewhat longer disease duration (6.2 vs 5.0 years), longer average duration of angioedema episodes (3.6 vs 2.5 days), and somewhat greater intensity of angioedema (moderate: 46% vs 44%; severe: 31% vs 22%). Disease control (UCT <12: 74% and 79%) and AE-QoL scores (41.9 [19.8] and 45.7 [24.2]) were similar in both populations, but A^+/W^- patients had lower DLQI scores (5.5 [6.2] vs 8.9 [7.4]). Emergency

% (n)	All (n:101)	Acute Urticaria (n:27)	Chronic Idiopathic Urticaria (n:46)	Control Group (n:28)	*P*
Gender, (female)	58.4 (59)	74.1 (20)	50 (23)	57.1 (16)	0.13
Age, year*	34.35 ± 12.68	32.96 ± 12	37.17 ± 15.05	31.07 ± 7.27	0.16
Previous under treatment		–	8.7 (4)	–	–

room visits were less common among A^+/W^- patients (36% vs 42%); visits to a general practitioner were more common (69% vs 58%). Both groups showed similar visit patterns to pharmacies (both 25%), hospitals (30% and 26%), specialised urticaria centres (both 32%), and dermatologists or allergist specialists (58% and 53%).

Conclusion: CSU is associated with a high frequency of angioedema. Such patients with and without wheals are largely similar in most aspects including high rates of poor disease control. Further studies in larger CSU angioedema only patient populations are needed to better understand this CSU subtype.

0038 | Thickener sensitivity in chronic urticaria

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Background: Food additives can trigger chronic urticaria. Thickeners are one of the most common additives used in packaged foods. In this study we aimed to evaluate thickener sensitivity in chronic urticaria patients.

Method: Twenty-four patients followed up for idiopathic chronic urticaria were included to this study. We performed skin prick tests for guar gum (cyamopsis tetragonolobus), carob (ceratonia siliqua), gum arabic (acacia spp), tragacanth (astragalus spp) which are used as thickener and we also evaluated spIgE levels for these additives.

Results: Average of symptom onset ages for 12 girls and 12 boys was 89 ± 53 months. Thirteen patients had perennial symptoms whereas 11 had recurrent attacks (4.5 ± 3.8 attack/year). Only one patient had seasonal symptoms. Family history of atopy was 21.7%. Four of the patients also had allergic rhinitis and two patients had asthma. Half of the patients' aeroallergen-specific IgE levels were positive and 27.3% had eosinophilia. Average aeroallergen-specific IgE and total IgE levels were 0.58 ± 0.5 kUA/l and 477 ± 243 IU/l respectively. In addition to routine treatments for chronic urticaria we performed omalizumab therapy to two patients with an excellent response.

Seven of the patients' symptoms were triggered with packaged food. One patient had (4.2%) multiple thickener sensitivity (guar gum-spIgE: 6.5 kUA/l carob-spIgE: 6.2 kUA/l, gum arabic-spIgE: 5.5 kUA/l, tragacant-spesifikIgE: 31.7 kUA/l) and one patient had minimal tragacanth sensitivity (tragacant-spIgE: 0.39 kUA/l). Their skin prick tests with these thickeners were also positive. The patient with multiple sensitivity had symptoms especially with chocolate (containing guar gum and gum arabic) and chips (containing tragacanth).

Conclusion: In conclusion thickener sensitivity must be questioned as a responsible agent in patients with idiopathic chronic urticaria.

0039 | Relevance of clinical and laboratory predictors in chronic spontaneous urticaria

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Background: Chronic spontaneous urticaria (CSU) is a common skin disorder in which approximately half of these patients are characterized by an auto reactive pathogenic state. 5%-50% of CSU patients may not reach controlled condition with antihistamines and these refractory patients require other treatment modalities beyond antihistamines[1].

Therefore, the need for reliable and safe parameters to link the pathogenesis and disease severity with reasonable therapeutic approaches has increased. Few studies have investigated the role of different parameters as predictor tools to evaluate disease severity of CSU patients.

Method: We assessed total severity score (TSS), ASST, total IgE, anti-thyroid antibodies basophil CD203c expression and BAT-CD203c using a two color flow cytometric method in 40 CSU patients and 40 normal controls.

Results: Our logistic regression analysis indicated that both BAT-CD203c and ASST were significant predictors of clinical severity of CSU ($P = 0.012$ and $P = 0.042$, respectively.

Conclusion: BAT-CD203c and ASST can be used as a potential predictor of clinical severity of CSU.

0040 | Clinical characteristics of patients with autoimmune chronic spontaneous urticaria and predictors of positivity of basophil activation test

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Background: Autoimmune urticaria (AIU) is described as having more severe symptoms, longer disease duration and lower responsiveness to therapy than non-AIU. However, in vitro tests to diagnose AIU are time consuming, expensive and require equipment not available in every centre.

Objectives: Clinical characterization of AIU and non-AIU patients (pts) and identification of clinical characteristics to predict a positive basophil activation test (BAT) result.

Method: Prospective study of 40 chronic spontaneous urticaria (CSU) pts evaluated in the Immunoallergology Department, according

to: demographic data, angioedema, nocturnal symptoms, autoimmune diseases, urticaria duration, hives duration, UAS7, DLQI and UCT scores, autologous serum skin test (ASST), anti-TG and anti-TPO antibodies, thyroid disease, serum total IgE, therapy for CSU control and BAT. AIU was defined as: CSU pts with positive BAT (BATp). Statistical analysis (SPSS V.23): descriptive statistics, chi-square, kappa correlation coefficient, odds ratio, Student test, binary logistic regression, ROC curve.

Results: 21 pts had negative BAT (BATn) and 19 BATp. BATp pts had higher number of positive ASST (74% VS 19%; $P < 0.05$; OR 11.9), angioedema (79% VS 43%; $P < 0.05$; OR 5), night symptoms (89% VS 52%; $P < 0.05$; OR 7.7), symptoms >5 days/week (95% VS 57%; $P < 0.05$; OR 13.5), Anti-Tg or TPO antibodies (53% vs 19%; $P < 0.05$; OR 4.4), UAS7 (24.0 ± 9.1 VS 14.1 ± 10.6; $P < 0.05$; OR 8.7), DLQI (9.9 ± 7.0 vs 5.8 ± 5.7; $P = 0.14$; OR 2.6) and lower serum total IgE (53.2 ± 67.2 vs 437.5 ± 975.9; $P = 0.11$) and UCT (7.4 ± 4.3 vs 9.6 ± 4.1; $P = 0.86$; OR 0.43). UAS7 > 16 had the highest negative predictive value for BATp (81.3%) followed by positive ASST (77.3%). Combined positive ASST and presence of angioedema had a sensitivity of 52.6% and specificity of 95.2% for BATp. Anti-Tg or TPO antibodies combined with positive ASST had a specificity of 100% for BATp. 13 (61%) BATn pts controlled their urticaria with <4 tablets of nonsedating antihistamines VS 9 (47%) of BATp. One (5%) BATn was proposed for omalizumab VS 6 (32%) of BATp. ROC curve (area under curve 0.897) shows that the characteristics ASST, angioedema, nocturnal symptoms, symptoms >5 days/week, Anti-Tg or TPO and UAS7 > 16 have a good discriminant power at identifying a positive BAT result.

Conclusion: ASST as routine test in association with in vitro thyroid tests and anamnestic/clinical data increases the likelihood of correctly diagnosing AIU. AIU diagnosis is important, as it has more severe CSU symptoms and is less responsive to therapy.

SUNDAY, 27 MAY 2018

OAS 08

ALLERGIC CONJUNCTIVITIS DIAGNOSIS AND MANAGEMENT

0041 | Topical cyclosporine A 1 mg/mL cationic emulsion improves in signs and symptoms of active severe vernal keratoconjunctivitis (VKC) in pediatric patients: Results of the phase III VEKTIS study

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Background: Cyclosporine A cationic emulsion (CsA CE) for topical ocular use is an oil-in-water emulsion that remains longer on the ocular surface, thus optimizing its therapeutic effects. The VEKTIS study, a phase III, multicenter, double-masked, vehicle-controlled trial, evaluated the efficacy/tolerability of CsA CE 1 mg/mL eye drops for treating severe vernal keratoconjunctivitis (VKC) in pediatric patients.

Method: 169 patients (4-18 years of age) with severe VKC and severe keratitis were randomized to 4 months treatment with 1 of 2 active doses of CsA CE 1 mg/mL (high-dose [4x/day] or low-dose [CsA CE 2x/day+vehicle 2x/day]) or vehicle alone, followed by an 8-months safety follow-up period when patients were re-randomized to 1 of the 2 active treatment arms. The primary endpoint (PE) was a mean composite score that reflected corneal fluorescein staining (CFS), need for rescue medication, and occurrence of corneal ulceration over the first 4 months. Patients were defined as responders at Month 4 if the mean CFS score of the last 3 months of treatment was ≤50% of Baseline, no withdraw, experience ulceration, or use rescue medication over the last 3 months.

Results: The PE was met; the difference in least-squares mean vs vehicle was significant for both the high-dose (0.76, $P = 0.007$) and low-dose groups (0.67, $P = 0.010$). CFS score improvements were the main driver, accounting for 70%, 78% of the treatment effect in the CsA CE high-dose and low-dose groups, respectively; reduced rescue medication use accounted for most of the rest (30% and 22%, respectively). There was no difference in mean number of corneal ulcer occurrences/month across treatment groups (0.001 for QID, 0.003 for BID; $P = 0.996$ for both vs vehicle). The efficacy responder rate was significantly higher in the high-dose (57.1%) and low-dose (61.1%), vs the vehicle group (34.5%) ($P = 0.015$ and $P = 0.004$, respectively). CFS reductions were stable over 8 months follow-up. CsA CE was well tolerated.

Conclusion: In the phase III VEKTIS study, treatment with CsA CE 0.01% (1 mg/mL) yielded substantial improvements in signs of severe VKC in a pediatric patients population over 12 months.

0042 | Correlations between clinical scales and the specific quality of life (QUICK) questionnaire in children with vernal keratoconjunctivitis

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Background: Vernal keratoconjunctivitis (VKC) is a severe inflammatory disease that appears in children and adolescents with seasonal recurrences. Severe signs and symptoms are associated to a reduced quality of life (QoL). A questionnaire for the evaluation of the quality of life in children with allergic keratoconjunctivitis (QUICK) has been proposed in clinical practice to evaluate the self reported QoL in these patients. The aim of the present study was to assess the correlation of the subjective results of the QUICK with signs and symptoms scores, and with the scoring systems for the assessment of the corneal epithelial damage.

Method: 26 active VKC consecutive patients were included (baseline) and evaluated by the QUICK questionnaire, signs and symptoms scoring scales (0-3), and corneal fluorescein staining (CFS) using the Oxford and the new VKC-CLEK scoring systems. The QUICK is a 16-items questionnaire (score 0-3) divided into 2-domains: symptoms (12 items) and activities (4 items). Patients were re-evaluated using the same parameters after 1 month (M1) of treatment. The scores from the worst eye were considered for the correlation between QUICK and signs and symptoms.

Results: At baseline, the 26 patients had an average score of CFS of 1.42 ± 1.6 (Oxford scale) and 3.0 ± 2.5 (VKC-CLEK). Concerning symptoms scores, the worst was photophobia (1.69 ± 1.2) and the better was pain (0.46 ± 0.9). QUICK dimensions were scored relatively high with a mean score for symptom of 69.6 ± 8.2 (on 100) and activities of 49 ± 9.7 (on 100). Oxford scale and VKC-CLEK scales were highly correlated ($r = 0.86$; $P < 0.0001$) and significantly correlated with photophobia, burning and tearing. The 2 staining scales were also correlated with QUICK dimensions. QUICK symptoms and activities were also significantly correlated with some of the single signs and symptoms scores.

Conclusion: QoL evaluated by a specific self-reporting questionnaire (QUICK) were correlated with signs and symptoms of the disease evaluated at the patient visits. Information on health-related QoL might be important for the choice of treatment in the current clinical practice.

0043 | Conjunctival allergen provocation tests may influence diagnosis and clinical decision

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Background: Allergen sensitization doesn't always correlate with clinical symptoms. Conjunctival allergen provocation test (CPT) can be used to further evaluate the association between symptoms and exposure.

Objective: To assess the clinical utility and safety of CPT with house dust mites and grass pollen in patients with rhinoconjunctivitis, and to correlate its results with skin prick tests (SPT) and specific IgE (sIgE).

Method: Patients with seasonal or perennial rhinoconjunctivitis from an allergy department of an University Hospital, with discrepancies between clinical history and allergen sensitization or dubious cases to decide immunotherapy, were referred to perform CPT. CPT was performed with Leti®, allergen extracts, according to the manufacturer recommendations: used only for 24 hours after preparation and performed at increasing concentrations (1/1000, 1/100, 1/10 and 1/1 HEP) until reaching the highest dose or eliciting a positive reaction. Total ocular symptom score (TOSS) was assessed and a positive result was considered if TOSS $\geq$ 5. Eighty patients, mean age 19 years, 63% female, were recruited from July 2016 to December 2017, of those 17 performed CPT to *D. pteronyssinus (Dpt)*, 16 to grass pollen and 47 to both.

Results: Of the 64 patients who underwent CPT to *Dpt*, 57 had positive challenge (median TOSS of 6 with interquartile range [5;6]). Grass pollen CPT was positive in 45 patients (TOSS 5[4;6]),. Anti-histamine was given to 9 patients due to nasal symptoms; ophthalmic corticosteroids were used in 14 cases. No severe or late phase reactions occurred. There was no correlation between concentration to elicit a positive challenge and sIgE, sIgE/total IgE and wheal size to *Dpt*. Grass pollen sIgE, sIgE/total IgE and wheal size correlated with the concentration to elicit a positive challenge (r = 0.434, P = 0.01; r = 0.327, P = 0.05; r = 0.514, P = 0.01, respectively). Considering CPT as the gold standard, sensitization measured by sIgE or skin tests demonstrated a sensitivity to *Dpt* of 94%, with a specificity of 43%; for grass pollen sensitivity was 96% and specificity 33%. The CPT´s influenced the decision to start or not immunotherapy in 40% of the patients.

Conclusion: CPT is currently underused in daily clinical practice. In our sample, it showed an additional diagnostic value, particularly in uncertain clinical situations and influenced allergen immunotherapy treatment decision.

0044 | Utility of the conjunctival allergen provocation test (capt) in patients with local allergy rhinitis (ral)

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Background: Ocular symptoms as comorbidities are often found in LAR (Local Allergic Rhinitis) patients. The existence of an ocular local allergic response has been demonstrated through specific conjunctival provocation tests and determination of sIgE in ocular secretions. Previous researches have reported a lack of correlation between serum sIgE and ocular allergy, suggesting that the sIgE detected in the eye is most likely produced locally. We try to determine the utility of the Conjunctival Allergen Provocation Test (CAPT) with allergens in patients with ocular symptoms in patients with Local Allergy Rhinitis, but with negative response to nasal multiple allergen provocation test.

Method: A prospective observational study that included 20 cases with signs and symptoms of perennial local allergic rhinitis and conjunctivitis with negative results to skin prick tests (SPT), specific IgE measurement and nasal allergen provocation test to common perennial allergen *Dermatophagoides pteronyssinus (D. PT)*. In addition, two control group, one positive control of 3 patients with signs and symptoms of the disease and CAPT positive to that allergen and a negative control of 3 patients without signs or symptoms of allergic conjunctivitis. The CAPT was performed with this allergen (D.PT) studying the conjunctival redness by photography and recording the total ocular symptoms score (TOSS) (range: 0–13 and positive over a cumulative score of 5).

Results: Of the 20 patients analyzed, 2 were positive for the CAPT. The mean age of the two CAPT positives was lower than those with TPC negative (P < 0.006), as well as the age of onset of symptoms which was earlier in the CAPT positive group, but without statistically significant differences. The analogical visual scale (AVE) questionnaire greater than 5 was obtained in 100% of patients with positive CAPT.

Conclusion: In a population with a very suspicious clinical history of perennial rhinitis and conjunctivitis, a CAPT may offers a new diagnostic opportunity to discover the sensitization, when other standard diagnostic tests have not given any positive results.

0045 | Comparison of symptoms during a conjunctival provocation test (CPT) and a controlled exposure to birch pollen in the Strasbourg Environmental Exposure Chamber (EEC) (ALYATEC)

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Background: As recommended by the task force (Pfaar O *et al*. Allergy 2017) we compared the results obtained during allergen exposure in EEC with the reference conjunctival provocation test. The aim of this study is to compare clinical scores obtained during the CPT and in the EEC.

Method: 16 patients with an allergic conjunctivitis to birch pollen were selected. They had a positive CPT to birch pollen. They were exposed on 2 consecutive days to nebulized birch pollen in the EEC. Abelson score were performed before and every ten minutes during the 240 minutes of exposure. Challenges were considered positive when Abelson score ≥5.

Results: Among 16 positive CPT, 12 subjects had a positive challenge in the EEC. The mean Abelson score was 6.2 with CPT and 5.8 on day 1 and 5.5 on day 2. A positive response was faster obtained with the CPT (36 ± 15 minutes) compared to EEC (92 ± 15 minutes) (P = 0.0001). The mean cumulative amount of Bet v1 inducing a positive CPT was 8759.5 ng ±7000 vs 0.2 ng ± 0.12 in the EEC (P = 0.0001).

Conclusion: 75% of positive CPT was also positive in EEC. There was a difference in amount of Bet v1 responsible of positive response in CPT and in EEC. The amount in the EEC is closer to the natural exposure (20 to 60 pollen grains) than the individual CPT. ALYATEC's EEC is a good tool to assess anti-conjunctival drugs.

0046 | Repeatability of the ocular response in patients with allergic rhinoconjunctivitis with repeated naturalistic allergen challenge in the environmental exposure chamber

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Background: Environmental exposure chambers (EECs) are used in allergic rhinitis models to assess clinical nasal and ocular symptom responses while circumventing the variability observed in outpatient field studies. In the present study, we evaluated the repeatability of total ocular symptom scores (TOSS) during repeated naturalistic exposure to ragweed allergen during separate EEC visits.

Method: Seventy-eight subjects with ragweed induced allergic rhinitis were exposed to airborne ragweed pollen in the EEC for 2 hours at an interval of 13 (+5) days between visits. During the EEC visits, subjects recorded their total ocular symptoms (sum of itchy eyes, watery eyes, and eye redness scored on a scale of 0 to 3) using an electronic diary (ePDAT®) prior to EEC entry and every 30 minutes throughout. The repeatability of ocular symptom response for each patient was assessed by Pearson correlation analysis and utilizing Bland-Altman approach.

Results: The mean TOSS levels at both visits 1 and 2 increased and peaked at similar levels with average maximum TOSS scores (1st visit = 5.15 ± 0.0.22; 2nd visit = 4.96 ± 0.23) and consistent ragweed pollen levels observed throughout the EEC visits. A highly significant correlation was shown of the overall average TOSS over time between visits 1 and 2 (r = 0.9941, P = 0.0005). When evaluating the average TOSS of each subject over 2 separate EEC visits, our data showed a correlation coefficient of r = 0.5720, $P < 0.0001$. In addition, a Bland-Altman plot had a mean difference of 0.4915 and 95% limits of agreement between –2.652 and 3.635 indicating a good agreement between the two visits.

Conclusion: The ability of naturalistic yet controlled allergen challenge in the EEC to elicit repeatable ocular symptoms at clinically significant levels in patients with allergic rhinoconjunctivitis suggests this a good approach to evaluate ocular treatments and gain insight toward their real-world efficacy.

SUNDAY, 27 MAY 2018
OAS 09
BIOLOGICALS: TARGETING TYPE 2 RESPONSES

0047 | Patient profile analysis (clusters definition) among respondent patients to omalizumab: Fenoma study

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Background: FENOMA study was performed among responders to omalizumab (OMA) in real world practice. In order to identify profiles of severe asthmatics responders to OMA, a cluster analysis was performed.

Method: A total of 345 patients diagnosed with severe asthma, responders to OMA therapy coming from FENOMA study were included. Patient profiling was conducted using partitioning-base clustering, an unsupervised, descriptive and summarizing way to obtain patients categorisation. Variables choice for clustering was done according to clinical criteria. Only variables shown on the total study population were included for data analysis.

Results: From the total starting population, 74.2% was considered for cluster analysis (n = 256). Two main response groups were identified: Cluster A (A) 141 patients, and Cluster B (B) 96 patients. Patients included in A had a mean age (SD) of 55.0 (12.5) years, being (75.2% female), while patients included in B had 40.2 (15.1) years (56.3% female). Family history of atopy was present in 27.7% of A patients and 46.9% in B. Diurnal symptoms for >2 days/week were shown in 14.9% of A patients (daily 58.2% and continuous 27.0%), and 51.0% in B (daily 37.5% and continuous 6.3%). Rescue medication was needed >2 days/week in 24.1% of A patients vs 51.0% (B), every day (52.5% vs 40.6%, respectively) and several times per day (22.7% vs 3.1%, respectively). Forced Expiratory Volume (FEV1) ≤80% was shown in 86.5% of A patients vs 42.7% in B. Previous to OMA initiation, patients included in A had a mean of 3.6 (2.5) exacerbations and 3.2 (2.7) visits to the emergency room. Positive skin tests were found in 68.1% patients and mean IgE value was 408.2 UI/mL (539.8). Peripheral blood eosinophilia was >5% of total blood count for 81.6% in group A. Considering B patients, 2.9 (2.2) exacerbations and 2.3 (5.3) emergency visits were reported. Positive skin tests were identified in 89.6% patients and mean IgE value was 620.0 UI/mL (644.0). Peripheral blood eosinophilia was >5% for 83.3% in group B.

Conclusion: Two main OMA responder patient profiles were identified: (A) women, mostly with lung function deterioration, frequent exacerbations and corticosteroids dependence, and (B) young population with better lung function and mostly positive results in allergy tests.

0048 | Response to omalizumab in urticaria & asthma; Is it 'a life-time together' treatment?

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Background: Omalizumab is a recombinant human-monoclonal-antibody that blocks IgE receptor. It is indicated in severe-asthma (SA) and chronic-idiopathic-urticaria (CIU). The aim of this study was to demonstrate how omalizumab differently effects SA and CIU.

Method: Follow-up data of 49 patients who were treated with omalizumab between march 2014-june 2017 were reevaluated one year apart. In SA, omalizumab were given according to total IgE/wt, whereas in CIU 300 mg/month.

Results: Most of the treated patients had CIU (n:38), whereas 23.1% (n:11) had SA, which has almost doubled compared to a year ago (20 and 6 patients, respectively). Although duration of CIU was similar, duration of the disease in asthmatics was significantly less a year later (25.33 ± 10.38 yr and 16.9 ± 3.81 yr). Compliance was significantly better in both groups after a year later, despite duration of omalizumab treatment wasn't much different. Almost half of CIU patients showed complete remission in both evaluation, complete remission observed in SA patients (18.2%) was statistically significant compared to last year (zero, $P < 0.05$). Treatment failure, as well as dropped to ~half in SA, but no change was observed in CIU. Likewise, recurrence rate following discontinuation of omalizumab was almost half in CIU (43.8% to 23.7%), but improved dramatically in SA (83.3% to 18.2% after one year, $P < 0.01$), which shows the power of compliance. No variables were related with remission/treatment failure/recurrence.

Conclusion: Omalizumab appears to be more effective in CIU in first year of treatment, and SA seems to equally improve in second year. However, as omalizumab treatment continues, not only the treatment failure, and the remission of the disease improves, but also –and maybe more importantly- recurrence vanishes in SA.

0049 | Real life treatment of cholinergic urticaria with omalizumab

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Background: Cholinergic urticaria (CholU), a frequent type of chronic inducible urticaria, presents with small itchy wheals upon

physical exercise or passive warming. Omalizumab has been licensed and shown to be very effective in patients with chronic spontaneous urticaria. Whether or not omalizumab is also effective in CholU is largely unknown.

Aim: To assess the effectiveness of omalizumab treatment in CholU.

Method: We retrospectively investigated the response of CholU patients upon Omalizumab treatment and analysed their response in correlation with the dosing and duration of the treatment as well as with clinical features of the patients.

Results: Of 16 CholU patients treated with omalizumab, 11 (69%) reported a major or complete response at their final dosing interval and drug dose, 2 patients reported a minor effect (12 %) and 3 patients (19%) showed no benefit. Treatment effects were linked to patient gender, with better responses in female patients, but not patient age, age at onset of disease, duration of disease, or total IgE serum levels. Omalizumab updosing led to a complete response in 4 of 6 patients, who did not achieve controlled disease on standard dosed omalizumab therapy.

Conclusion: Omalizumab treatment is effective in the majority of CholU patients, especially in female patients. Most non-responders to standard doses benefit from updosing of omalizumab. Our findings call for controlled clinical trials of omalizumab in CholU.

0050 | Dupilumab improves health related quality of life in uncontrolled, moderate-to-severe asthma patients with comorbid allergic rhinitis from the phase 3 LIBERTY ASTHMA QUEST study

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Background: Allergic rhinitis (AR) is a frequent type 2 comorbidity in asthma, associated with worse asthma-specific outcomes such as asthma control, symptoms, and health-related quality of life (HRQoL). Dupilumab (DPL), a fully human anti-interleukin (IL)-4R-α monoclonal antibody that inhibits IL-4 and IL-13, key drivers of type 2 inflammation, is approved in the EU, USA, and other countries for treatment of adults with inadequately controlled moderate-to-severe atopic dermatitis (AD). In a double-blind, placebo (PBO)-controlled phase 3 study (NCT02414854), asthmatics aged ≥12 years, without a minimum baseline eosinophil requirement, uncontrolled with medium-to-high-dose inhaled corticosteroids plus up to two additional

	PBO (N = 194)	DPL 200 mg q2w (N = 390)	Difference vs PBO (95% CI)	P value vs PBO	PBO (N = 214)	DPL 300 mg q2w (N = 409)	Difference vs PBO (95% CI)	P value vs PBO
Baseline RQLQ total score, mean (SD)	1.95 (1.02)	2.01 (1.16)			1.95 (1.20)	1.90 (1.12)		
Change from baseline in RQLQ total score at Week 12, LS mean (SE); N	−0.49 (0.07); 167	−0.67 (0.05); 332	−0.17 (−0.33, −0.02)	0.0286	−0.46 (0.06); 192	−0.68 (0.05); 348	−0.22 (−0.37, −0.08)	0.0030
Change from baseline in RQLQ total score at Week 24, LS mean (SE); N	−0.52 (0.07); 164	−0.71 (0.05); 332	−0.20 (−0.36, −0.03)	0.0200	−0.53 (0.07); 182	−0.62 (0.05); 342	−0.09 (−0.25, 0.07)	0.2530
Change from baseline in RQLQ total score at Week 52, LS mean (SE); N	−0.42 (0.08); 129	−0.84 (0.05); 263	−0.42 (−0.61, −0.24)	<0.0001	−0.45 (0.07); 149	−0.83 (0.05); 274	−0.39 (−0.56, −0.21)	<0.0001

CI, confidence interval; LS, least-squares; SD, standard deviation; SE, standard error.

controllers, received add-on DPL 200/300 mg or matched PBO every 2 weeks (q2w) for 52 weeks. For the overall intent-to-treat population, both DPL regimens significantly reduced annualized severe exacerbation rates during the 52-week treatment period, improved pre-bronchodilator forced expiratory volume in 1 second (FEV_1) at Week 12, improved asthma symptoms/HRQoL measures, and were generally well tolerated. This pre-specified analysis assessed the effect of DPL over time on AR-specific HRQoL.

Method: Patients with self-reported medical history of AR completed the validated standardized Rhinoconjunctivitis Quality Of Life Questionnaire (RQLQ) at Weeks 12, 24, and 52.

Results: 63.5% (n = 1207) reported medical history of comorbid AR. DPL 200 mg q2w vs PBO showed significant and sustained improvements in total RQLQ scores ($P < 0.05$) (Table). DPL 300 mg q2w vs PBO also showed improvements over time that had statistical significance at Weeks 12 and 52 ($P < 0.01$). The most frequent adverse event (AE) occurring at higher frequency in the DPL-treated groups vs PBO was injection-site reactions (15%/18% vs 5%/10%, respectively). Conjunctivitis AEs were similar between DPL and PBO, in contrast to DPL studies in AD.

Conclusion: Dupilumab significantly improved AR-specific HRQoL in patients with uncontrolled, moderate-to-severe asthma with comorbid AR, and was generally well tolerated.

0051 | Novel therapeutic anti-beta common monoclonal antibody, csl311, affects gene transcriptional pathways contributing to the pathogenesis of chronic rhinosinusitis with nasal polyps

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Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is an inflammatory disease of the nose and paranasal sinuses characterized by eosinophilia, local IgE formation, mucus production and a type 2 inflammatory cytokine profile. Treatment includes comprehensive sinus surgery and oral corticosteroids. Corticosteroids are effective at controlling NPs but cannot be used long term due to side effects; NP recurrences are common. IL-3, GM-CSF and IL-5 are type 2 inflammatory cytokines that signal via a receptor composed of a cytokine-specific a chain and the b_c receptor. They are implicated in CRSwNP pathogenesis and co-ordinate infiltration of lymphoid and myeloid cells, airway mucosal remodeling and mucus hypersecretion. We have identified a therapeutic antibody, CSL311, that is specific for the human $ß_c$ receptor and showed that it is a potent antagonist of IL-3, GM-CSF and IL-5 signaling. Here we utilize a humanized mouse model of NP to assess efficacy of CSL311 compared to standard of care, prednisolone, and dissect the molecular pathways responsible for its efficacy.

Method: *Rag2*$^{-/-}$*Il2rg*$^{-/-}$hIL-3/GM-CSF knock-in mice were implanted with pieces of non-disrupted human NPs. After 1 week mice were injected with CSL311 (5 mg/kg), isotype control (5 mg/kg), prednisolone (1 mg/kg) or saline vehicle (0.9%) weekly for 4 weeks and size of NPs monitored externally. After 5 weeks NPs were excised and examined by histology, flow cytometry and RNA sequencing.

Results: Both CSL311 and prednisolone reduced NP xenograft size. CSL311, but not prednisolone, reduced eosinophil, neutrophil, macrophage and mast cell number in the xenografts. Both agents reduced mucus in polyps; however, this effect was more pronounced in CSL311-treated samples. Ingenuity pathway analysis of differentially expressed gene transcripts shows CSL311 decreases genes associated with granulocyte/monocyte recruitment and function, and identified pathways downstream of GM-CSF, IL-4 and IL-13 as affected by CSL311. Consistent with CSL311 affecting type 2 inflammation the top disease associations with the differentially expressed genes included hypersensitivity and atopic disease.

Conclusion: Administration of CSL311 attenuated growth, mucus production and inflammatory cell infiltrate in NP xenografts and was more effective than prednisolone. Gene transcripts regulated by CSL311 included those involved with type 2 cytokine signaling and myeloid cell function.

0052 | AllergoOncology: An immunologically relevant rodent model demonstrates safety of a tumour-specific IgE therapy

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Background: To-date all monoclonal antibody cancer therapeutics are of one antibody class, IgG. We hypothesized that efficacy may be improved by the development of tumour-antigen specific IgE antibodies due to the superior tissue bioavailability and higher cognate Fc receptor affinity of this antibody class (AllergoOncology). Safety assessment of such novel agents requires the design of biologically-relevant *in vivo* model systems that consider class-specific immunological functions and safety.

Method: Differences in human and murine Fcε receptor expression and structure render the murine system less relevant to human IgE biology, whereas FcεRI expression and cellular distribution in rats mirrors that of humans. Therefore, we designed and implemented a surrogate immunocompetent rat model bearing a syngeneic rat

tumour. Rats were treated with a surrogate rat IgE antibody; engineered with rat Fc regions to provide immunological insights more likely to recapitulate human IgE-FcεR interactions, and equivalent to the human chimeric MOv18 IgE specific for the tumor associated antigen folate receptor alpha.

Results: Tumour growth restriction was detected in immunocompetent tumour-bearing rats treated with rodent MOv18 IgE compared with rats given the corresponding rat MOv18 IgG antibody or buffer control. No clinical, histopathological or systemic metabolic signs of moderate or severe toxicities, nor physiological or immunological evidence of cytokine storm or signs of a type I hypersensitivity reaction were detected. We observed i) marked elevation of immune cell infiltration into the tumour lesions of rats treated with rat MOv18 IgE; ii) activation of immunological pathways in tumour-bearing rat lungs, and iii) elevated serum concentrations of TNFα, a mediator previously associated with IgE-mediated anti-tumour and anti-parasitic functions.

Conclusion: Our findings indicate both efficacy and safety of MOv18 IgE and support translation of this novel therapeutic approach to the clinic. MOv18 IgE is now the first IgE immunotherapy to be evaluated in man in a first-in-class, first-in-man clinical trial.

SUNDAY, 27 MAY 2018
OAS 10
FROM CONTACT METAL ALLERGY TO MASTOCYTOSIS

0053 | Usage of nickel containing writing utensils does not cause specific hand eczema in nickel allergics

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Background: There are many nickel-containing daily life articles with repetitive skin contact. We asked whether using nickel-containing writing utensils, i.e. pens lead to a local skin reaction in nickel allergic individuals. Therefore we performed an application study with 100 nickel allergic patients using nickel and non-nickel-containing pens in blinded fashion.

Method: 100 individuals (93 female, 22-78 years) with a positive patch test to nickel participated in three 2-hour periods of handwriting a standardized text. There was no systemic or local immunomodulatory medication or actual hand eczema. For each individual there was one nickel-releasing pen in 2 writing phases and one not nickel-releasing "control" pen in one writing phase. Before and after writing there was a clinical examination of the hands (erythema, scaling, dryness, potential eczema) and each individual was asked to document the "subjective" skin symptoms (itching and dryness). Local skin reactions were assessed by optical coherence tomography (OCT) (reference: normal skin), and compared to reference OCT value of allergic contact dermatitis. Since pens were code-numbered, eluates could be made and extent of nickel release in vitro was assessed for those pens that provoked skin symptoms.

Results: 29 individuals reported symptoms like local (hand) or generalized itching and some clinical findings were noted like local redness/erythema. No eczema was found. When relating the grading of the symptoms to the status "nickel releasing/non releasing" pen, there was almost equal symptoms extent and distribution in both conditions. OCT-analysis of the writing hand showed rather irritant reaction. There was no relation between nickel release data and reported skin symptoms.

Conclusion: No significant difference was found between clinical reactivity when using nickel-releasing pen and non-nickel-releasing pen. In addition there was no relation to the amount of nickel release. If symptoms were reported/ seen, it occurred most of the time in the same person with both types of the pen. Thus, no increased specific, i.e. allergic skin reaction seemed to occur upon usage of nickel containing writing utensils.

0054 | Allergic contact dermatitis caused by dexpanthenol - a not so rare sensitizer

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Background: Dexpanthenol (technical name, panthenol - INCI name, pantothenol - synonym; CAS 81-13-0) is the stable alcohol of pantothenic acid, a water-soluble vitamin (B5) that is required for the biosynthesis of coenzyme A in cells. It is commonly used in pharmaceutical and cosmetic industries, because of its moisturizing, anti-inflammatory and wound-healing properties.

Method: We performed a retrospective study of patients with positive (1 + or more intense) reactions to dexpanthenol among 1832 consecutively patch tested patients at our clinic from 2010 to 2017. Dexpanthenol 5% pet. (Trolab®, SmartPractice Europe) was included in the Department's baseline series after several positive reactions to Bepanthene® cream (Bayer, Diegem, Belgium), which was also tested *as is* in 125 cases. Patch tests methodology followed the European Society of Contact Dermatitis recommendations.

Results: We observed 26 positive reactions to dexpanthenol (1.4% of consecutively tested patients) and all the 11 patients that were also tested to bepanthene® were positive. The majority were women (69%), with a mean age of 50.3 years and suffered from hand, lower limb or facial eczema (73%) or chronic leg ulcer (27%). Further patch testing with cosmetic (85%), topical drugs (38%) and fragrance (31%) series showed other positive reactions, in average, to 7.3 allergens. The most common were amerchol L101 50% pet. (46%), fragrance mix I 8% pet. (38%), chlorhexidine 0.5% aq., methylchloroisothiazolinone/methylisothiazolinone 0.02% aq. and cetearyl (cetostearyl) alcohol 20% pet. (23% each). With regard to the causal products, 17 subjects (65%) were exposed to Bepanthene® and 9 (35%) to various cosmetic products containing lower concentrations of dexpanthenol, particularly other wound-healing creams, shampoos and lipsticks.

Conclusion: ACD to dexpanthenol is considered to be rare, with positive reactions ranging from 0.2% to 0.7% in large series of aimed testing. Our study demonstrated a higher incidence (1.4%), especially in patients with several reactions to other allergens from cosmetics and topical drugs and considerable use of cosmetic or pharmaceutical products (facial and hand dermatitis and leg ulcers). Although formulations with dexpanthenol are mostly considered 'safe' wound-healing products, in the appropriate clinical settings ACD for this component must be suspected and excluded.

0055 | Metal allergy related to the use of orthopaedic prosthesis after trauma and normal aging process

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Background: Metal hypersensitivity is very common, especially to nickel sulphate, which is contained in many objects and substances we use on a daily basis. The steady increase of metal allergies related to the use of orthopaedic prosthesis after trauma and normal aging process, led us to carry out this study.

We performed some tests before patients underwent hip, knee, shoulder replacement intervention. Those tests aim at evaluating if there might be a risk for metal allergy post intervention.

Method: We have developed a protocol based on the medical history and patch testing (Lofarma SpA - Milano), so as to evaluate metal hypersensitivity. Primary care checks have been carried out with x-ray and clinical evaluation, the latter via visual analogic scale (VAS).

Results: Out of 94 patients awaiting prosthesis, a high level of allergy has been found out, indeed: 15 patients were positive to nickel suphate, cobalt sulphate and chromium sulphate, 6 patients were positive only to nickel sulphate and cobalt sulphate, 2 patients were positive only to titanium oxide (Patch Test Lofarma SpA - Milano). The follow up was carried out on four years. No patient reported any reaction related to hypersensitivity or complications after implant.

Conclusion: With a preventive activity performed via patch tests, it is therefore possible to diagnose not only a specific allergy, but to lead surgeons towards the best choice of prosthesis for the needs of patients. Prostheses are composed of sliding surfaces that inevitably produce friction: depending on the materials they are made of, cobalt, nickel, chromium, titanium, these can release particles that may trigger an inflammatory reaction (local or generalized) or an allergic reaction. In fact, allergies are among the main complications that arise after a prosthetic operation, sometimes making a second intervention necessary. A timely and careful diagnosis in cases of doubtful sensitivity, can avoid this type of problems, which cause discomfort to the patient.

The choice of modern hypoallergenic implants can help prevent any kind of potential reactions.

0056 | The effect of omalizumab in mastocytosis patients. prospective double-blind, placebo-controlled multicentre study

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Background: Patients with mastocytosis often suffer from a variety of symptoms caused by mast cell mediators. Besides H1-blockers, treatment remains difficult. Omalizumab, a monoclonal anti-IgE-antibody has been postulated to have a positive impact on mastocytosis-associated symptoms such as flush, vertigo, gastrointestinal troubles or anaphylaxis.

Method: The effect of omalizumab was investigated in 17 patients with various forms of mastocytosis in a multicenter prospective double-blind placebo-controlled trial. Seven patients were randomised to the omalizumab group, 9 to placebo. Omalizumab was dosed according to total serum IgE and body weight as in allergic asthma. Primary endpoint of the study was the change in the AFIRMM score after 6 months of treatment. Groups were age-balanced (45.4 y ± 8.8 in the placebo vs 47.7 ± 13.8 in the verum), whereas 66.6% in the omalizumab and 85.7% in placebo group were female. Median disease duration was 4.5 y ± 2.9 in the placebo and 10.0y ± 5.1 in the verum group. A variety of laboratory parameters were also analyzed.

Results: After 6 months the median AFIRMM score improved from 104.0 to 102.0 for the placebo and from 52.0 to 26.0 for the Omalizumab group, respectively. The amount of reduction was not significantly different in the treatment groups ($P = 0.941$). Regarding the secondary endpoints—including changes in the AFIRMM score at the end of the study, the number of allergic reactions, changes in VAS for major complaints, pressure-induced wheal and flare and the frequency of the use of mastocytosis-specific drugs such as antihistamines or cromoglycates showed a considerable, but again not significant improvement in the omalizumab group. Adverse events (AE) events like urticaria, bronchospasm, anaphylactic shock showed no significant difference between both groups. Expression of FceRI on basophils reduced in patients receiving omalizumab but not with placebo.

Conclusion: In our small group omalizumab seems to improve mastocytosis symptoms corresponding to effects described in the literature, involving mainly diarrhea, dizziness, flush, and anaphylactic reactions. A tendency to improve in the AFIRMM score and in the secondary endpoints was seen. AE were few in number and equally distributed. To our knowledge, this is the first double-blind placebo

controlled study for omalizumab in mastocytosis patients. Further larger studies would be advisable to confirm our findings.
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0057 | Severe bullous cutaneous mastocytosis

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Case Report: Mastocytosis is a heterogeneous disease affecting both children and adults. In children the disease is nearly always restricted to the skin and often shows spontaneous regression whereas in over 80% of adults, involvement of other organs, predominantly of bone marrow, is seen in this chronic disease. Although life threatening progressions of the disease have been described even in infants, such cases remain very rare.

Case Presentation: A 5-months-old girl presented with flushing, large yellow plaques and blisters. The plaques showed edema, erythema and itching after rubbing and scratching, a phenomenon known as Darier's sign. Flushing of the body first appeared at the age of 3 weeks. Followed by yellow plaques and blisters. Episodes of abdominal cramping, diarrhea or vomiting were not reported. Laboratory findings at first visit: Tryptase value elevated to 29.4 µg/l (≤11.4 µg/l). IgE-level (4.55 kU/L) and liver parameters were in the normal range. Hemoccult test was negative. Lymphadenopathy and hepatosplenomegaly investigated clinically and by ultrasonography were not seen.

Management and Outcome: Within the next month the girl developed severe generalized large blisters. Blisters often appeared several hours after episodes of flushing and became hemorrhagic although a therapy with dimetindene/desloratadine was initiated. Blisters were sterile punctured and different non-adhesive wound dressings were applied. Clear improvement occurred under oral application of prednisolone (2.5 mg/kg/d) combined with dimetindene/desloratadine. Tryptase levels decreased to 11.55 µg/l. Therefore, prednisolone was reduced to 0.5 mg/kg/d and after one year the episodes of severe flushing and blistering stopped.

Discussion: Bullous diffuse mastocytosis is rare. It is described almost exclusively in children. It can be a life-threatening disease since infants are at risk to develop severe generalized blisters and wound infections. In addition, severe anaphylactic reactions caused by e.g. drugs, temperature changes or physical triggers have been reported. Still the prognosis is very good. After some years blistering usually stops, dermographism, thickening of the skin and hyperpigmentation may persist into adulthood. In some exceptional cases an involvement of bone marrow was also seen in children, indicating progression to a systemic mastocytosis. Therefore, for this severe form of cutaneous mastocytosis, regular medical examinations by a specialist should be performed.

0058 | Clinical features and time-related prognosis of mastocytosis among children in Kazakhstan

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Background: We aimed to study clinical features, natural evolution and response to treatment, time-related prognosis of cutaneous mastocytosis among children in Kazakhstan.

Method: 32 children (19 males, 13 females, male-female ratio 1.46:1) aged 3 months-17 years with diagnosed mastocytosis were under our observation in allergological center Unit, Astana, Kazakhstan in 2002-2017. Only those with more than 3 mastocytomas were included in the study population. All patients underwent physical examination with detected positive Darier's sign. Serum tryptase and skin biopsy were performed initially. Follow up visits were performed twice a year either until complete resolution, or until the end of the study. Written informed consent for publication was taken from all patients or guardian (parents).

Results: There were 22 cases of urticaria pigmentosa (68.8%), 4 cases of mastocytoma (12.5%) and 6 of diffuse cutaneous mastocytosis (18.7%). Mean serum tryptase level were 15.4 ± 7.8 ng/mL. Among our patients only 11 (34.4%) did not have disease resolution after fifteen years follow up. Good prognostic factors were male gender, early age of onset - under 3 years and smaller lesions with fewer affected areas. According to the Children's Dermatology Life Quality Index, only in 4 patients there was a significant effect on quality of life. There were no reported cases of anaphylaxis even after Hymenoptera stings among our patients.

Conclusion: Childhood mastocytosis, most common type of which is urticarial pigmentosa, is a relatively rare disease with good prognosis and minimal or only moderate effect on quality of life.

SUNDAY, 27 MAY 2018

OAS 11

ANAPHYLAXIS AND EOSINOPHILIC ESOPHAGITIS

0059 | Clonal mast cell disease (C-MCD) may be missed if baseline tryptase is not assessed in all anaphylaxis

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Background: Anaphylaxis is a frequent manifestation in patients with systemic c-MCD, being a common clinical presentation in patients with indolent systemic mastocytosis without skin involvement (ISM -/-) and monoclonal mast cell activation syndrome (MMCAS). Main specific elicitors are hymenoptera stings, followed by drugs and food. Nevertheless, most anaphylaxis clinical guidelines recommend to rule out c-MCD only in case of hymenoptera sting and in idiopathic anaphylaxis.

Objective: To assess the usefulness of the baseline tryptase determination to diagnose systemic c-MCD in patients whom anaphylaxis lead to their diagnose.

Method: Patients diagnosed of c-MCD and controlled in our department from 2007 to 2017 were selected. Non-monoclonal MCD were excluded of the analysis. The diagnosis of c-MCD was based on the bone marrow study using the WHO criteria. Anaphylaxis episodes were carefully assessed, including triggers, cutaneous involvement and baseline serum tryptase levels (sBT). The Spanish Network on Mastocytosis (REMA) score to assess the probability of systemic c-MCD (≥2) was performed.

Results: Data from a total of 86 patients with a diagnosis of systemic c-MCD was collected. Anaphylaxis lead to their diagnosis in 25 cases (29%), 15 female and 10 male, 2/25 (8%) ISM, 18/25 (72%) ISM-/- and 5/25 (20%) MMCAS. 15 (60%) out of the 25 patients had a REMA score ≥2. sBT was elevated (>11.4 mcg/dL) in 22 (88%) out of 25 patients. The 3 patients with normal sBT were allergic to Hymenoptera sting. In 7 patients sBT was <20mcg/dL. All patients suffered a grade III anaphylaxis. 8 (32%) lacked cutaneous symptoms during the anaphylaxis. The most frequent triggers were drugs in 48%, followed by Hymenoptera sting 28% and food 12%. Co-existence of IgE mediated allergy was confirmed in 44% patients. Idiopathic anaphylaxis was diagnosed in 3 (12%) of patients. c-MCD would have been missed in 60% of cases if sBT had not been assessed (those with drug or food induced anaphylaxis).

Conclusion: Our data suggest that a sBT determination should be performed in all patients with an anaphylaxis, independently of the existence of a known trigger. When elevated sBT and/or REMA score ≥2, a complete bone marrow study should be performed.

0060 | Anaphylaxis to pumpkin seed is caused by a 2S albumin

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Background: Pumpkin is a member of the *Cucurbitaceae* family, but unlike melon, few cases about allergic reactions to this food have been described. 2S albumins, a storage protein family, are relevant allergens in many foods and, despite it has been described in pumpkin seed, its allergenicity had not been proved yet. The aim of the present study is to evaluate IgE-mediated sensitivity in four patients with a clinical history of anaphylaxis after eating pumpkin seeds, and identify and characterize allergens involved.

Method: The purification from protein extract was performed using chromatographic methods. The peptide sequence was done by mass spectrometry. Molecular characterization was conducted by electrophoretic methods (1- and 2-DE). Its secondary structure and its stability were studied by circular dichroism spectroscopy. Immunoassays were performed using allergic patient' sera.

Results: Pumpkin seed extract was tested with allergic sera by immunoblotting, revealing that all recognized mainly proteins of low molecular mass around 14 kDa. They were isolated and purified by means of gel filtration in Sephadex G-50 and reverse phase in HPLC. Purified 2S albumin has 14 kDa and present two chains of 8 and 4 kDa under reducing conditions in SDS-PAGE. The protein shows helicoidal secondary structure, stable at 85°C. The purified protein was recognized by allergic patients' sera in immunoblotting. Inhibition immunoassays revealed cross-reactivity with melon seeds but not with other food extracts.

Conclusion: Isolated protein showed similar structural characteristics than other 2S albumins described. Inhibition assays revealed the cross-reactivity with melon seeds. 2S albumin from pumpkin seed could be responsible for the severe allergic symptoms as anaphylaxis. This protein can be used as clinical tools for in component-resolved diagnosis (CRD).

0061 | Genetic variation at IL-18 gene is associated with wheat-dependent exercise-induced anaphylaxis in Chinese Han population

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Background: Wheat-dependent exercise-induced anaphylaxis (WDEIA) is severe IgE-mediated food allergy which triggered by wheat ingestion with physical activity, resulting from interaction of environmental and genetic factors. Interleukin-18 (IL-18) is a proinflammatory cytokine involved in allergic inflammatory reactions by influence the balance of Th1/Th2 immune response. This case-control study aimed to identify the possible association of the genetic variation in the IL-18 gene with WDEIA in Chinese Han patients.

Method: Four tag SNPs (rs5744280, rs360729, rs360717 and rs1946518) were selected in the IL-18 gene, and SNP genotyping was conducted among the 130 WDEIA patients and 600 normal participants by MassARRAY platform. The total and specific IgE (sIgE) antibody levels of WDEIA group were measured using an ImmunoCAP system.

Results: There were statistically significant differences of allele frequencies distribution of rs1946518 (OR = 1.547, 95% CI = 1.177–2.025, $P = 0.0015$) and rs5744280 (OR = 1.508, 95% CI = 1.148–1.969, $P = 0.0027$) between WDEIA and control group. The genotype distributions of rs1946518 and rs5744280 were significantly different between the WDEIA and control groups under the log-additive model of inheritance ($P = 0.0014$ and $P = 0.0027$, respectively). At position rs1946518 in the IL-18 promoter region, individuals with the GG genotype have increased risk for WDEIA compared with those with TT genotype (OR = 1.56, 95% CI = 1.18–2.04, $P = 0.0014$), and log transformed total IgE values was significantly higher in the heterozygous GT group than in major allele homozygous TT group ($P = 0.0036$). The frequency of haplotype AAGG was significantly different between WDEIA and control group (OR = 1.498, 95% CI = 1.144–1.963, $P = 0.0030$). All other association studies showed no statistically significant ($P > 0.05$).

Conclusion: Our study indicated that the genetic variation at IL-18 gene was associated with the risk of WDEIA. At position rs1946518, the minor allele G might be a potential risk factor accounting for WDEIA, and GT genotype might be associated with the higher production of total IgE in WDEIA patients.

0062 | Predictive factors for early vs delayed response to budesonide orodispersible tablets in eosinophilic esophagitis: Results from the pivotal trial EOS-1

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Background: Twice-daily treatment with a newly-developed budesonide 1 mg orodispersible tablet formulation (BUL) was highly superior to placebo to induce clinical and histological remission in adult patients with active eosinophilic esophagitis (EoE) after only six weeks (1). An additional 6-week open label induction was offered to those patients allocated to active drug with incomplete response at week 6 assessment.

Method: To identify potential baseline predictors for early (i.e., within 6 weeks) vs delayed (i.e., within 12 weeks) clinico-histological remission in patients with EoE treated with budesonide 1 mg orodispersible tablets BID. Baseline disease and demographic characteristics of 34 and 16 patients achieving clinico-histological remission at week 6 or 12 respectively were analyzed in this subgroup analysis.

Results: Baseline characteristics are presented in table 1. ROC curve analysis of baseline worst grade of dysphagia experienced within the last 7 days, using a 0-10 point NRS, was performed for budesonide treated patients in clinico-pathological remission at wk 6 vs wk 12, and showed a cut-off of ≤ 5 points as a predictor for 'early remitter' with a Youden Index of 0.305, being moderately associated with an AUC of 0.6664, a sensitivity of 61.8%, and a specificity of 68.8%.

Conclusion: Patients with a delayed response to treatment were younger and showed a delayed disease diagnosis as well as higher

	Remission week 6 (LOCF)N = 34	Remission week 12 (LOCF)N = 16	*P*-value (two-sided)
Sex, n (%)			
Male	29 (85.3%)	14 (87.5%)	1.0000*
Age [years] at baseline			
Mean (SD)	39.2 (12.04)	34.8 (10.39)	0.2125**
Time interval since first EoE symptoms [months]			
Mean (SD)	136 (112.4)	[N = 15]167 (102.5)	0.3596**
Total modified EEsAI endoscopic instrument score (0-9)			
Mean (SD)	3.6 (1.54)	4.2 (1.28)	0.2044**
Dysphagia NRS (last 7 days)			
Mean (SD)	5.2 (2.11)	6.4 (1.75)	0.0486**
Pain during swallowing NRS (last 7 days)			
Mean (SD)	3.0 (2.55)	4.3 (3.16)	0.1311**
Patient's Global Assessment concerning the severity of EoE symptoms NRS			
Mean (SD)	5.6 (1.52)	6.6 (1.45)	0.0314**
Weekly EEsAI-PRO score			
Mean (SD)	[N = 33] 51.8 (15.2)	[N = 15] 59.4 (13.28	0.1045**

clinical and endoscopic disease activity at baseline. However, only higher clinical (dysphagia) and patient's global assessments of EoE activity at baseline were significantly associated with a delayed clinico-histologic remission of EoE.

Reference: (1) Lucendo AJ, et al. Gastroenterology 2017;152 (5, Suppl.1):S-207.

0063 | Baseline serum tryptase in severe anaphylaxis

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Background: Diagnosis of anaphylaxis is based on the assessment of symptoms according to the patient's history. Baseline serum tryptase (BST) can be measured as biomarker and is used to identify mast cell dependent diseases. The aim of this analysis was to determine the frequency of BST detection and the association of elevated BST with various types of elicitors and severity of anaphylaxis.

Method: The Network for Online Registration of Anaphylaxis (NORA) collects data on elicitors, symptoms and severity, treatment and the diagnostic workup of patients who have experienced at least one episode of severe anaphylaxis, as documented within medical records of participating tertiary referral centres. Recording of BST was implemented in 2010.

Results: Between 2010 and 2017, data of 7694 cases were obtained. BST values were given in 4551 (59.1%) cases. BST was measured in the group of insect venom anaphylaxis in 87.8%, in drug anaphylaxis in 47.4%, in food anaphylaxis in 34.3% and in the group of idiopathic anaphylaxis in 67.3%. BST was more frequently available from adults (70.0%) than children (31.5%). No difference of the measurement frequency between the severity grades according to Ring&Messmer (grade II: 61.9%, grade III: 55.5%, grade IV: 65.5%) were found. Of 4551 available BST values 429 (9.4%) were above the normal range (>11.4 µg/l) with a median of 17.9 [11.4-221.0] µg/l. An underlying mastocytosis was reported in 94 (21.9%) of the 429 patients with elevated BST.

Conclusion: BST is more often determined in adults than children. In adults, BST is measured more frequently in patients who experienced anaphylaxis to insect venom or to an unknown allergen, although it is not rarely elevated in food or drug induced anaphylaxis. An underlying known mastocytosis was only present in a fifth of patients with elevated BST suggesting that a large proportion of patients had increased BST due to unknown mastocytosis or other reasons. Taken together, BST is an important biomarker in anaphylaxis and should be determined in all patients who experienced severe anaphylaxis.

MONDAY, 28 MAY 2018
OAS 12
CHALLENGES IN ALLERGY DIAGNOSTICS

0064 | Skin Prick Test (SPT) and specific IgE may measure different IgE reactivities and be complementary in allergy diagnosis

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Background: Skin Prick Test (SPT) and serum specific IgE (sIgE) are supposed to be qualitatively concordant. Therefore, clinical practice employs either of the tests to be positive to determine immediate type sensitization. In allergen immunotherapy (AIT) clinical trials, however, both tests have been required to assess patient eligibility. This analysis evaluates the quantitative relationships between the tests from large adult grass-pollen (GRP) and dust mite (HDM) trials.

Method: SLIT tablet trials included SPT and sIgE testing with non-identical extracts. Positive SPT (ALK-Abelló) wheal diameters and sIgE levels (ImmunoCAP Singleplex, ThermoFisher) from GRP (n = 226) and HDM-allergic subjects (n = 424) were compared retrospectively. Further analysis included an adult cohort from Copenhagen (grass n = 72, birch n = 74), tested by SPT and sIgE (ADVIA Centaur, Siemens) with identical birch pollen (Bet v) and timothy grass pollen (Phl p) extracts.

Results: Across trials there was a lack of quantitative correlation between SPT and sIgE with r = 0.21 between log mm Phl p SPT and log Phl p sIgE (US-GPR study P05238) and r = 0.08 between log mm Der p SPT and log Der p sIgE (EU-HDM studies MT-02, MT-03 pooled). Except, in the Copenhagen Allergy Study, a correlation of r = 0.7 and r = 0.48 between log mm Phl p SPT and log Phl p sIgE and between log mm Bet v SPT and log Bet v sIgE were noted.

Additional variables on top of sIgE may shape final biological responses (i.e. SPT) to allergens:

> total IgE concentration, regulation of high affinity IgE receptors, and sIgE/total IgE ratio individual mediator response (intrinsic sensitivity), cellular pre-activation (i.e. Syk levels), inhibitory effects by competing ("blocking") antibodies number/tissue distribution of effector cells, and end organ responses.

Conclusion: SPT and sIgE may show acceptable qualitative concordance, but poor quantitative relation. Using identical allergen extracts and single center data sets may improve the quantitative correlation. Noteworthy, each test measures different IgE reactivity responses and hence may complement each other when selecting patients for AIT.

0065 | Diagnosis of genuine grass pollen allergy in Mediterranean polysensitized children with pollinosis by skin testing with a recombinant hybrid molecule of the major timothy grass pollen allergens

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Background: Skin testing of allergic patients represents a commonly used first diagnostic method in clinical practice but allergen extracts often contain cross-reactive allergens and therefore do not allow to identify precisely the sensitizing allergen source. Our aim was to investigate the suitability of a recombinant hybrid molecule, consisting of the four major allergens from timothy grass pollen (Phl p 1, Phl p 2, Phl p 5, Phl p 6) for *in vivo* diagnosis of genuine grass pollen allergy in polysensitized Greek children suffering from pollinosis.

Method: Sixty-four children aged from 6 to 17 years with a positive skin reaction and/or specific IgE to grass pollen extract and 9 control children with allergy to allergen sources other than grass pollen were studied. Skin prick testing was performed with the recombinant hybrid molecule, the single recombinant grass pollen allergens, grass pollen extract, and panel of other pollen extracts. Specific IgE reactivity to 176 micro-arrayed allergen molecules was determined using ImmunoCAP ISAC technology. IgE reactivity to the hybrid was detected by non-denaturing RAST-based dot blot analysis.

Results: Genuine grass pollen sensitization could be confirmed in 92% of the patients by SPT and IgE reactivity to the hybrid molecule whereas for 8% of the patients lacking hybrid-specific IgE, sensitizations to nPhl p 4, nCyn d 1, rPhl p 11, and to the profilin marker Phl p 12, which explained the positive SPT to grass pollen extract despite lack of genuine grass pollen sensitization.

Conclusion: The recombinant hybrid molecule represents a useful tool for *in vivo* diagnosis of genuine grass pollen allergy.

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0066 | Proteomic profiling of mite skin prick test solutions commercially available in India

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Background: Skin prick test (SPT) solutions are important diagnostic tools for diagnosing allergy. The prick test solutions are based on natural extracts where the allergen composition and content is highly dependent on the raw material and subsequent production processes. It is known that allergen extracts from different companies can vary significantly which also may affect their allergenic activity and in turn influences the result of the diagnostic assay. In India one European and several Indian Companies are marketing products for allergy prick test diagnosis. However, no in-depth analysis has been performed with skin prick test solutions available on the Indian market. The aim of this study was to compare the quality of the different products with regard to source identity, allergen content and composition.

Method: SPT mite (Der f, Der p, Blo t) solutions (from one European and 3 Indian providers, all commercially available on the Indian market) were analyzed by tandem mass spectrometry (LC-MS/MS), SDS-PAGE with silver staining and immunoblots using both patients' sera and monoclonal antibodies.

Results: Significant quantitative and qualitative differences in allergen/protein compositions, as well as different degrees of contaminations with other mite species and human proteins were observed. *Dermatophagoides pteronyssinus*: only in one SPT product the relevant Der p-allergens were detected. Other products were either prepared from a wrong mite species (*Suidasia medanensis*), or contained mostly human proteins. *Dermatophagoides farinae*: again just one product contained all relevant Der f-allergens, whereas the others either missed some Der f-allergens and showed signs of significant human contaminations, or were even prepared from the wrong mite species. *Blomia tropicalis*: only one SPT product was available which was once more prepared from the wrong mite species. No Blo t-allergens were detected in this product.

Conclusion: Mass spectrometry is a powerful tool to prove the identity of the source material, to determine the allergen profile (number of allergens present) with quantitative comparison of single allergens, as well as to detect contaminations in SPT solutions. It is evident from this data that quality control of some providers needs to be significantly improved, as some products contain different allergens/proteins than specified. Consequently, a reliable diagnosis using these products is not possible.

0067 | Simultaneous quantification of eight purified food allergens using fluorescent multiplex array

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Background: Quantification of food allergens is increasingly important for dose assessments of food preparations used in oral immunotherapy (OIT), food allergy prevention, and monitoring safety in the food industry. Our aim was to develop a validated multiplex immunoassay capable of simultaneously measuring eight major food allergens.

Method: Using the Luminex xMAP system, microspheres were coupled to specific monoclonal antibodies for allergen capture. Biotinylated specific monoclonal or polyclonal antibodies were used for detection. Reference standards were formulated from natural or recombinant allergens, with purity established by mass spectrometry. A full method validation determined parameters of linearity, range, limits of quantification and detection, accuracy and precision of the multiplex food immunoassay.

Results: Full method validations were completed for peanut (Ara h 3, Ara h 6), cow's milk (Bos d 5) and shrimp tropomyosin, with egg (Gal d 2), cow's milk (Bos d 8, casein) cashew (Ana o 3), hazelnut (Cor a 9) and soy (Gly m 5) in final development stages. The standard curves for all analytes allow for quantification over a large dynamic range (3 logs). The lower limits of detection (LLOD) were as low as 0.02 ng/mL and 0.06 ng/mL. Intra- and inter- assay accuracy and precision results for three samples assayed in triplicate on four occasions passed acceptance criteria within the range of 70-130% recovery and a coefficient of variation of $\leq$15%.

Conclusion: The fully validated multiplex array provides a quantitative, accurate and precise tool for the simultaneous measurement of eight specific food allergens with the potential application in food immunotherapy formulations and for risk assessment in the food industry.

0068 | Local IgE in subjects with allergic and non-allergic rhinitis (LISA)

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Background: The aim of this prospective study (LISA) was to evaluate the incidence of 'local allergic rhinitis', which may affect

individuals previously diagnosed with non-allergic rhinitis in a non-selected group of young students.

Method: A total of 131 students (age 24.97 ± 5.08 years) with possible allergic rhinitis and 25 non-allergic controls without rhinitis (age 21.96 ± 1.95 years) were recruited by public posting.

97 of 131 students with possible allergic rhinitis were positive (≥ 3 mm) to at least one common allergen by prick testing at visit 1.24 classical cases with house dust mite (HDM) allergy, 21 students with non-allergic rhinitis and 18 non-allergic controls were further investigated at visit 2. Blood and nasal secretion (NC) were obtained, and in students with HDM allergy and non-allergic rhinitis a nasal provocation test (NPT) with HDM was performed (ClinicalTrials.gov Identifier: NCT02810535).

Results: Total IgE and specific IgE (sIgE) in serum and nasal secretion (kU/L) differed significantly between groups (Kruskal-Wallis test $P < 0.001$). Students with HDM allergy: serum total IgE median 207.56 (15.27-4868.00), sIgE HDM median 27.99 (0.11-311.48) and in NC total IgE median 14.13 (0.10-53.57), sIgE HDM median 1.19 (0.10-14.93) vs students with non-allergic rhinitis: serum total IgE median 21.93 (2.23-621.17), sIgE HDM median 0.05 (0.05-1.47) and in NC total IgE median 0.38 (0.10-71.86), sIgE HDM median 0.10 (0.10-0.10) and controls: serum total IgE median 24.89 (2.15-74.70), sIgE HDM median 0.05 (0.05-0.32) and in NC total IgE median 0.10 (0.10-2.04), sIgE HDM median 0.10 (0.10-0.10). The NPT with HDM was positive in patients with HDM allergy only.

Conclusion: Local IgE is present to a substantial amount in patients with HDM allergy, but not in non-allergic rhinitis. In a non-selected population, exclusive local production of IgE is absent, and our findings are challenging the emerging concept of local allergic rhinitis.

0069 | A biomarker of bradykinin angioedema attacks usable in clinical practice: The d-dimers/tryptase ratio

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Background: There are two major etiologies of angioedema (AE): mast cell-mediated AE (mast-AE), which accounts for 95% of patients admitted to emergency departments for AE, and bradykinin-mediated AE (brad-AE), less frequent but with more severe prognosis. The distinction is clinically based and no biological markers are currently validated. Previous studies have suggested potential targets such as markers of fibrinolysis and coagulation, inflammatory and coagulation contact phase proteins.

Method: We conducted a prospective study evaluating coagulation marker concentrations (TP, TCA and fibrinogen), fibrinolysis markers (D-dimers), complement, inflammation (high-sensitivity C-reactive protein or hs-CRP) and mast cell activation (tryptase) during an AE episode. Three groups of patients were tested: those with mast-AE, those with brad-AE and patients with abdominal pain of known etiology, neither bradykinin or mast cell-mediated, at the time of the attack (D0) and then seven days later (D7).

Results: 120 AE attacks were included involving 108 patients including 44 mast-AE, 35 brad-AE and 41 controls. The blood concentration of D-dimers was higher during the brad-AE (2.02 vs 0.635 ng/mL, $P < 10^{-3}$) and mast-AE (1.49 vs 0.62 ng/mL, $P < 10^{-3}$) attacks than D7 with a higher concentration in brad-AE vs mast-AE ($P = 0.01$) or vs controls ($P = 0.009$). For brad-AE, there was a correlation with the severity of the attack ($P = 0.04$). Concentrations of complement, coagulation markers and CA125 were not modified during the course of the attack. Serum tryptase concentrations were higher in mast-AE than in brad-AE (6.195 vs 4.58 μg/L, $P = 0.01$). A D-dimer/tryptase ratio with a threshold of 0.75 would confirm the diagnosis of brad-AE with 100% specificity and a positive predictive value of 100%.

Conclusion: Exploration of fibrinolysis abnormalities (in particular D-dimers) and the D-Dimer/tryptase ratio could be used as a promising biomarker of a brad-AE attack, especially for abdominal forms or isolated forms with difficult diagnosis.

MONDAY, 28 MAY 2018

OAS 13

NOVEL CONCEPTS IN MECHANISMS IN ALLERGY IMMUNOTHERAPY

0070 | Immunological characterization of Polistes dominula venom and identification of the hyaluronidase Pol d 2

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Background: Venom specific immunotherapy is highly effective in patients at high risk of severe or fatal anaphylactic reactions to insect stings, but selection of the right venom for therapy is a prerequisite. In regions where *Vespula spp.* and *Polistes spp.* coexist, discrimination between allergy to one or both insects is difficult due to the limited information about *Polistes venom* components and the high degree of cross-reactivity between the venom allergens. Prominent candidate allergens, leading to cross-reactivity between the venoms of Hymenoptera species, are the hyaluronidases. The hyaluronidases of honeybee and *Vespula* venom are well characterized, but no information about the hyaluronidase of *Polistes dominula* venom and its cross-reactivity were available.

Method: *Polistes dominula* venom was analyzed by mass spectrometry. Genomic data of Polistes dominula were used to identify venom proteins. Identified proteins were cloned from venom gland cDNA, recombinantly produced in insect cells, characterized by immunoblotting and assessed for IgE reactivity with sera of venom-allergic patients.

Results: Amongst other proteins, *Polistes dominula* hyaluronidase was successfully identified in the venom, and produced recombinantly in insect cells together with its homologues from honeybee and *Vespula* venom. The analysis of specific IgE in sera from honeybee, *Vespula* and *Polistes* venom-allergic patients showed IgE reactivity of all hyaluronidases with diverse cross-reactivity patterns.

Conclusion: The *Polistes* venom hyaluronidase proved to be IgE reactive with sera of venom allergic patients, independent of cross-reactive carbohydrate determinants. Hence, it might be able to complete the panel of *Polistes* venom allergens for improved molecular diagnostics in the future. Due to its allergenic properties, the new *Polistes* venom allergen was designated as Pol d 2.

0071 | A longitudinal comparison of IL-5 and IL-10-producing antigen-specific clonal T cell repertoires throughout AIT

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Background: Allergy is a chronic disease affecting more than to 20% of the North American population. It is associated with potentially debilitating symptoms. Allergen-specific immunotherapy (AIT) is a disease-modifying treatment associated with Treg induction and elevated IL-10 production. To date, it is unknown if IL-10-producing T cells are induced *de novo* or if AIT modulates allergen-specific Th2 cells to become tolerogenic.

Method: To investigate whether IL-5 and IL-10-producing antigen-specific T cells share a common T cell repertoire, we performed a longitudinal TCRβ sequence analysis of Timothy grass (TG)-specific T cells from donors who received AIT for 15 months. As a control, TCRβ sequencing was also performed on TG-specific T cells from an allergic donor. TG-specific cells were expanded by 14-day *in vitro* culture with a pool of immuno-dominant TG-derived T cells epitopes (TG pool). A pertussis epitope pool was used in parallel as a control. Subsequently, cells were restimulated with the same pool and allergen-specific cells were isolated by cytokine capture with column enrichment, sorting IL-5 and IL-10 -producing cells. The TCRβ repertoire was sequence from DNA isolated from sorted cells.

Results: Preliminary results revealed that in the TG pool-specific cells, some clonal overlap was observed between IL-10 + and IL-5 + cells, suggesting that a fraction of IL-10 + cells may originate from IL-5 + cells, but some are generated *de novo*.

Conclusion: Longitudinal analysis of the allergen-specific TCR repertoire is a reliable method to determine if tolerogenic T cells are induced *de novo* or if they are modulated T cells that originate from the same repertoire as allergen-specific Th2 cells.

0072 | Butyrate and fructo-oligosaccharides support oral immunotherapy by suppressing basophil and mast cell activation and induce possible epigenetic changes in mast cell progenitors

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Background: In previous work, we showed that a fructo-oligosaccharide (FOS) supplemented diet enhanced oral immunotherapy (OIT) efficacy in a mouse model for cow's milk allergy, which led to reduced mast cell activation upon allergen challenge. Fermentation of FOS by intestinal bacteria leads to production of short-chain fatty acids (SCFA, e.g. butyrate). Increased levels of butyrate were found in the caecum of OIT+FOS mice. The exact contribution of FOS and/or butyrate in dampening the allergic response is however unknown.

Objective: Investigating the effect of combining OIT with butyrate or FOS supplementation on the development and IgE-mediated activation of mast cells and basophils.

Method: C3H/HeOuJ mice were sensitized to the cow's milk protein whey and subjected to OIT with or without FOS or butyrate supplementation. Bone marrow was collected during and after OIT and cultured with IL-3 and SCF into bone marrow-derived mast cells (BMMC). c-Kit and FcεRI expression on BMMC was analyzed using flow cytometry and IgE-mediated degranulation was determined by measuring β-hexosaminidase. After OIT, whole blood samples were used to perform a Basophil Activation Test (BAT) and intradermal (i.d.) and intragastric (i.g.) challenges were conducted to measure the acute allergic skin response (delta ear swelling) and mucosal mast cell degranulation (mMCP-1 in serum). After challenge, caecum content was collected to measure SCFA levels.

Results: BMMC developed from bone marrow of FOS exposed mice showed reduced expression of c-Kit and FcεRI and IgE-mediated activation was also reduced. Allergen-induced basophil activation was reduced in OIT+butyrate blood samples compared to OIT samples. These findings were in accordance with the observed reduction in the acute allergic skin response and the reduction in mast cell degranulation in OIT+FOS and OIT+butyrate mice compared to sensitized controls. A significant increase in butyrate in the caecum content was observed in OIT+FOS mice compared to sensitized controls and OIT mice.

Conclusion: FOS and butyrate either or not combined with OIT have profound inhibitory effects on allergic effector cells like mast cells and basophils. These inhibitory effects may partly be explained by the induction of epigenetic changes, because *in vitro* development of mast cells from bone marrow progenitors is affected. Further research is needed to investigate if this approach may improve treatment strategies for food allergies in the future.

0073 | Interleukin (IL)-35 producing T regulatory cells (iTR35) suppresses type II innate lymphoid cell (ILC2) and T helper 2 (Th2) cell function and are induced following grass pollen immunotherapy

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Background: IL-35 producing T cells (iTR35) are a novel subset of regulatory T cells with immunomodulatory properties. These inducible regulatory T cells produce IL-35, which is a dimeric protein with two subunits, IL-12A and Epstein-Barr virus induced 3 (EBI3). The effect of IL-35 on innate and adaptive type II allergic responses is yet to be determined. We hypothesized that IL-35 inhibit IL-5 and IL-13 production by ILC2 and Th2 cells. We further hypothesized that IL-35 inhibit IgE production by B cells.

Method: In a prospective controlled cross-sectional study of allergen immunotherapy, the proportion of ILC2s, IL-5 + , IL-13 + and dual IL-5 + 13 + ILC2s were enumerated in non-atopic controls (NAC, n = 16) and grass pollen allergics (SAR, n = 16) during the peak pollen season. The effect of IL-35 on ILC2, Th2 cells and IgE production by B cells was evaluated by flow cytometry, multiplex Luminex system and ImmunoCAP System. iTR35 cells were enumerated in NAC, SAR and sublingual immunotherapy-treated patients (SLIT, n = 16).

Results: ILC2s were higher in SAR compared to NAC during grass pollen season ($P = 0.002$). Moreover, SAR had elevated proportions of $IL5^+$ ($P = 0.042$), $IL13^+$ ($P = 0.042$) and dual $IL5^+IL13^+$ILC2s ($P = 0.003$) compared to NAC. In the presence of IL-25 or IL-33, IL-35 inhibited the production of IL-5 and IL-13 (both, $P = 0.031$) by ILC2s. In addition, IL-35 suppressed the production of allergen-driven IL-4, IL-5 and IL-13 by Th2 cells. Interestingly, CD40L/IL-4/IL-21-mediated IgE production by B cells was inhibited in the presence of IL-35 ($P = 0.015$). iTR35 cells suppressed Th2 cell proliferation ($P = 0.003$) and IL-4 ($P = 0.004$), IL-5 ($P = 0.004$) and IL-13 ($P = 0.001$) production. The proportions of allergen-driven iT_R35 cells and IL-35 levels were found elevated in SLIT (both, $P < 0.001$) and NAC (both, $P < 0.001$) when compared to SAR.

Conclusion: For the first time, we have shown that IL-35 inhibit type II cytokine production by ILC2s and allergen-driven Th2 cells. Restoration of IL-35 production by inducible Tregs (iTR35) is a prerequisite for tolerance induction during sublingual immunotherapy.

0075 | A comparison of microcrystalline tyrosine (MCT) and aluminium as adjuvants for allergen immunotherapy in mice

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Background: Allergen immunotherapy (AIT) is potentially disease-modifying, however low efficacy, long treatment duration, and frequently occurring side effects limit usage. One strategy to improve AIT has been to develop new adjuvant systems. In this study, we compared AIT efficacy using microcrystalline tyrosine (MCT) or aluminium adjuvants. We also tested the mode of action of MCT as an adjuvant in AIT or immunisation.

Method: Female BALB/c mice were sensitised by four weekly low-dose intraperitoneal injections with cat allergen extract. After a dormant period of four weeks, the mice received subcutaneous AIT comprising recombinant cat major allergen (rFel d 1) using MCT or aluminium as adjuvants. The AIT was repeated twice with two-week intervals. Cat-dander- and Fel d 1-specific antibodies were tested in serum and AIT efficacy was tested in an anaphylaxis model in which the mice received a systemic challenge with high-dose cat dander allergen extract. Prior to and over a period of 2-3 hours after the challenge, the body temperature was monitored, hypothermia being a measure for anaphylaxis. Mechanisms of the adjuvant action of MCT were studied using mice deficient in Toll-like receptor (TLR) and inflammasome signalling. The mice were immunised subcutaneously with two-week intervals with ovalbumin adjuvanted with MCT or aluminium. B- and T-cell responses were assessed by antibody and cytokine ELISAs and by flow cytometry.

Results: AIT with rFel d 1 and either MCT or aluminium induced comparable IgG1 and IgG2a antibody responses. Antigen-specific IgE response slightly decreased as measured after AIT with MCT compared to aluminium-based AIT. MCT and aluminium reduced the anaphylaxis in sensitised mice. Moreover, the analysis of T-cell responses showed that both MCT and aluminium induced cytokine secretion as measured by IL-2, IFN-g and IL-10. B- and T-cell responses induced with MCT-based vaccines were not dependent on MyD88 or TRIF signalling associated with TLRs, nor were they dependent on inflammasome activation as assessed in ASC-deficient mice.

Conclusion: MCT and aluminium are effective as adjuvants in immunisation and in AIT. The induced B- and T-cell responses are comparable and these responses were independent of IgG induction. Hence, MCT represents a suitable alternative depot adjuvant in aluminium-based immunisation and AIT.

MONDAY, 28 MAY 2018

OAS 14

INNOVATIVE PRIMARY IMMUNE DEFICIENCY MANAGEMENT

0076 | Trec and krec levels as a predictors of flow cytometry results

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Background: Flow cytometry is a common but expensive diagnostic technique for Primary immunodeficiency diseases (PIDD) detection. It requires a significant amount of training and therefore cannot be used as a screening tool.

T-cell recombination excision circles (TREC) and kappa-deleting element recombination circle (KREC) in a dried blood spot and in peripheral blood using real-time polymerase chain reaction (PCR) are usually used as a tool for severe combined immune deficiency. TREC and KREC represents an attractive and cheap target for a more extensive use in clinical practice.

This pilot study aims to assess TREC and KREC measurement ability to predict flow cytometry results.

Method: We carried out analysis of data from children assessed by clinical immunologists at Speransky Children's Hospital, Moscow, Russia with a suspected immunodeficiencies between May 2013 and August 2016. Peripheral blood samples were sent for TREC/KREC and flow cytometry analysis (levels of CD3, CD4, CD8 and CD19 were assessed).

Results: A total of 1005 samples were analysed using both methods: flow cytometry and TREC/KREC assay and were included into the analysis. We assessed ability of TREC to predict the abnormal levels of CDs 3, 4, 8 and a combination of TREC and KREC to predict the abnormal levels of all lymphocyte subsets (CD 3, 4, 8, 19) analysed.

TREC demonstrated AUC of 0.66 (95% CI 0.63—0.70) while a combination of TREC and KREC provided AUC of 0.65 (95% CI 0.62—0.69).

A cut-off point of a probability of 0.4 resulted in 59% sensitivity and 65% specificity for TREC, while a probability of 0.3 for a combination of TREC and KREC showed 93% sensitivity and 24% specificity, respectively.

We observed a moderate correlation between the levels of TREC and CD4 ($r = 0.55$, $P < 0.01$) and KREC with CD19 ($r = 0.56$, $P < 0.01$). Moderate to low correlation was found between TREC with CD19 ($r = 0.34$, $P < 0.01$) and KREC with CD4 ($r = 0.33$, $P < 0.01$).

Conclusion: In this pilot study, we assessed ability of TREC, KREC and their combined measurements to predict abnormal levels of lymphocyte subsets. The models did not show convincing predictive value, however, combined use of TREC and KREC resulted in a modest ability to rule out abnormal numbers of lymphocyte subsets. Further investigations on a larger cohort are needed to evaluate TREC/KREC abilities to be used as a screening tool on a wider scale.

0077 | Malignancies as the main cause of death in CVID: An Italian long-term multicenter study

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Background: In the last decade, CVID life expectancy increased due to improvements in surveillance, prevention and treatment of recurrent and severe infections, whilst cancer mortality did not change.

Method: In this large CVID Italian cohort (400 patients), we assessed cancer prevalence over a thirty years follow-up and we estimated mortality rate in hematological, gastrointestinal malignancies and in other cancers to assess the quality of the current prevention strategies.

Results: Patients with malignancies were significantly older at CVID-diagnosis (44 ± 2 vs 22 ± 12 years) than cancer-free CVIDs. The prevalence of hematological, gastric and other cancers were 15%, 6% and 20%, respectively. Hematological malignancies were generally non-Hodgkin's B-cell lymphomas and often involved extra nodal sites. The overall survival in hematological cancer, gastric cancer and other malignancies group was: (1-yr) 67%, 54%, 88%; (2-years) 61%, 36%, 80%; (20 years) 61%, 27%, 29%. Treatment of CVID-associated cancer was similar to the treatment of cancers in other settings and included anti-CD20 monoclonal antibodies as a therapeutic agent in B-lymphoid malignancies. The rate of infections during chemotherapy was low, whilst the incidence of severe malabsorption in patients who underwent gastrectomy was high. Moreover, patients who died were significantly more likely to have suffered from cancer.

Conclusion: In our cohort, cancer appeared to be the main cause of death in CVID. The prevalence of CVID-associated malignancies recorded was higher in comparison to other study and rising over the time. CVID with cancer should receive full therapy regimen, due to a risk of infection similar to the non-CVID cancer population.

0078 | Bacterial colonization of respiratory tract in primary antibody deficiencies

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Background: Primary antibody deficiencies (PAD) are characterized by defective Ig production resulting in high susceptibility to bacterial infections, especially caused by S. pneumoniae (Sp) and H. influenzae (Hi). There is a limited evidence on the rate of microbial airway epithelial colonization and on the role of bacterial carriage on the development of recurrent respiratory tracts infections in such populations. The aim of this study was to investigate the prevalence of *Sp* and *Hi* colonization in Common Variable Immunodeficiency (CVID) and Unclassified Antibody Deficiencies (Hypogamma) in Italy and their clinical and immunological correlates.

Method: Nasopharyngeal and oropharyngeal swabs were obtained from 93 CVID and 16 Hypogamma adults under Ig replacement treatment during the period October 2016-April 2017. Presence of *Sp* and *Hi* was investigated using cultural methods and RT PCR. *Sp* isolates were serotyped by the Quellung reaction; capsular type of *Hi* isolates was determined by PCR. Clinical phenotype and respiratory infections' rate over 6 months of follow up (FU) were recorded.

Results: Among CVID, prevalence of carriage assessed by culture was 11% and 27% for *Sp and Hi*, respectively. RT PCR allowed to identify a higher rate carriage of *Sp* and *Hi* compared to standard culture. CVID and Hypogamma had not different rate of *Sp* colonization, whereas CVID had higher rate of *Hi* carriage identified by both culture and RT PCR. No synergistic association between *Sp* and *Hi* colonization was observed. Among CVID, *Sp and Hi* carriage were associated to low IgA/IgM levels. RT PCR merely identified low IgM as risk factors for *Hi* carriage. Bronchiectasis and chronic lung disease were not associated with carriage of *Sp* and *Hi*. The carrier status did not interfere with infections' rate at FU. Twenty percent of CVIDs received antibiotic prophylaxis, most of them macrolides (86%). CVID receiving antibiotic prophylaxis were not more likely to be colonized by *Sp* or *Hi*. All *Hi* isolated were resistant to macrolides. Prophylaxis with Azithromycin increased the risk of *Sp* resistance to macrolides.

Conclusion: IgM and IgA attenuate the risk to be colonized by *Sp* and *Hi*. Passively administered IgG are not transported to the surfaces of epithelial cells, so replacement treatment cannot completely prevent colonization and infections by encapsulated bacteria. Further strategies to replace the mucosal immunity defect are much to be desirable in PADs.

0079 | Sensitisation to Staphylococcus aureus in atopic dermatitis, STAT3-, and DOCK8-hyper-IgE syndrome

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Background: Severe atopic dermatitis (AD) overlaps with hyper-IgE syndromes (HIES) regarding eczema, eosinophilia, elevated serum-IgE levels, and recurrent *Staphylococcus aureus (S. aureus)*infections. HIES are primary immunodeficiencies due to monogenetic defects such as in the genes *DOCK8* and *STAT3* and may serve as human model diseases to better understand pathomechanisms of *S. aureus* sensitization.

Method: We assessed the specificity of serum IgE of AD and HIES patients in the context of clinical and immunological findings.

Results: As previously reported, total serum-IgE levels were similarly elevated in STAT3-HIES, DOCK8-HIES, and AD patients, whereas sensitization pattern differed between the disease groups. AD patients were mainly sensitized against aeroallergens, whereas DOCK8-HIES patients showed clinical allergy and specific IgE against food allergens. STAT3-HIES patients had food- and aeroallergen-specific IgE comparable to non-allergic controls and overall no clinical allergy or TH2-shift. Our preliminary unpublished data show that all patient groups presented with significantly higher serum concentrations of IgE specific to *S. aureus* enterotoxin A, B, C, and TSST compared to healthy controls. The ratio of IgE specific to *S. aureus* toxins was highest in STAT3-HIES patients.

Conclusion: Though total serum IgE is elevated in HIES and AD patients, all disease groups show a different and specific IgE-based sensitization pattern correlating with specific clinical disease and T cell subset phenotypes while we will further elucidate the role of STAT3 signaling as well as of *S. aureus* in regards to allergic disease.

0081 | Efficacy and safety of recombinant human C1 esterase inhibitor treatment for hereditary angioedema attacks: Interim analysis of a European registry

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Background: Recombinant human C1 esterase inhibitor (rhC1-INH) is approved for treatment of hereditary angioedema (HAE) attacks due to C1 inhibitor deficiency (C1-INH-HAE). A European treatment registry was established to review the adverse event (AE) profile and efficacy of rhC1-INH.

Method: Patients with C1-INH-HAE were enrolled following a decision to treat with rhC1-INH. Treatment decisions were at the discretion of the patients' healthcare professionals (HCPs). Using a web-based questionnaire, the HCPs entered data about the attack, response to therapy, and AEs following treatment. An interim analysis of the ongoing registry was performed.

Results: From 01 July 2011 to01 December 2017, 52 C1-INH-HAE patients (23 male/29 female; mean age, 45.3 years; age range, 22-76 years) located in 7 countries reported 1602 attacks and were treated with rhC1-INH within the registry. The mean age at HAE diagnosis was 24.5 years (range, 4-68 years). Before registry entry, patients, including 17 (32.7%) who were on maintenance therapy/prophylaxis at registry enrollment, experienced a mean of 28 HAE attacks in the prior year. There were 732 (45.7%) abdominal, 646 (40.3%) peripheral, 252 (15.7%) oro-facial-pharyngeal, 35 (2.2%) urogenital, and 27 (1.7%) laryngeal attacks; among these were 86 attacks that involved 2 locations and 2 attacks that involved 3 locations. Mean rhC1-INH dose was 3308 IU (44 IU/kg). Patients reported relief of 98.0% of attacks (1570/1602) with rhC1-INH within 4 hours; most attacks (99.9%; 1600/1602) required only a single dose. Two attacks treated with an initial dose of 2100 IU (33 IU/kg and 28 IU/kg) received a second dose of 2100 IU. No hypersensitivity or thrombotic/thromboembolic events were reported. No patients had any related serious AEs.

Conclusion: The rhC1-INH treatment registry provides real-world data that are consistent with previous reports on the safety and efficacy of rhC1-INH.

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MONDAY, 28 MAY 2018

OAS 15

PATHOPHYSIOLOGY IN UPPER AIRWAY DISEASES

0082 | Epithelial barrier dysfunction facilitates IgG1 and IgE responses and inflammation in a murine model

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Background: Epithelial barrier dysfunction has been associated with allergic rhinitis and asthma. We hypothesized that disruption of the barrier facilitates allergic sensitization, and that a barrier restoring drug, fluticasone propionate (FP), will prevent sensitization.

Method: Mice were intranasally exposed to a barrier disturbing agent, chitosan, and OVA as a model-allergen every other day for 12 days +/- nasal treatment with FP or azelastine (AZE) starting 2 days before and continued during the sensitization phase. Outcome parameters were OVA-specific IgG1, total IgE, blood eosinophilia, serum and bronchoalveolar lavage (BAL) β-hexosaminidase and BAL IL-5 levels. To study permeability changes, naïve mice were pretreated with FP or AZE and exposed once to chitosan, followed by OVA.

Results: Chitosan-OVA mice had increased levels of OVA-specific IgG1, total IgE, β-hexosaminidase, eosinophilia, and IL-5 compared to sham-OVA mice. Treatment with FP or AZE each reduced OVA-specific IgG1 and total IgE levels. FP decreased eosinophilia, serum and BAL β-hexosaminidase, and IL-5. AZE reduced BAL β-hexosaminidase levels. One nasal application of chitosan-OVA in naïve mice resulted in elevated levels of OVA and β-hexosaminidase in serum.

Conclusion: Chitosan induces epithelial barrier dysfunction and facilitates sensitization to OVA. Both FP or AZE each reduces sensitization, possibly due to a combination of barrier function preservation and an anti-inflammatory effect by FP, or by the histamine-1 receptor antagonizing effect of AZE.

0083 | Role of IL-13Ra2 in modulating IL-13 induced MUC5AC and ciliary changes in healthy and CRSwNP mucosa

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Background: The IL-13 receptor α2 (IL-13Rα2) is a receptor for IL-13 which has conflicting roles in mediating IL-13 responses in the lower airway; with little known about its impact on upper airway diseases. We sought to investigate the expression of IL-13 receptors, IL-13Rα1 and IL-13Rα2, in chronically inflamed nasal epithelium, and explore IL-13 induced signaling pathways in an *in vitro* model of human nasal epithelial cells (hNECs).

Method: The protein and mRNA expression levels of IL-13 and its receptors in nasal biopsies of patients with nasal polyps (NP) and healthy controls were evaluated. We investigated goblet cell stimulation with mucus hypersecretion induced by IL-13 (10 ng/mL, 72 hours) treatment in hNECs using a pseudo-stratified epithelium in air-liquid interface (ALI) culture.

Results: There were significant increases in IL-13, IL-13Rα1 and IL-13Rα2 mRNA and protein levels in NP epithelium with healthy controls as baseline. MUC5AC mRNA positively correlated with IL-13Rα2 ($r = 0.5886$, $P = 0.002$) but not with IL-13Rα1 in primary hNECs. IL-13 treatment resulted in a significant increase in mRNA and protein levels of IL-13Rα2 only in hNECs. IL-13 treatment induced an activation of extracellular signal-regulated kinases (ERK)1/2 and an upregulation of C-JUN; where the IL-13 induced effects on hNECs could be attenuated by ERK1/2 inhibitor (50 μMol/L) or dexamethasone (10^{-4}-10^{-7} Mol/L) treatment.

Conclusion: IL-13Rα2 has a potential role in IL-13 induced MUC5AC and ciliary changes through ERK1/2 signal pathway in the nasal epithelium. IL-13Rα2 may contribute to airway inflammation and aberrant remodeling which are the main pathological features of CRSwNP.

0084 | Lactobacillus casei AMB-R2 restores nasal epithelial barrier integrity in chronic rhinosinusitis

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Background: A defective epithelial barrier has been demonstrated in patients with chronic rhinosinusitis with nasal polyps (CRSwNP). Lactobacilli are thought to restore epithelial barrier dysfunction, though its effect on barrier function in CRSwNP has not been studied. We here evaluated the barrier restoring capacity of *Lactobacillus casei* AMB-R2 in CRSwNP.

Method: Sinus and/or nasal polyp tissue from patients with CRSsNP (n = 9) and CRSwNP (n = 24) were collected during functional endoscopic sinus surgery. The inferior turbinate from non-allergic patients was used as control (n = 12). The collected tissue was mounted in Ussing chambers and epithelial integrity was evaluated by measuring trans-tissue resistance (TTR) and paracellular flux of FITC-dextran 4 kDa (FD4). Nasal epithelial cells (NECs) from controls (n = 5) and CRSwNP patients (n = 5) were isolated and grown in air-liquid interphase. NECs were stimulated with *L. casei* AMB-R2 (10^7 CFU/mL) for 6 hours and epithelial integrity was evaluated by measuring trans-epithelial resistance (TER). Male BALB/c mice (n = 5/group) were endonasally pretreated with *L. casei* AMB-R2 (10^9 CFU/mL) prior to 3 consecutive applications of *Staphylococcus aureus* enterotoxin B (SEB) (10 μg/mL) to induce barrier dysfunction. One hour later, FD4 (50 mg/mL) was applied to evaluate nasal mucosal permeability.

Results: TTR of sinus tissue and nasal polyps from CRSwNP patients was significantly decreased (10 ± 0.8 vs 32 ± 4, $P < 0.0001$) compared to controls and CRSsNP, associated with an increased FD4 passage ($P < 0.0001$) in CRSwNP patients. *In vitro*, stimulation of CRSwNP cultures with *L. casei* AMB-R2 for 6 hours significantly increased TER (422 ± 43 vs 642 ± 61, $P < 0.05$), and had no effect in control. *In vivo*, FD4 mucosal permeability was decreased in mice treated with *L. casei* AMB-R2 compared to positive control (10 ± 3 vs 19 ± 4 pmols, $P < 0.01$).

Conclusion: The sino-nasal epithelial barrier is disrupted in CRSwNP compared to controls and patients with CRSsNP. Treatment with *L. casei* AMB-R2 restores nasal epithelial barrier integrity in CRSwNP *in vitro* and *in vivo*. Further research is needed to unravel the beneficial effect of *L. casei* AMB-R2 on barrier function.

0085 | Human IL-17-producing type 2 innate lymphoid cells govern neutrophilic inflammation in cystic fibrosis

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Background: Almost half of cystic fibrosis (CF) patients develop chronic rhinosinusitis with nasal polyps (CRSwNP). CRSwNP is characterized by eosinophilia, high IL-5 and IgE concentrations in nasal polyp tissue, whereas in CF-CRSwNP, inflammation is neutrophil driven and sinus cultures typically grow *P. aeruginosa* or *S. aureus*. We have previously shown that the IL-5 producing type 2 innate lymphoid cells (ILC2s) are enriched in NP of non-CF CRSwNP. In CF-CRSwNP, the frequency of ILC2 is diminished, whereas the frequency of $NKp44^-$ ILC3 is increased, as compared to CRSwNP. Here, we identify epithelium-derived TGF-β, IL-1 β, and IL-23 as inducers of ILC2 transdifferentiation in CF-CRSwNP into IL-17-, GM-CSF-, TNF-, and IL-8-producing ILC3s-like cells and their role in neutrophil recruitment and maintenance in the local tissue. We also identify novel markers for ILC plasticity.

Method: NP-derived ILC2 and ILC3 were exposed to: TGF-β, IL-1 β, and IL-23, or co-cultured with NP-epithelium to induce their plasticity. We analysed the progeny of single NP-ILC2 in the presence of IL-1b, IL-23, and TGF-b. Cytokines were measured by ELISA and intracellular staining. Genome-wide expression changes in ILC2s were determined with microarray. Granulocytes composition in NP was determined by FACS.

Results: Increased frequencies of IL-17-producing ILC3 in CF is a consequence of ILC2 plasticity. We show that key cytokines in CF: TGF-β, IL-1β, and IL-23 govern ILC2 transdifferentiation towards ILC3-like IL-17-producing cells. This can be reversed by IL-4, as ILC3 from CF-CRSwNP exposed to IL-4 restored the CRTH2 marker expression and capability to produce IL-5. Single-cell progeny expansion of NP-ILC2 reveals that ILC2s are a heterogeneous population and that a fraction of ILC2s can transdifferentiate into-IL-17 secreting cells, whereas part of these cells remains resilient to conversion. Epithelium is a source of TGF-β, IL-1β, and IL-23 as co-cultures of ILC2 with *P. aeruginosa*-challenged NP-epithelium resulted in IL-17 production by ILC2s. These IL-17 producers may enhance neutrophil recruitment and maintenance, as transdifferentiated ILC2s also produce TNF, GM-CSF, and IL-8. Microarray revealed new markers that may help identify the plastic sub-population of ILC2s.

Conclusion: 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.

0086 | Mucosal innate immunity of the upper airways in children with recurrent bacterial ENT infections

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Background: State of mucosal immunity might play an essential role in pathogenesis of recurrent bacterial acute otitis media (AOM) and rhinosinusitis (ARS). The study objectives were to investigate innate immune response in the upper airways in association with mucosal microbiology in children with AOM / ARS.

Method: 214 children (6.0 (3.7; 12.0) years) with bacterial AOM (31.8 %) or ARS (68.2 %) were enrolled to the study: 128 children with episodic bacterial AOM / ARS (group I) and 86 children with recurrent (≥ 4 episodes a year) course of AOM / ARS (group II). The controls were 30 healthy children. The study investigated patterns of antibiotic use, nasopharyngeal or middle ear exudates cultures and concentrations of antimicrobial peptides (AMPs) - human cathelicidin (hCAP-18/LL-37), lactoferrin (La) and lysozyme (Lys) - in oropharyngeal secretions at early and late disease, and after recovery.

Results: 100.0 % of parents in group II practiced self-prescription of antimicrobials for their child vs only 25.8 % in I group ($P < 0.0001$). II group demonstrated lower rate of typical pathogens of AOM / ARS, but higher rates of microbiology failures and resident *Haemophilus parainfluenzae* and *Staphylococcus aureus* isolates (41.1 % and 50.1 % vs 11.5 % and 35.6 % of all positive cultures in group I respectively, $P < 0.05$). Ampicillin susceptibility of *Str. pneumoniae* and *St. aureus* strains was lower in group II ($P < 0.05$).

In both groups the direction of change in the AMP concentrations was similar: hCap-18/LL-37 and La peaked at the onset of bacterial infection, Lys levels were maximal in the recovery period, and minimal concentrations of all given AMPs were registered in a healthy state. Group II vs group I showed reduced hCap-18/LL-37 and La levels at early disease ($P < 0.01$), reduced hCap-18/LL-37 levels at late disease ($P < 0.05$), and lower Lys levels in all phases of the study ($P < 0.001$). Lys in controls exceeded group II values ($P < 0.001$). Levels of AMPs demonstrated the inverse correlation with disease symptoms duration.

Non-causative bacteria residence was associated with low mucosal AMP levels beyond acute infection, but seems to be linked to temporarily activated local immune response at the onset of bacterial infection, in a specific for each study group way.

Conclusion: In children with recurrent bacterial AOM / ARS malfunction of airway mucosal innate immune mechanisms is present, along with high proportion of resident *St. aureus* and *H. parainfluenzae* in mucosal microbiota.

0087 | Mislocalization and/or absence of RSPH1, RSPH4A and RSPH9 are associated with motile cilia abnormalities in nasal polyps

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Background: Ciliary dysfunction is an associated complication commonly found in patients with nasal polyps (NPs). However, the factors and mechanisms leading to such complications are still poorly understood and remains to be further investigated for their relevance in nasal polyp pathogenesis. Therefore, we sought to assess the expression patterns of radial spokes protein 1, 4A and 9 (RSPH1, RSPH4A and RSPH9) in the nasal mucosa of patients with nasal polyps, in order to establish their role in contributing to ciliary dysfunction in nasal polyps.

Method: Biopsies were obtained from polyp tissues of 86 NP patients and inferior turbinate tissues of 32 healthy controls and analyzed for their expression patterns using immunofluorescence staining. We classified the patterns of RSPH1, RSPH4A and RSPH9 localization as pattern A (presence throughout the axoneme), pattern B (undetectable in distal axoneme), and pattern C (complete absence of throughout the axoneme); where pattern A is the normal baseline. Following which, we developed a semi-quantitative scoring system for which 0 = (pattern A > 70%); 1 = (patterns A + B > 70%); and 2 = (pattern C ≥ 30%) to assess the correlation between pattern score and nasal polyp characteristics.

Results: Based on our scoring system, the median (1st and 3rd quartile) score of RSPH1, RSPH4A and RSPH9 was 0.20 (0.05 and 0.40), 0.20 (0 and 0.68) and 0.23 (0 and 0.80), respectively, for samples from healthy controls; and significantly higher at 1.10 (0.60 and 1.63), 1.25 (0.80 and 1.80) and 1.40 (0.80 and 1.80) for samples from NPs ($P < 0.001$ for all comparisons). Furthermore, compared with patients with non-eosinophilic NPs, the RSPH1, RSPH4A and RSPH9 scores were significantly higher in patients with eosinophilic NPs ($P = 0.010$, 0.044 and 0.005).

Conclusion: The mislocalization and/or absence of RSPH1, RSHP4A and RSPH9 from motile cilia may be associated with the development of NPs, particularly eosinophilic NPs. This finding suggest that these factors may be clinically relevant in the future diagnosis and treatment of NPs.

MONDAY, 28 MAY 2018
OAS 16
LINKING INFECTIONS AND IMMUNE RESPONSES

0088 | Helminth products limit type 2 inflammation by directly suppressing granulocyte activation

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Background: Helminth parasites can modulate immune responses, thus suppressing type 2 inflammation e.g. in allergy. Granulocyte activation is a central event in allergy, but if and how helminth parasites may directly interfere with granulocyte recruitment and activation is unclear.

Method: Mice were sensitized to house dust mite (HDM) intranasally. Helminth products were co-administered locally during the sensitization and challenges. Granulocytes in the bronchoalveolar lavage fluid were enumerated microscopically and by flow cytometry. Chemotaxis of human granulocytes *in vitro* was assessed using trans-well assays. Eicosanoid production or surface marker expression by granulocytes was analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) or flow cytometry, respectively.

Results: We show that products of a helminth parasite suppress granulocyte recruitment both *in vitro* and during house dust mite allergy *in vivo*. Mixed granulocytes or isolated eosinophils from healthy human donors or patients with eosinophilia showed reduced expression of chemotactic receptors (e.g. CCR3) after treatment with helminth products. In addition, the lipid mediator profile of human granulocytes was substantially altered after helminth product treatment, resulting in a pronounced suppression of pro-inflammatory leukotrienes.

Conclusion: Our findings suggest that helminths may regulate type 2 immune responses by directly modulating granulocyte activation and recruitment. Thus, treatment with helminth products could be a promising immunomodulatory approach to treat inflammatory diseases with aberrant granulocyte activation.

0089 | Transcriptomic analysis of a nasal In vitro model from multiple individuals revealed nasal initiated tissue specific signatures in an influenza metagenomics analysis

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Background: *In vitro* and *in vivo* research based on cell lines and animals are likely to be insufficient in elucidating certain biological and physiological phenomena mimicking human systems, especially for generating pre-clinical trial data. There is an obvious demand for a model that can further bridge the gap between experimental data and pre-clinical/clinical trials to elucidate the differential responses, especially at the primary target site of respiratory viruses, the nasal epithelium.

Method: Our lab has previously generated an *in vitro* differentiated human nasal epithelial cells (hNECs) model. We showed that these cells, the primary target of respiratory viruses, can display the repertoire of immune responses against respiratory viruses such as influenza A virus and rhinovirus, which is useful for identification of processes initiated at the nasal epithelium. Building on this, we performed microarray analysis of influenza infected hNECs to elucidate nasal responses; followed by metagenomic analysis to show that hNECs is comparable to human clinical models. We then further identified nasal initiated pathway from the primary infection site as a result of influenza infection

Results: Metagenomic analysis showed that hNECs transcriptomic changes following influenza infection is comparable with clinical array data, including datasets with high sample sizes. The primary target site for influenza infection is found to express innate and adaptive immune genes, which was initiated via pathogen sensing through RNA sensors, followed by immune signaling via the JAK-STAT, interferon and interleukin pathways, that leaned heavily towards Th1 activation. Additionally, our model also elucidated down-regulation of metabolic processes not present in blood serum analysis.

Conclusion: In conclusion, this study supports the utility of the hNECs model as a complementary model to acquire differential responses against various respiratory virus infections in the nasal epithelium, which is found to participate heavily in the initiation of immune responses and other pathways leading to the disease burden and resolution of infections.

0090 | Activation of inflammasome upon rhinovirus infection in asthmatic airway epithelium is enhanced by house dust mite exposure

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Background: Inflammasomes are important biological complexes participating in the pathophysiology of several inflammatory disorders. There is limited knowledge of the role of inflammasome activation in human asthmatic bronchial epithelium. We aimed to understand the involvement of inflammasome activation during exposures to house dust mite (HDM) and human rhinovirus (HRV16) in the pathogenesis of asthma and upon viral infections.

Method: Primary human bronchial epithelial cells (HBE), bronchoalveolar lavage fluid (BAL) samples and bronchial biopsies from control and asthmatic patients at baseline and/or after infection with HRV16 were analysed using next generation sequencing, multiplex immunoassays and confocal microscopy. Air-liquid interphase cultures of HBE treated with HDM and HRV16, mouse models of HDM-induced allergic airway inflammation and poly-IC-induced lung inflammation mimicking viral-induced lung inflammation were used to examine inflammasome activation and expression of inflammasome-related molecules.

Results: We found striking changes in the expression of inflammasome-related genes in HBE after HRV16 infection in healthy and asthmatic subjects. Changes in inflammasome- and virus-related pathways and their functions were accompanied with full activation of inflammasome, represented by formation of ASC specks, increased secretion of IL-1ß, which was blocked with caspase-1 inhibitor, but not with a specific NLRP3 inflammasome inhibitor. Notably, release of mature IL-1ß was limited to the apical surface of polarized cells, which corresponded with apical expression of ASC in human lung biopsies and increased IL-1ß secretion into BAL of asthmatic patients. Upregulation of *DDX58* (RIG-I) gene expression upon HRV16 infection and HDM exposure in HBE, altogether with *ex vivo* apical expression of RIG-I protein in epithelium in human lung biopsies highlight that RIG-I is a candidate for inflammasome sensor of viral infections in HBE. Additionally, increased expression of several inflammasome-related genes such as *Aim2*, *Casp1*, *Asc* and *Il1b*confirms inflammasome signature of lungs from mouse models of HDM-induced asthma and poly-IC-induced lung inflammation with specific upregulation of viral sensors namely *Ddx58* and *Mda5* only in poly-IC model mimicking viral-induced lung inflammation.

Conclusion: These data demonstrate house dust mite priming enhances inflammasome activation and apical accumulation of inflammasome-related molecules in asthmatic bronchial epithelium after rhinovirus infection.

0091 | The characteristics of serum hydroxy-eicosatetraenoic acids in allergic bronchopulmonary aspergillosis

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Background: Hydroxy-eicosatetraenoic acids (HETEs) are a class of monohydroxy fatty acids that are metabolized by arachidonic acid (AA). They may be involved in the pathogenesis of asthma, but little is known about their potential roles in allergic bronchopulmonary aspergillosis (ABPA). This study was performed to evaluate serum HETEs in ABPA patients and to observe the changes of HETEs levels during treatment.

Method: *S*era from ABPA (N = 20), fungal associated asthma (FAA, N = 15), non-fungal associated asthma (NFAA, N = 23) patients and the healthy controls (N = 12) were assessed for 14 eicosanoids by using ultra-high performance liquid chromatography-quadruple-time of flight/MS (UHPLC-Q-TOF/MS). In addition, sera from 12 ABPA patients were collected at 1, 2, 3, 6 months after treatment in our hospital.

Results: Compared with FAA and NFAA subjects, ABPA subjects expressed lower levels of serum AA, 5(S)-HPETE, 12(S)-HPETE and 5(S)-HPETE ($P < 0.05$). However, their levels of serum 15(S)-HETE and 12(S)-HETE were higher than in NFAA subjects and HC groups ($P < 0.05$). After treatment, the serum levels of 15(S)-HETE, 12(S)-HETE, 11(S)-HETE, 8(S)-HETE and 5(S)-HETE in ABPA subjects all decreased with the decrease of *Af.* sIgE concentration. Additionally, in APBA subjects, there was a positive correlation between serum AA and tIgE (rho = 0.649; $P < 0.05$). Serum 11(S)-HETE was positively correlated with *Af.* sIgE, FVC and FEV1 respectively (rho =

0.659, $P < 0.05$; rho = 0.753, $P < 0.01$; rho = 0.800, $P < 0.01$). Serum 15(S)-HETE was positively correlated with the ratio of FEV1/FVC (rho = 0.632, $P < 0.05$). Simultaneously, both 12(S)-HETE and 15(S)-HETE showed a positive correlation with peripheral blood eosinophil count (rho = 0.683, $P < 0.05$; rho = 0.681, $P < 0.05$).

Conclusion: *Our data suggest that the increased release of* 15(S)-HETE, 12(S)-HETE and 11(S)-HETE in ABPA subjects might, at least partially, be a mechanism underlying ABPA. Furthermore, the novel finding of serum levels of HETEs in ABPA patients during treatment indicates that HETEs may help in future in monitoring the progression of ABPA.

0093 | Functional basophil and lymphocyte assays for *Aspergillus fumigatus*-related disease in lung transplant recipients

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Background: Lung transplantation (LTx) is nowadays a valid therapeutic option for selected patients with end-stage lung disease despite an optimal medical therapy. Infection with *Aspergillus fumigatus* (AF) increases the risk of chronic lung allograft dysfunction (CLAD) which is the main cause of death after 3 years post-LTx. AF may responsible for various clinical presentations ranging from bronchial colonization to invasive pulmonary aspergillosis, aspergilloma, or allergic bronchopulmonary aspergillosis (ABPA). However, the diagnosis of AF-related disease (ARD) remains challenging. We conducted a pilot study to assess the potential value of functional basophil and lymphocyte activation tests (BAT, LAT) for ARD after LTx.

Method: Nineteen lung transplant recipients (LTR) (11 cystic fibrosis (CF), 4 idiopathic pulmonary fibrosis, 3 emphysema/COPD, 1 diffuse bronchiectasis) and 10 non transplanted CF patients were evaluated. Four groups were defined according to AF status: AF colonization (AFC) without ABPA or AFS; AF sensitization (AFS): detectable AF-specific IgE; ABPA as defined by ISHAM 2013; no ARD. BAT was performed with the Flow2CAST kit and AF allergen extract (Bühlmann Laboratories, Switzerland). For LAT, after a 24-hour incubation with AF extract, lymphocyte activation was evaluated with CD69 expression.

Results: Both LTx and AF status did not impair basophil activation by anti-FceRI antibodies (median > 90% of CD63 + basophils in all groups). LTx was associated with lower levels of fMLP-induced basophil activation (43% of CD63 + basophils in LTR vs 57% in non-transplanted patients, *P* 0.02). AF-induced basophil activation was found in 2/3 ABPA non transplanted CF patients, in 3/3 LTR with ABPA-like symptoms, in 2 LTR with a past history of ABPA preceding LTx, and in 1 LTR who developed ABPA 3 months later. AFC status did not interfere with BAT outcome. TCD4 and TCD8 from both LTR and non-transplanted patients displayed similar responses to phytohemagglutinin, and did not react to AF extract in ABPA or AFS patients.

Conclusion: Our findings show that BAT and LAT are promising diagnostic tools for ARD workup in LTx recipients. The feasibility of the assays is demonstrated. BAT results show promising sensitivity for ABPA diagnosis. LAT results show the absence of impairment by immunosuppression suggesting that regardless of the transplant status, ARD is independent from lymphocyte activation.

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OAS 17
FOOD ALLERGY AND BEYOND

0094 | Efficacy and safety of low-dose oral immunotherapy for patients with severe cow's milk allergies: A 3-year follow-up

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Background: Continuous intake of 3 mL of cow's milk (CM) enable patients to consume 25 mL, which allows the patient to consume some processed foods containing CM (Yanagida; IAA. 2015). Our current study aimed to investigate low-dose oral immunotherapy (LDOIT) using 3 mL of CM during a 3-year follow-up.

Method: Patients who were >5 years old with systemic symptoms during an oral food challenge (OFC; 3 mL of CM) were enrolled and divided into an LDOIT group (n = 18) and a control group (n = 15). Starting from a dose without symptoms, patients gradually increased the dose to 3 mL. One year after starting the LDOIT and after avoiding CM for 2 weeks, the subjects underwent a 2-day OFCs with 3 mL and 25 mL of baked CM. Subjects with positive results continued the 3-mL LDOIT, and the OFC was repeated 1 year later. Subjects who passed the 3-mL and 25-mL OFCs were allowed to consume processed foods that included 25 mL of baked CM in their home on a daily basis. Subjects who remained asymptomatic after consuming the processed foods with baked CM were considered to have achieved sustained unresponsiveness to the 25-mL challenge (25-mL SU). The control group included subjects who reacted to the 3-mL OFC, completely eliminated CM, and underwent the OFC 3 years later.

Results: The median baseline ages/CM-specific IgE titers/threshold reactivity dose were 8.8 years/48.7 kUa/L/1.6 mL in the LDOIT group and 5.7 years/26.1 kUa/L/2.4 mL in the control group. In the LDOIT group, 25-mL SU was achieved by 3 patients (17%) after 1 year, 6 patients (33%) after 2 years, and 9 patients (50%) after 3 years. Only 1 patient (6.6%) in the control group achieved 25-mL SU after 3 years ($P = 0.007$). After 3 years, the LDOIT group had significantly decreased CM-specific IgE titers ($P < 0.01$), although no significant change was observed in the control group. The LDOIT group experienced moderate and severe adverse reactions during 1.2% of all intakes (143/12 268) and intramuscular adrenaline treatment was rare (0.02%, 2/12 268).

Conclusion: Prolonged LDOIT may be safe and effective for patients with severe CM allergies, and could improve their quality of life.

0095 | Clinical features and prognostic factors in children with food protein induced allergic proctocolitis: A multicenter study

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Background: The frequency of food protein-induced allergic proctocolitis (FPIAP) has increased in the last decade, however there is limited information regarding the features, risk factors and prognosis of the disease.

Method: The study was conducted in five different allergy outpatient clinics and included children less than one year of age who were admitted with rectal bleeding and had a diagnosis of FPIAP and also who were under follow-up with FPIAP during the study period between September 2016 and August 2017. All patients were evaluated for clinical features, prognosis and prognostic factors.

Results: Overall, 257 infants were evaluated and 50.2% (n = 129) were female. The onset age of the symptoms was 3 months [Interquartile range (IQR): 2-4]. The frequency of cesarean delivery rate, prematurity, and breastfeeding were 61.6%, 13.6% and 97.2%, respectively. Cow's milk (99.2%) was the most common trigger, while 24.5% of the patients had multiple food allergies. Other offending foods were egg (22.9%), beef (5.5%), walnut (3.2%), wheat (2.4%), peanut (2.4), chicken meat (2.4%) and fish (2%). 12.8% (n = 33) of our patients had atopic dermatitis. Tolerance developed before the age of one year in 28.7 % of the patients. Tolerance developed between the age of 1–2 years in 60.3%, between the age of 2–3 years in 10.3% and after the age of 3 years in 0.7% of the patients. In the multivariate logistic regression analysis, multiple food allergy [3.679 (95% CI: 1.278-10.593, $P = 0.016$), presence of colic [7.3 (95% CI: 2.27-23.8, $P = 0.001$] and skin prick test (SPT)/sIgE positivity [3.964 (95% CI: 1.424-11.034, $P = 0.008$) were found to be risk factors for the persistence of allergy.

Conclusion: SPT/sIgE positivity, multiple food allergy and presence of colic were found to be risk factors for persistent disease in patients with FPIAP.

0096 | The effect of sleep deprivation and exercise on peanut allergy thresholds: Insights from the Thresholds of Reactivity and Clinical Evaluation (TRACE) Study

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Background: Peanut allergy is a public health concern affecting 2% of the US population and can cause fatal allergic reactions upon accidental peanut consumption. Population reactivity thresholds to peanut are currently being defined to protect the allergic population and improve food labelling. It is currently unknown how common co-factors such as stress or exercise impact these thresholds which may have crucial implications for allergen risk management.

Method: In a cross-over study we investigated how sleep deprivation (mimicking stress) and exercise influence reaction threshold in 101 peanut allergic adults. Following confirmation of allergy by double-blind, placebo-controlled peanut challenge, participants underwent three further open challenges in a randomly assigned order: One with exercise following each dose, one with sleep deprivation on the night preceding challenge and one with neither co-factor (no intervention). The primary endpoint was threshold eliciting dose at each challenge. We estimated the difference in average log-threshold between challenges with and without a co-factor using a linear mixed effects model.

Results: 64 subjects (mean age 25y) completed all challenges. The median (confidence interval) eliciting doses for 1%, 5% and 10% of the population during no intervention challenge were 1.5 mg (0.8, 2.5), 4 mg (2.4, 6.4) and 6.7 mg peanut protein (4.1, 10.5), respectively. The estimated changes in log-threshold for exercise and sleep (95% confidence intervals) were -0.576 (−0.938, −0.215; $P = 0.002$) and −0.615 (−0.976, −0.253; $P = 0.001$) respectively.

Conclusion: Exercise and sleep deprivation significantly reduce reaction thresholds to peanut. Accounting for this variation due to co-factors is critical in population threshold modelling for enhanced protection of peanut allergic consumers.

0097 | Threshold of peanut reactivity: An international multi-centre survey of hospital-based open challenges

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Background: Although management of food allergies typically involves avoidance of the food, many specialist allergy centres now have programs of desensitisation to milk, egg and increasingly peanut. This international survey aimed to determine the threshold of peanut to which children reacted during hospital-based open peanut oral challenges.

Method: Anonymised data from clinical records were retrospectively collated from six specialist paediatric allergy centres in the UK, one in Ireland and from the HealthNuts study from Australia. Demographic information, allergy test results and outcome of oral challenges to peanut were recorded. For patients failing their peanut challenge, the amount of peanut to which they reacted and nature of the reaction were also recorded.

Results: 1604 children aged 1 to 18 years old underwent open peanut challenges. 271 (22%) had a previous clinical history of possible or likely allergic reaction to peanuts, 509/777 (66%) had never reacted to peanut but had a positive skin prick test and/or a positive peanut specific IgE. 590 (37%) reacted during challenge with objective clinical signs of allergy. Of these, 58 (10%) had evidence of anaphylaxis, mainly wheeze. 38% reacted to 100 mg or less of peanut, while 48% of patients reacted only after consuming 1 g or more.

Conclusion: Half of the children in the cohort tolerated up to one peanut (equivalent to one peanut) before developing objective clinical evidence of allergy and therefore would be excluded from current trial based immunotherapy protocols. Other modalities of immunotherapy need to be considered for such children.

0098 | Evidence of an abnormal epithelial barrier in active, untreated and corticosteroid-treated eosinophilic esophagitis

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Background: Eosinophilic esophagitis (EoE) is a chronic, immune/antigen-mediated disease characterized by symptoms related to esophageal dysfunction and an eosinophil-predominant inflammation. This study has aimed to investigate whether the recently observed sensitization to *Candida albicans* in EoE patients is owing to pre-existing disease and its underlying abnormal epithelial barrier or, alternatively, is linked to corticosteroid (CS) therapy.

Method: Medical histories, as well as serum and tissue samples of 60 EoE patients (15 CS-naive, 45 with current or previous CS therapy) and 20 controls, stored in the Swiss Eosinophilic Esophagitis Database (SEED) and Biobank, were analyzed. We applied ImmunoCAP to measure IgE levels and immunofluorescence techniques to examine epithelial barrier components.

Results: EoE patients had higher total IgE levels and were more frequently sensitized to *Candida albicans* than controls. In EoE tissue specimens, increased numbers of eosinophils and mast cells, a higher expression levels of thymic stromal lymphopoietin (TSLP), cathelicidin, proteases, i.e. the kallikreins (KLK)-5 and KLK-7, were observed as compared with controls, while reduced expression of lympho-epithelial Kazal-type-related inhibitor (LEKTI), filaggrin, E-cadherin, claudin, occludin, demoglein-1 was found, independent of CS therapy. In CS-treated EoE, significantly lower numbers of CD1a+ cells and cathelicidin expression were noted as compared to CS-naive EoE.

Conclusion: This study provides further evidence that EoE is associated with an abnormal epithelial barrier and postulates that CS therapy, by reducing innate immune mechanisms, may promote *Candida albicans* colonization and likely subsequent sensitization.

0099 | Biomarkers in profilin-mediated food allergy: Platelet exhaustion as a characteristic of severe allergic profile

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Background: The prevalence of allergic diseases and their severity have increased worldwide, so it is necessary to improve the classification of allergic patients in order to give them an effective treatment. In some areas of Spain with high levels of grass pollen, allergic patients become sensitized to minor allergens, such as profilin, and surprisingly, some of them develop severe profilin-mediated food allergic reactions. Our objective was to identify genetic biomarkers useful to improve the classification and treatment of patients with a higher probability of developing severe reactions.

Method: 22 patients were classified in 4 groups, according to their reactions to purified profilin oral challenge test: Non-allergic (n = 6), mild (n = 5), moderate (n = 6) and severe (n = 7). RNA extraction was performed on ficoll-isolated PBMCs using the RNeasy® Mini Kit (Qiagen) and its quality was assessed with Experion RNA StdSens analysis kit (Bio-Rad). The gene expression profile of all samples was analyzed using GeneChip® WT PLUS Reagent Kit (Thermo Fisher Scientific) and two specific softwares: Affymetrix® Expression Console™ and Affymetrix® Transcriptome Analysis Console (TAC). Pathways and gene regulators involved in this allergic process were analyzed performing a Gene Set Enrichment Analysis (GSEA) and using Ingenuity Pathway Analysis (IPA) software. The microarray results were validated by quantitative real-time PCR (qPCR).

Results: A total of 596 transcripts were identified as being significantly different among all experimental groups. The biggest differences were observed between mild and severe groups and moderate and severe groups. In order to characterize the severe profile, we carried out a GSEA and an IPA analysis of these comparisons. The results showed that most representative pathways and functions were related to platelets. Moreover, these pathways were significantly downregulated and presented a negative Z-score in severe patients compared to mild and moderate groups.

Conclusion: In the course of our study, we found out that severe patients have a different transcriptomic profile compared to mild and moderate patients and there are transcripts involved in platelets functions that should be considered as potential biomarker as they change in relation to the degree of allergic inflammation. These findings might lead to improve the diagnosis and treatment of this type of allergic patients.

MONDAY, 28 MAY 2018
OAS 18
MECHANISMS OF ASTHMA

0100 | Identification of novel genes associated with asthma exacerbations in Puerto Ricans

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Background: Asthma is a chronic inflammatory disease of the airways whose symptoms may be controlled in most patients. However, many of them manifest asthma exacerbations, which may threaten their life. Among populations from the United States, childhood asthma prevalence and exacerbation rates are highest in Puerto Ricans and lowest in Mexicans. Although it is known that both environmental and genetic factors are involved in asthma exacerbations, no previous study has identified genes associated with this outcome in Latino populations. Here, we aimed to identify genetic variants associated with asthma exacerbations among Puerto Ricans and to assess whether they are also relevant in Mexicans.

Method: We performed a genome-wide association study of asthma exacerbations among 1140 Puerto Ricans children and young adults with asthma from the GALA II study. Imputation of genetic variants was carried out using the Haplotype Reference Consortium as reference panel by means of the Michigan Imputation Server. Association of 10.6 million genetic variants with minor allele frequency ≥1% was tested by means of logistic regression models, including as outcome the presence of exacerbations during the past 12 months (hospitalizations, emergency room visits or use of oral corticosteroids) and adjusting by age, sex and genetic ancestry. A population-specific empirical threshold was applied to declare genome-wide significance ($P \leq 1.83x10^{-7}$), and variants suggestively associated with asthma exacerbations among Puerto Ricans ($P \leq 5x10^{-6}$) were examined for replication in 658 Mexicans from the GALA II study.

Results: A total of 4 variants were genome-wide significantly associated with asthma exacerbation in Puerto Ricans (minimum *P*-value = $9.1x10^{-8}$) and 43 additional were suggestively associated. These variants were located at 11 different loci, including some potential relevant genes, such as *TADA2B*, *GRPEL1*, *DMRT1*, and *FBXO9*. Interestingly, a recent genome-wide association study also identified a variant in the putative promoter of *DMRT1* as associated with childhood asthma with sex specific effects. However, none of the variants was significantly associated in Mexicans.

Conclusion: We identified several novel genes suggestively associated with asthma exacerbations in Puerto Ricans that might be population-specific. Validation will be performed in further independent studies.

0101 | The expression and function of miR-146a/b in human bronchial epithelial cells

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Background: Asthma is a chronic inflammatory disease of the airways that can be categorized into eosinophilic, neutrophilic and paucigranulocytic inflammatory phenotypes, defined by the presence and/or absence of airway eosinophils and neutrophils in the airways. Patients from all asthma phenotypes occasionally experience exacerbations, which are frequently caused by human Rhinovirus (RV) infections. RVs replicate in human airway epithelial cells, where they activate interferon response and the NF-κB pathway. microRNAs (miRNAs) are post-transcriptional gene expression regulators that are involved in many biological processes, including inflammation. Our study is focused on investigating the expression and functions of miR-146a and miR-146b (miR-146a/b) in human bronchial epithelial cells (HBECs).

Method: Differential cell counts were measured in bronchoalveolar lavage fluid. Quantitative real-time PCR was used to analyse gene expressions of IL-8, CCL5, CXCL1, IRAK1, CARD10 in HBECs from asthmatic patients. miR-146a and miR-146b expression was quantified by RT-qPCR. To study miR-146a/b expression and functions during RV infection, HBECs were infected with RVs for 24 or 48 hour (MOI 0.01).

Results: We show that miR-146a/b expression was downregulated in HBECs from asthma patients brush biopsy samples when compared with non-asthmatic subjects. In particular, the expression of miR-146a/b was downregulated in HBECs of patients with neutrophilic asthma compared with eosinophilic and paucigranulocytic

inflammatory phenotypes. In contrast, upregulated expression of miR-146a/b-influenced pro-inflammatory chemokines, IL-8 and CXCL1, was detected in HBECs from patients with neutrophilic asthma.

When HBECs were infected with RV 1B and A16, we detected increased expression of miR-146a/b and miR-146a-influenced chemokines CCL5, IL-8 and CXCL1. When HBECs were transfected with miR-146a before the infection with RVs, the expression of IL-8, CCL5 and CXCL1, as well as miR-146a direct targets from the NF-κB pathway, IRAK1 and CARD10, was significantly reduced.

Conclusion: In conclusion, we show that miR-146a expression is altered in HBECs from neutrophilic asthma patients and during RV infection. In addition, our results demonstrate the capacity of miR-146a to suppress direct target genes from the NF-κB pathway and pro-inflammatory chemokines in HBECs and indicate that reduced expression of miR-146a/b might be associated with neutrophilic asthma.

0102 | Analysis of T cell-induced bronchoconstriction

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Background: To investigate a role of helper T (Th) cells in asthma, culture supernatants of activated T cells were analyzed for the constriction of cultured bronchial smooth muscle cells.

Method: Ovalbumin (OVA) specific Th clones were derived from either the regional lymph nodes of Balb/c mice immunized with OVA/CFA or splenocytes of DO11.10 transgenic mice expressing T cell receptor specific for OVA/H-2d. Th clones were adoptively transferred into unprimed mice. Upon antigen challenge, airway resistance was continuously monitored by either unrestrained whole body plethysmography (BUXCO) or resistance/compliance analyzer under anesthetized condition. Bronchoalveolar lavage was performed 48 hr after antigen challenge. Supernatants of stimulated Th clones were analyzed for contractile activity using collagen gels embedded with murine primary bronchial smooth muscle cells. Effects of antagonists against various mediators were analyzed both *in vitro* and *in vivo*.

Results: When unprimed mice were transferred with Th clones, T5-1, T6-2, T6-4, and T6-7, Penh values were significantly increased 6 hr after OVA challenge. In contrast, mice transferred with other Th clones, BF7, T6-1, or T6-10 did not show any change. Airflow limitation was confirmed by a direct measurement of airway resistance under anesthetized, restrained, and intubated conditions. Contractile activity was detected in the supernatants of T6-2 stimulated with immobilized anti-CD3. T cell-induced contraction was not affected by H1R or LTR1 antagonist.

Conclusion: T cell activation caused airflow limitation in addition to eosinophilic inflammation, AHR, and mucous hyperplasia. T cell-derived bronchoconstriction seems a good target for treatment-resistant asthma.

0103 | Generation of neutrophil mediators during an experimental infection with rhinovirus-16 in asthmatic patients

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Background: Rhinovirus (RV) accounts for the large majority of viral infections causing asthma exacerbations among children after 3 years of age and among young adults. RV infections are also known to stimulate the recruitment of neutrophils into the airways and these cells have also been reported to aggravate airway inflammation in asthma. We postulate that mediators generated from neutrophils during an experimental infection with RV-16 will be increased in nasal washes (NW's) from asthmatics compared to non-asthmatic controls during the course of infection.

Method: Sixteen subjects (ages 19-33) were inoculated with RV-16 (dose = 300 $TCID_{50}$). They included 9 allergic-asthmatics (total IgE levels 596-1989 IU/mL) and 7 controls (total IgE levels 5-42 IU/mL). Neutrophil mediators (both neutrophil elastase (NE) and myeloperoxidase (MPO)) were measured by ELISA in NW's obtained before virus inoculation (on Day 0) and during the infection (i.e., on Days 1, 2, 3, 4, 7, 14, 21 following RV inoculation). The results were compared to symptom scores.

Results: Both NE and MPO levels peaked in NW's on Day 3 of the infection, paralleling the development of peak cold symptoms in both asthmatics and controls. Compared to baseline values before inoculation, mediator values by Day 3 increased 7 and 5-fold for NE and MPO, respectively, among the asthmatics, compared to 5 and 3-fold, respectively, among controls. Cumulative values (summed over the first 3 days following RV inoculation) trended higher in asthmatics compared to controls (e.g., geometric means for NE = 3775 ng/mL and 2167 ng/mL, respectively, $P = 0.45$; MPO = 1375 ng/mL and 917.3 ng/mL, $P = 0.66$), but this trend was no longer apparent during resolution of the infection (days 14 and 21).

Conclusion: During the early (innate) phase of the infection, a consistent trend in higher levels of neutrophil mediators was observed in nasal washes from asthmatic subjects compared to non-asthmatic controls. This increase was no longer seen during resolution of the infection.

0104 | Determination of potential biomarkers in innate immune and inflammasome signaling for the prediction of childhood wheeze

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Background: Asthma is known as most common chronic airway inflammatory disease in children worldwide, with increasing prevalence. In preschool-age, asthma presents as wheezing triggered by different factors (multitrigger/MTW and viral/VW). An early prediction and their subsequent risk for asthma is currently difficult and underlying immune mechanisms of distinct wheeze phenotypes are unknown. Therefore, we aimed to define the course of wheeze phenotypes based on innate and inflammasome-associated immune mechanisms at birth.

Method: Cord blood mononuclear cells (CBMCs) from newborns (n = 283) of the PAULINA/PAULCHEN birth cohort were isolated, kept unstimulated or were stimulated with phytohemagglutinin (PHA) or lipid A (LpA) for 72 hour. Selected genes related to the innate immune system and to the inflammasome were analyzed by qRT-PCR and cytokine levels by Multiplex Immunoassay. Wheeze phenotypes and healthy controls (HC) were defined via a follow-up by parental questionnaires at 3 and 6 years of age. Data analyses were performed with *R* software, using parametric ANOVA and/or nonparametric Wilcoxon test (two groups) or Kruskal-Wallis test (three groups). Statistical significance was defined as $P < 0.05$.

Results: Overall, children with MTW present a common pattern characterized by increased gene expression of innate- and inflammasome pathway genes at birth, while VW rather showed a downregulation, respectively. NLRP3 was increased in unstimulated cells of MTW ΔC_T = 11.85 [10.71;12.88] vs HC ΔC_T = 12.69 [12.21;13.57], $P = 0.081$ and in PHA-stimulated cells ΔC_T = 14.30 [13.39;15.65] vs HC ΔC_T = 15.26 [14.74;16.54], $P = 0.015$. In contrast, VW showed a downregulation in unstimulated cells ΔC_T = 12.84 [12.28;15.18], $P = 0.028$ and PHA-stimulated cells ΔC_T = 16.07 [15.34;18.67], $P = 0.005$ and LpA-stimulated cells ΔC_T = 13.90 [13.19;14.30], $P = 0.014$. Identical significant effects were observed for IL-1R1, and for genes involved in innate immunity (TLR5, TLR7, MDA5, CLEC7A). This indicates different disease entities, characterized by distinct immune regulation at birth for subsequent wheeze phenotypes.

Conclusion: At birth, we identified activated gene expressions of the innate and inflammasome-pathways in children with subsequent MTW. Further analyses including the 10 year-follow up may indicate whether innate and inflammasome pathways may serve as early biomarkers for a trajectory of childhood asthma.

0105 | Aerobic exercise inhibits asthma phenotype involving inhibition of purinergic signaling and lymphoid organs hyperactivation

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Background: Asthma is a chronic inflammatory airway disease, in which purinergic signaling has a central role, controlling activation of structural and hematopoietic cells, involving lymphoid organs hyperactivation. Aerobic exercise (AE) present anti-inflammatory effects to the airways, but the cellular and molecular mechanisms are unknown. Thus, this study evaluated the effects of AE on purinergic signaling and on lymphoid organs hyperactivation in a model of asthma induced by house dust mite (HDM).

Method: AE was performed in a treadmill at moderate intensity, 5x/week, 1 h/session, for 4 weeks, beginning 2 weeks after HDM administration. HDM (*dermatophagoides pteronyssinus*; 100 mg/mouse) was administered 3x/week, for 6 weeks.

Results: The results demonstrated that AE reduced adenosine triphosphate (ATP) accumulation ($P < 0.001$), IL-1beta, IL-4, IL-5, CXCL1/KC, IL-13, IL-17, IL-23, IL-33 and TNF-alpha ($P < 0.001$), while increased IL-1ra, IL-2 and IL-10 in bronchoalveolar lavage (BAL). Total number of leukocytes, eosinophils, lymphocytes and neutrophils in BAL and the number of eosinophils, neutrophils and lymphocytes in the airway wall ($P < 0.01$) were reduced by AE. Airway collagen, elastin, smooth muscle and mucus were reduced by AE ($P < 0.01$). TGF-beta, IGF-1 and VEGF levels was reduced by AE ($P < 0.001$). Lung mechanics (Resistance, Elastance, GTIS, HTIS, RAW) and airway hyperresponsiveness (AHR) to methacholine was ameliorated by AE ($P < 0.01$). IL-4, IL-5 and IL-13 production by re-stimulated mediastinal lymph nodes, splenocytes and bone marrow cells was also reduced by AE. The expression of P2X7, P2Y2 and P2Y6 by peribronchial leukocytes ($P < 0.01$) and also by airway epithelial cells ($P < 0.01$) were reduced by AE.

Conclusion: AE reduces asthma phenotype by inhibiting purinergic signaling and lymphoid organs hyperactivation in a model of HDM-induce asthma.

MONDAY, 28 MAY 2018
OAS 19
DRUG HYPERSENSITIVITY AND CROSS-REACTIVITY

0106 | Cefprozil cross-reactivity in amoxicillin-allergic children

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Background: A label of penicillin allergy is associated with an increased use of alternative antibiotics such as cephalosporins. Cefprozil is a second-generation cephalosporin widely used in North America for common childhood infections and it shares an identical R1 side chain with amoxicillin. However, to this date there is no published data on the tolerance of cefprozil in amoxicillin-allergic patients. Thus, the aim of the present study was to determine the rate of cross-reactivity to cefprozil in amoxicillin-allergic Canadian children.

Method: Sample consisted of consecutive children evaluated at the CHU Sainte-Justine allergy clinic for a history suggestive of hypersensitivity to amoxicillin or amoxicillin/clavulanic acid between February 2015 and May 2017. Investigation was first performed by prick and intradermal skin tests with penicilloyl-polylysine (PPL), benzylpenicillin (BP) and amoxicillin, and intradermal skin tests with PPL and BP. Patch tests with amoxicillin were also performed in patients with a suspicion of severe delayed hypersensitivity. Patients then underwent drug provocation test (DPT) to the culprit drug, amoxicillin or amoxicillin/clavulanic acid (45 mg/kg), in the allergy outpatient clinic. Finally, cefprozil DPT (15 mg/kg) was offered to all amoxicillin-allergic patients. If negative, each DPT was followed by a four-day ambulatory course with the concordant agent.

Results: We evaluated 1 156 children with a mean age of 7.2 years (0.9—18.0). Mean time elapsed between the index reaction and the allergy investigation was 4.1 years (0.1-14.3). Amoxicillin allergy was confirmed in 53 patients (4.6%; 95% CI 3.5-6.0) and selective clavulanic acid allergy in one patient. All patients presented a mild to moderate delayed hypersensitivity, 53 confirmed by DPT and one by patch test. Skin tests were positive in one patient only. Cefprozil DPT was performed in 39 amoxicillin-allergic patients and resulted in a delayed reaction in three (7.7%; 95% CI 1.9-21.0).

Conclusion: We describe here the first systematic assessment of clinical cross-reactivity between amoxicillin and cefprozil, a cephalosporin widely used in North America and that shares an identical R1 side chain with amoxicillin. Moreover, our cohort strengthens the idea that DPT is the most reliable test for delayed type hypersensitivity.

0107 | Low cross-reactivity between cephalosporins and penicillin: Results from a Danish allergy center

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Background: Patients with suspected allergy to antibiotics are abundant and constitute a therapeutic problem in all hospital settings. Reactions to penicillins are by far most often reported, and cephalosporins are in these cases often considered therapeutically beneficial as substitute for penicillin. We investigated a possible cross reactivity between patients reacting to cephalosporins and penicillin.

Method: The study was retrospective including patients evaluated at the Allergy Center, Odense University Hospital, Denmark from January 2003—May 2017 for allergy to cefuroxime. Data was obtained from the ORCA database. Inclusion criteria were measurement of specific IgE to cefuroxime and/or skin test (ST) with cefuroxime; 320 patients fulfilled the criteria and were included.

Results: Measurement of specific IgE to cefuroxime was performed in 284 patients and in 252/284 specific IgE to penicillin was also measured; six patients were IgE positive to both cefuroxime and penicillin, four patients were positive to cefuroxime only, and 17 patients were IgE positive to penicillin only.

Skin test with cefuroxime was performed in 197 patients and in 171/197 ST with penicillin was also carried out; one patient had delayed positive ST to both cefuroxime and penicillin, seven patients had positive ST to cefuroxime, one had a delayed positive reaction occurring several hours after testing with cefuroxime, and 10 patients had positive ST to penicillin only. Both ST and specific IgE to cefuroxime were positive in 3 patients who all had a case history of anaphylaxis, and one of them also had positive IgE to penicillin, however, penicillin challenge was negative. In total, 145 patients were challenged intravenously (up to 1500 mg) with cefuroxime, and 7 were positive. Of patients challenged with cefuroxime, 72 were also challenged with penicillin and 3 (all negative cefuroxime challenge) were positive.

Conclusion: Allergy to cefuroxime was confirmed in 22/320 patients (6.9%). In patients evaluated for both cefuroxime and penicillin, there were only few overlapping positive tests: IgE in six patients and ST in one patient. This study indicates low clinical cross reactivity between cefuroxime and penicillin.

0108 | Hypersensitivity reactions to antiepileptic drugs in pediatric population

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Background: Antiepileptic drugs are frequently associated with hypersensitivity reactions mainly cutaneous such as maculopapular exanthems and bullous and pustular eruptions on the basis of their clinical, cellular and molecular pathophysiology.

The purpose of our study was to confirm or rule out the diagnosis of hypersensitivity to antiepileptic drugs in children.

Method: Of 96 children referred to the Drug Allergy Unit of University Children's Hospital of Belgrade, in the last 15 years, 45 (46.87%) were boys and 51 (53.13%) were girls. The ages ranged from 1 years to 17 years (mean age 8.63). Various clinical reactions were described as being induced by drugs: Maculopapular rash with or without fever and lymphadenopathy 68 (70.83%), urticaria with or without fever and/or angioedema 26 (27.08%), SJS 1 (1.04%), erythema multiforme 1 (1.04%). The time period that had elapsed from the occurrence of reaction to the performance of *in vitro* and *in vivo* testing varied from 1 months to 3 years.

Patch tests were performed with culprit drug such as lamotrigine (in 41 children), valproate (in 12 child), carbamazepine (in 35 children), phenobarbital (in 6 children) and topiramate (in 2 children). In children with a history of mild hypersensitivity reactions and negative patch tests we performed intradermal tests with delayed reading. We did not performed LTT.

Results: Out of the total of 96 tested children we found 56 (58.33%) positive patch tests and 7 (7.29%) positive intradermal tests. Positive patch tests were at 19 out of 41 tested with lamotrigine (46.34%), 21 out of 35 tested with carbamazepine (60%), 10 out of 12 tested with valproate (83.33%) and 6 out of 6 tested with phenobarbital (100%). Positive intradermal tests were at 5 out of 22 tested to lamotrigine (22.73%), 1 out of 14 tested to carbamazepine (7.14%) and 1 out of 2 tested to valproate (50%).

In all children (33) with negative allergy work-up, we performed serologic screening for viral (adenovirus, parvovirus B19, EBV, enteroviruses) or Mycoplasma pneumoniae infection. Out of 33 tested children 19 (57.58%) were positive : 9 (47.37%) to Mycoplasma pneumoniae, 5 (26.32%) to parvovirus B19, 3 (15.79%) to enterovirus and 2 (10.53%) to adenovirus.

Conclusion: In all children with suspected hypersensitivity reactions to anticonvulsive drugs it is necessary to perform complete allergy work-up and in all children with negative allergy work-up should perform infectious agent studies to confirm coincidental reactions.

0109 | The reliability of ALDEN scores for the prediction of drug-specific T cells in Stevens-Johnson syndrome

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Background: ALDEN, an algorithm for assessment of drug causality in Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), is a clinical tool to identify the suspected culprit drugs. Unexpectedly, the reliability of culprit drug assessment using ALDEN score has not been validated against the results of in vitro diagnostic testing. This study was to explore whether the ALDEN scores could accurately predict the culprit drugs confirmed by interferon-γ (IFN-γ) enzyme-linked immunospot assay (ELISpot).

Method: The suspected culprit drugs were assessed according to ALDEN algorithm in 26 patients with SJS/TEN. Frequencies of drug-specific IFN-γ release cells were measured by ELISpot after incubating patient's peripheral blood mononuclear cells (PBMCs) with the suspected culprit drugs. Positive ELISpot response was defined as ≥20 spot-forming units (SFU)/ 106 PBMCs.

Results: Fifty-nine suspected drugs were tested. The common suspected drugs were anticonvulsants (27.1%), sulfamethoxazole-trimethoprim (18.6%), beta-lactams (11.9%), antituberculosis drugs (11.9%), and allopurinol (8.5%). Based on ALDEN drug causality assessment, 44.1%, 22.0%, and 33.9% of the tested drugs were classified in category I, II, and III (i.e., very probable, probable, and very unlikely to possible causalities, respectively).

A weak but significant correlation between ALDEN scores and drug-specific IFN- γ release cells was observed ($r = 0.27$, $P = 0.03$). The average ALDEN score (mean±SD) was 4.4 ± 2.0. Those with positive ELISpot responses (5.7 ± 0.5) had significantly higher ALDEN score than those with negative responses (3.9 ± 2.1, $P < 0.001$).

The average frequencies of drug–specific IFN-γ releasing cells in the suspected drugs for category I, II, and III were 128.8 ± 45.0, 90.2 ± 41.9, and 0.4 ± 0.3 SFU/106 PBMCs, respectively. Positive IFN-γ ELISPOT responses were detected in 30.5% (18/59) of the tested drugs. Approximately 46.2% of the suspected drugs in category I and II had positive responses; while none of drugs in category III had any. According to the Youden's index, ALDEN score of 4.5 was the optimal cutoff value for culprit drug confirmation.

Conclusion: Drug-specific IFN-γ releasing cells in SJS/TEN subjects were rarely detected among the suspected drugs with possible or lower causality categories defined by ALDEN algorithm; while almost

half of drugs in probable/very probable categories showed positive ELISpot results.

0110 | Bullous fixed drug eruption (BFDE): A case series

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Background: Fixed drug eruption (FDE) is a common cutaneous adverse reaction, characterized by the recurrence of single or multiple round erythematous plaques that typically regress leaving residual. There are different clinical features of FDE. BFDE is a rare and severe form that may be misdiagnosed with other forms of bullous drug eruptions.

We describe herein an original case-series of BFDE. Culprit dugs were identified using skin test and/or a positive rechallenge with the suspected drug.

Method: We reviewed medical records of patients with suspected FDE in our department of pharmacovigilance of Monastir during the period from 2004 to 2017 and we included all cases of BFDE. The drug imputability was established according to Begaud and *al* method. Skin tests were performed in the involved skin according to the ENDA recommendations.

Results: Among 35 cases of FDE, recorded in our database, 11 patients (5 men and 6 women) had the bullous type. The median age of our patients was 50 years. Ten patients have multiple hyperpigmented lesions. The median delay between drug intake and eruption onset was 72 hours. Paracetamol was the most common causative agents (n = 5) followed by non steroidal anti-inflammatory drugs (n = 4) (mefenamic acid in two cases and piroxicam in two others) and antibiotics (n = 2) (doxycycline and levofloxacin).

The determination of the culprit drug was made possible by a positive patch test in 7 out of 11 patients. For the remaining cases, accidental rechallenge or oral provocation helped to establish the culprit drug.

Conclusion: We demonstrated in our study that paracetamol is the most causative drug associated with BFDE and pointed out the usefulness of drug patch tests in identifying the culprit drug. In case of BFDE, it is recommended to look for the concept of taking paracetamol given the frequency of consumption of this drug.

MONDAY, 28 MAY 2018
OAS 20
MANAGEMENT OF FOOD ALLERGY

0111 | Quantitative risk reduction through peanut immunotherapy: Assessment for the peanut-allergic population undergoing epicutaneous immunotherapy (EPIT)

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Background: Peanut allergy is a common, global disease currently without therapeutic options. The clinical relevance of increasing the peanut-allergic population's threshold by immunotherapy has not been previously characterized. In this study, we quantify the clinical benefits of Epicutaneous Immunotherapy (EPIT) in peanut-allergic populations through risk reduction studies.

Method: In this study, we utilized four primary inputs for the quantitative risk assessment: the peanut-allergic individual's clinical threshold value pre- and post-immunotherapy, consumption data from the Dutch or United States national food consumption surveys regarding the amount of food consumed per eating occasion for selected packaged food products and the concentration of peanut protein in the consumed packaged food product.

Clinical data from the VIPES pediatric (6-11 yo) population (Viaskin Peanut 250 µg Phase IIb study) were utilized to calculate the population risk reduction geometric mean for the active and placebo groups. Further, data from the VIPES study was applied to the overall peanut-allergic population and quantitative risk reduction assessments were performed to investigate predicted risk reductions of EPIT immunotherapy outside of the VIPES population.

Results: Using the VIPES study clinical data in combination with currently available consumption and packaged food contamination data, geometric mean risk reductions for children consuming packaged cookies were calculated to be greater than 95% in the active treatment group and less than 15% in the placebo group. Extending to the overall peanut-allergic population, an overall risk reduction of 80 to 86% would be predicted for the child age group after EPIT treatment when consuming packaged cookies, as well as other selected foods.

Conclusion: It is concluded that Epicutaneous Immunotherapy with Viaskin Peanut 250 µg provides a clinically relevant benefit to the peanut-allergic population. This includes a significant reduction in risk due to unexpected exposures to peanut protein in packaged foods within the VIPES study population as well as an expected similar reduction in risk if applied in the larger peanut-allergic population.

0112 | Efficacy and safety of AR101 in peanut allergic patients aged 4-55: Results from an international phase 3, randomised, double-blind, placebo-controlled trial (PALISADE)

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Background: A phase 2 study indicated that the novel, investigational oral biologic drug AR101 could be a potential immunomodulatory treatment for peanut allergic patients. The PALISADE Phase 3 study was conducted to confirm the efficacy and safety signals observed in Phase 2.

Method: Eligible peanut allergic subjects aged 4-55 years were randomized in a 3:1 ratio into a double-blind, placebo controlled trial of oral immunotherapy with AR101 in Europe, Canada and the USA. Key entry criterion included reaction at ≤100 mg peanut protein in a screening double-blind, placebo-controlled food challenge (DBPCFC). The trial included initial escalation and up-dosing phases, approximately 6 months of 300 mg/day maintenance, and an exit DBPCFC.

Results: 842 subjects were screened; 554 were randomized and 551 received ≥1 dose of study drug. The study population averaged 11.3 years (range 4-49), was 57% male, and 79% Caucasian. 72% had allergic rhinitis, 54% asthma, 59% atopic dermatitis, and 65% were allergic to other foods. 407 (74%) had a previous history of anaphylaxis with peanut. Baseline median (IQR) values were: peanut skin prick wheal diameter 11.5 (9-15) mm; peanut-specific IgE 61.75 (16.7–179) kUA/L; and maximum tolerated dose at screening DBPCFC 10 (3-30) mg. 97% of AR101 subjects and 94% of placebo subjects had at least one treatment-emergent adverse event, none of which was life-threatening, with the majority being either mild or moderate. 442 subjects (80%) completed the study (76% of the AR101 arm, 92% of the placebo arm). Discontinuations were treatment-related in 11% of the AR101 arm and 1.4% of the placebo arm. 64.6% of AR101 subjects vs 5.1% of placebo subjects tolerated a single highest dose of at least 600 mg of peanut protein (1043 mg

cumulative) with no more than mild symptoms in the exit DBPCFC. During the exit DBPCFC, 53% of the placebo subjects required at least one epinephrine, compared with 9.8% of the AR101 subjects.

Conclusion: The results of PALISADE are consistent with the AR101 phase 2 results, and indicate that AR101 could be an immunotherapeutic option for peanut allergic patients, by reducing the risk of allergic reaction following accidental peanut exposures.

Peanut protein at the exit DBPCFC	300 mg	600 mg	1000 mg
AR101 (n = 413)	73.4%	64.6%	48.7%
Placebo (n = 138)	10.9%	5.1%	3.6%
95% CI difference	(53–72%)	(49.9–69.2%)	(35.7–54.4%)
P-value	<0.00001	<0.00001	<0.00001

0113 | Hypoallergenic maternal elimination diets and vegetarian diets are associated with vitamin B12 deficiency in infants

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Background: Amongst infants treated in the Allergy Dept. for food allergy, we have identified several infants with subclinical vitamin B12 deficiency. Vitamin B12 deficiency may cause significant morbidities, including growth failure, anaemia and neurodevelopmental impairment. We aimed to identify clinical risk factors for vitamin B12 deficiency in infants.

Method: From November 2009 to May 2012, we conducted a retrospective case-control study. All patients <24 months of age who had undergone vitamin B12 (holotranscobalamin) testing at the Royal Children's Hospital, Melbourne, were identified from a pathology database. For each vitamin B12-deficient index case, two aged-matched vitamin B12-replete patients were enrolled. Medical records were reviewed for the main diagnosis, presence of food allergies, eczema, breastfeeding in first month of life, maternal elimination diets, restricted infant diets, vegetarian/vegan diets and formula feeding. Possible risk factors for vitamin B12 deficiency were identified by chi-square analysis and logistic regression, including adjustment for potential confounders.

Results: 803 children <24 months had undergone holotranscobalamin testing. Of these, 54 (6.7%, 95% CI 5.1-8.7%)) were vitamin B12-deficient. 52 patients and 104 age-matched controls (mean age 10.1 mo) were included. Vitamin B12 deficient infants were more likely than controls to suffer from food allergy (38.5% *vs* 16.4%; $P = 0.002$) and eczema (34.6% vs 17.3%; $P = 0.012$). Other associations included breastfeeding for at least 1 month (94.2% *vs* 69.2%; $P < 0.001$), hypoallergenic maternal elimination diets (28.3% *vs* 3.8%; $P < 0.001$) and restricted infant diets (36.5% *vs* 7.7%; $P < 0.001$). Cases with vitamin B12 deficiency were less likely than controls to have been formula fed (9.6% *vs* 75%, respectively; $P < 0.001$). 25% of vitamin B12-deficient patients *vs* 8.8% of controls belonged to families with vegetarian/vegan diets ($P < 0.001$). On logistic regression analysis, breastfeeding ($P = 0.02$), hypoallergenic maternal elimination diets ($P = 0.02$) and vegetarian/vegan diets ($P = 0.06$) were identified as likely independent risk factors for vitamin B12 deficiency, whereas food allergy was not independently associated ($P = 0.97$).

Conclusion: While the association of vegetarian/vegan diets with vitamin B12 deficiency is widely recognised, this is the first study to demonstrate an association of infantile vitamin B12 deficiency with hypoallergenic maternal elimination diets.

0114 | Oral immunotherapy in walnut-allergic patients

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Background: Oral immunotherapy (OIT) for food allergy is currently recommended as a treatment option for patients with milk, egg or peanut allergy. However data on OIT for walnut allergy is limited. The purpose of this study was to examine the efficacy and safety of walnut OIT.

Method: Patients aged ≥4 years diagnosed with walnut allergy, based on a positive skin prick test (SPT) and a positive oral food challenge were enrolled in walnut OIT. Patients with asthma were stabilized and included. OIT consisted of repeated cycles of in-hospital up-dosing separated by monthly fixed daily doses. The goal was to achieve 4000 mg of walnut protein. Patients with walnut allergy undergoing standard of care of elimination diet were challenged 6 months apart and served as controls. SPT and basophil activation test (BAT) were performed at enrollment and at follow-up.

Results: A total of 54 patients began walnut OIT between 5/2016 and 12/2017 and 32 completed treatment. 29 patients (90.6%) were successfully desensitized at a mean duration of 5.4 ± 3.6 months, and 3 patients failed (1 with late anaphylactic reactions and 2 for food aversion). Adverse reaction requiring epinephrine were experienced by 5 patients (15.6%) for in hospital up-dosing and 2 patients (6.3%) at home. Patients requiring epinephrine either in hospital or at home were all successfully desensitized. None of the 12 control patients were desensitized to walnuts (interval, 6.6 ± 2.7 months). In desensitized patients, SPT wheal size decreased from a mean of 9.2 ± 3.1 to 4.3 ± 3.1 mm and %CD63 from 35.5 ± 26.3 to 2.5 ± 6.3 ($P < 0.001$ for both), but not in the control group 8.8 ± 3.9 to 7.1 ± 3.2 mm ($P = 0.11$) for SPT and from 47.7 ± 27.2 to 38.9 ± 19.7 ($P = 0.1$) for %CD63.

Conclusion: The efficacy of OIT in walnut-allergic patients is high. Adverse reactions occur but do not prevent subsequent successful desensitization. Desensitization is associated with immunologic changes including reduced skin sensitization and basophil reactivity.

0039 | Relevance of clinical and laboratory predictors in chronic spontaneous urticaria

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Background: Chronic spontaneous urticaria (CSU) is a common skin disorder in which approximately half of these patients are characterized by an auto reactive pathogenic state. 5%-50% of CSU patients may not reach controlled condition with antihistamines and these refractory patients require other treatment modalities beyond antihistamines[1].

Therefore, the need for reliable and safe parameters to link the pathogenesis and disease severity with reasonable therapeutic approaches has increased. Few studies have investigated the role of different parameters as predictor tools to evaluate disease severity of CSU patients.

Method: We assessed total severity score (TSS), ASST, total IgE, anti-thyroid antibodies basophil CD203c expression and BAT-CD203c using a two color flow cytometric method in 40 CSU patients and 40 normal controls.

Results: Our logistic regression analysis indicated that both BAT-CD203c and ASST were significant predictors of clinical severity of CSU ($P = 0.012$ and $P = 0.042$, respectively.

Conclusion: BAT-CD203c and ASST can be used as a potential predictor of clinical severity of CSU.

0116 | Identification of eosinophil derived neurotoxin binding peptide by Phage display and its diagnostic potential for Eosinophilic esophagitis

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Background: Eosinophilic esophagitis (EoE) is an inflammatory condition of the esophagus characterized by the presence of large numbers of eosinophils. Currently, EoE diagnosis is based on invasive endoscopic procedures, histopathological examination and the patients' clinical history. Hence, the identification of novel biomarkers is highly desirable for EoE diagnosis and monitoring. In this study, our aim was to select short peptides by Phage Display and validate them as novel biomarkers for EoE detection.

Method: Firstly, we have performed a comparative proteomic analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) of esophageal biopsies from pediatric patients with eosinophilic esophagitis, gastroesophageal reflux disease and healthy individuals. Then, the Phage Display technology was used to select peptides against one of the most up-regulated protein in EoE patients. Two phage clones were selected after three selection cycles, and their reactivity were evaluated by phage-ELISA using a commercial human recombinant protein. Furthermore, peptide sequences were determined by DNA sequencing and peptides' binding to their protein target was analyzed by *in silico* prediction tools.

Results: Mass spectrometry results showed that eosinophil-derived neurotoxin protein (EDN) was highly up-regulated in EoE patients, an eosinophil granule protein that is deposited on diseased tissues. Through ELISA assays, two highly reactive EDN- binding peptides (D3 and C5) presented similar or greater specificity when compared with commercial polyclonal antibodies to EDN.

Conclusion: This is the first study that demonstrates the detection of eosinophil-derived neurotoxin protein using peptides identified by phage display. These peptides may become useful diagnostic tools for detection of EoE patients.

MONDAY, 28 MAY 2018
OAS 21
NOVEL INSIGHTS IN PEDIATRIC DERMATOLOGY

0117 | Clinical features of Hereditary angioedema due to C1-INH deficiency in pediatric population. A prospective study

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Background: Hereditary angioedema due to C1-INH deficiency (C1-INH-HAE) usually debuts in pediatric age. The clinical course in adulthood is well known but less data are available in children. Our objective was to evaluate prospectively the clinical features in our pediatric population whit C1-INH-HAE.

Method: We prospectively studied 13 patients (53.85% males) aged 1 to 18 years, with C1-INH-HAE who were regularly followed up in our Allergy department during a 9-year- period (2008-2017). We determined onset of the disease, frequency and severity of HAE attacks. Genetic study and laboratory analysis were performed in order to establish diagnosis.

Results: 92.3%(n12) of the children had positive family history of C1-INH-HAE. 69.2%(n9) children were diagnosed when clinically debuted with symptoms. 30.8%(n4) children were screened for mutations due to positive family history. The median age of onset of symptoms was 5.7 years (interquartile range [IQR], 1-10). One child (7.6%) diagnosed was asymptomatic, 38.5% (n5) had less than 3 attacks per year, 38.5% (n5) had 3 to 5 attacks per year and 15.4% (n2) had more than 6 attacks per year. The most common angioedema attack sites were peripheral (84.6%) and abdominal (53.8%) . Facial attacks were 38.46%. Genital and laryngeal attack were rare, 15.4%, and 7.7%, respectively. Four children (30.76%) presented severe HAE attacks, one of them presented severe repeated laryngeal attacks.

Conclusion: In our pediatric C1-INH-HAE population, symptom onset occurred at a median age of 5.7 years. The majority of children with C1-INH-HAE were diagnosed after presenting symptoms. Peripheral and abdominal attacks were more common than facial, laryngeal and genital attacks.

0118 | Recombinant human C1 esterase inhibitor for the acute treatment of hereditary angioedema attacks in children

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Background: Hereditary angioedema (HAE) attacks, related to C1 esterase inhibitor (C1-INH) deficiency, are typically observed during the first 2 decades of life. However, limited data are available regarding treatment of children. Ruconest is a recombinant human C1 esterase inhibitor (rhC1-INH) approved for acute treatment of HAE attacks in adolescents and adults. This study evaluated rhC1-INH for HAE attacks in children.

Method: Patients (2-13 years) with functional C1-INH levels <50% of normal were eligible for enrollment and could be treated when presenting with an HAE attack, symptom onset <5 hours, of at least moderate severity (investigator score [range, 0-5] of ≥3). Patients were treated with a rapid (~5-minute) intravenous infusion of rhC1-INH 50 IU/kg (maximum, 4200 IU); a second dose could be administered at investigator discretion. Efficacy (time to beginning of relief; time to minimal symptoms) was assessed by patient (or caregiver) using a visual analogue scale (range, 0-100 mm).

Results: Twenty children (aged 5-13 years) were treated with rhC1-INH. Seventy (95.9%) of 73 attacks were treated with a single dose. Seven (35.0%) patients were treated for ≥4 attacks. Overall median time to beginning of relief was 60.0 minutes (95% confidence interval [CI], 60.0-65.0 minutes). Median time to minimal symptoms (attack remission) was 122.5 minutes (95% CI, 120.0-126.0). No children withdrew from the study due to an adverse event. No treatment-related serious adverse events or hypersensitivity reactions were reported; no neutralizing antibodies were detected.

Conclusion: rhC1-INH was efficacious, safe, and well tolerated in children. These data support published adult data that rhC1-INH for HAE attacks is efficacious and well tolerated across various age groups.

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0119 | Clinical evaluation of children with cutaneous mastocytosis and c-kit mutation screening

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Background: Cutaneous mastocytosis (CM) is a heterogeneous disease which commonly presents with skin lesions in childhood. In this study, we aimed to determine c-Kit mutation from peripheral blood samples, which might be responsible for the etiopathogenesis of pediatric mastocytosis, as well as to ascertain prognostic factors by using the patients' long term follow-up results.

Method: The clinical observation data of 32 children, who had been diagnosed with CM, were retrospectively researched. Exon 8, 9, 11, 13 and 17 c-Kit gene locations were analyzed from DNA material that was obtained from peripheral blood samples of all the patients using PCR analysis and automatic DNA sequencing.

Results: The tryptase level was higher in familial cases and in cases with who had gastrointestinal mediator releasing symptoms ($P = 0.017$, $P = 0.038$). The use of clarithromycin and vitamin D was determined as triggers for mediator release. Hypogammaglobulinemia was found in 6 (18.8%) cases. Indoor tobacco exposure was seen to be higher in non-remission patients than in remission patients (59.1%, 20%, respectively) ($P = 0.040$). Allergic diseases were observed in 80% of complete remission patients and 22.7% of non-remission patients ($P = 0.002$). Concomitant allergic diseases were found to be a good prognosis marker among pediatric CM patients. No c-Kit mutation was discovered in any of the patients.

Conclusion: In this study, tobacco exposure would seem to be a barrier for remission and concomitant allergic diseases were seen to be a good prognosis marker. Evaluation of peripheral c-Kit mutation has no diagnostic contribution among pediatric CM patients in contrast to adults.

0120 | Exposure to phthalates is associated with acute urticaria in children

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Background: Urticaria is a heterogeneous disorder that has a wide spectrum of clinical presentations, and is one of the most common skin disorders. However, few studies have examined the effect of phthalates on the development of acute urticaria. We investigated the association between urticaria and urinary metabolites of phthalates in young Korean students.

Method: We prospectively recruited students, aged 7 to 8 years, who participated in the Seongnam Atopy Project (SAP_{2016}), performed by the Seongnam City Government in Korean children. We developed a questionnaire to assess the relationship of symptoms of urticaria with demographic and clinical variables, such as age, sex, and allergic diseases. Children who had experienced symptoms of urticaria lasting <6 weeks during the previous 12 months were classified as having acute urticaria. Urinary metabolites of phthalates, mono-isobutyl phthalate (MiBP), mono [2-ethyl-5-oxohexyl] phthalate (MEOHP), and mono-isobutyl phthalate (MEHHP), were measured using gas chromatography/tandem mass spectroscopy.

Results: We prospectively examined 149 Korean children, 28 (18.8%) with acute urticaria and 121 (81.2%) without acute urticaria. These 2 groups had no statistical differences in sex ($P = 0.294$), age ($P = 0.972$), or BMI z score ($P = 0.787$). We divided all subjects into quartiles according to their levels of MiBP, MEOHP, and MEHHP. Relative to subjects in the lowest quartiles, the aORs for acute urticaria were significantly greater for subjects in the highest quartiles of MiBP (4.622; 95% CI, 1.181 to 19.770; $P = 0.039$), MEOHP (11.720; 95% CI, 1.762 to 77.967; $P = 0.011$), and MEHHP (5.338; 95% CI, 1.072 to 26.566; $P = 0.041$). Moreover, there were significant linear trends across quartiles (MiBP, $P = 0.009$; MEOHP, $P = 0.018$; MEHHP, $P = 0.035$).

Conclusion: we found that increased urinary concentration of phthalates correlates with acute urticaria in young children. This finding suggests that efforts should be made to reduce exposure to phthalates, chemicals that are widely used in adhesives, food packages, and cosmetics, in children. Future longitudinal studies are warranted to confirm the association between exposure to phthalates and urticaria.

0121 | Therapeutic effect of tyndallized Lactobacillus rhamnosus IDCC 3201 on atopic dermatitis in children: A 12-week, double-blind, randomized, placebo-controlled study

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Background: Probiotic (mainly live form of bacteria) therapies have been proven to be effective in treating atopic dermatitis (AD), while there are some controversies on the therapeutic effect on AD. Several studies suggested that killed probiotics would have immunomodulatory effect in allergic diseases including AD. This study was performed to evaluate the therapeutic effect and safety of tyndallized *Lactobacillus rhamnosus* (IDCC 3201, isolated from Korean

breast-infants, repeated heat treated and incubated) in children with AD.

Method: In a randomized, double-blind, placebo-controlled study, tyndallized *Lactobacillus rhamnosus* (IDCC 3201, isolated from Korean breast-milk fed infants) at a dose of 1.0×10^{10} CPU/day or placebo was given in children (aged 12 months to 12 years) with moderate AD, once a day for 12 weeks. During the study periods, minimal use of steroids and hydrating lotion was permitted, and the amounts of both items were adjusted for statistical analysis. SCOring of AD (SCORAD) scores, allergic inflammatory markers, and safety parameters were evaluated.

Results: For the safety analysis, a total of 100 subjects (50 in treated, 50 in control group) were evaluated, and there were no significant differences in safety parameters between two groups. For evaluating the therapeutic effects of IDCC 3201, 33 subjects in each group were finally analyzed. The decrement of SCORAD score at 12 week was significantly greater in the treated group (10.04 ± 8.08) compared to the control group (5.25 ± 7.75). Levels of eosinophil cationic protein (ECP) and Interleukin (IL)-31, a cytokine related with itching, were significantly lower in the treated group. The improvement of SCORAD scores, ECP and IL-31 levels were remarkable in AD children for 50 months of disease duration or longer and in children aged 5 years or more. [Funded by Ildong Pharmaceutical Co. Ltd.]

Conclusion: In children with moderate AD, oral administration of tyndallized *Lactobacillus rhamnosus* (IDCC 3201) showed the therapeutic effect on AD, and the effects correlated with decrement of ECP and IL-31, and the effect was remarkable in subgroups analysis.

0122 | Plasma periostin levels and it's relationship disease severity in children with atopic dermatitis

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Background: Periostin is an extracellular matrix protein was recently characterized that it plays an important role in the pathogenesis of AD via release of proinflammatory cytokines and chemokines like TSLP, IL25, IL 33 from activated keratinocytes.
The aim of this study to evaluate the relationship between plasma periostin levels with severity and chronicity of AD in children.

Method: The children with diagnosis of only atopic dermatitis as study group and healthy children in control group were enrolled to the study. The diagnosis of atopic dermatitis was made according to Hanifin-Rajka criteria by a physician. Data on demographic features (age, gender, family history of atopy, age of onset and duration of symptoms and laboratory values of serum eosinophil, total IgE and skin prick test results were collected through patient's medical records. These verity of the disease was assessed by SCORing Atopic Dermatitis index. Serum Periostin levels were measured with human Periostin ELISA kit from Bioassay Technology Laboratory (Shanghai, China) according to the manufacturer's instructions.

Table 1- Some demographic and clinical values of patient and control group

	AD patients (n = 29)	Control group (n = 31)	*P* value
Age months (mean, ±SD)	80.7 ± 52.8	90.3 ± 41.6	>0.05
Gender (M/F)	15/14	18/13	>0.05
SCORAD (mean, ±SD)	35.1 ± 12.6	–	
Total Ig E > 100 Iμ/L (n[%])	17 (59%)	ND	
Eosinophils >4% (n[%])	18 (62%)	ND	
Atopy (n[%])	17 (59%)	ND	
Food allergy	3 (10%)	-	
Aeroallergen sensitivity	9 (31%)	ND	
Treatment (n[%])			
Emollient or moisturizer only	9 (31%)		
Topical steroid +emollient or moisturizer	18 (62%)\		
Calcineurin inhibitors + emollient or moisturizer	2 (7%)		

ND, not done.

Results: The study group consisted of 15 boys and 14 girls with AD and the control group consisted of 18 boys and 13 girls. The mean ages of the AD patients and the control group participants were 80.7 ± 52.8 and 90.3 ± 41.6 months, respectively. No significant differences in age or gender existed between the groups ($P > 0.05$). Mean plasma periostin levels were 63.0 ± 19.0 ng/mL in AD patients, and 23.6 ± 7.3 in healthy controls. There were statistically significant difference between groups (*P*: 0.001). Plasma periostin levels did not vary according to gender and atopy. Age of onset, duration of symptoms also were not correlated with plasma periostin levels. Although there was a positive relationship between plasma periostin levels and SCORAD index of patients, a correlation analysis did not show a statistical significance ($r = 0.19$, $P > 0.05$).

Conclusion: Plasma periostin levels were found to be significantly higher in patients with atopic dermatitis. Based on this, periostin was thought to play a role in the pathogenesis of atopic dermatitis. On the other hand we think that periostin is not a useful marker for predicting severity and chronicity of atopic dermatitis.

MONDAY, 28 MAY 2018

OAS 22

IMMUNOTHERAPY: MEASURES AND CLINICAL OUTCOME

0123 | The efficacy and safety of allergen immunotherapy in patients with mast cell disorders and hymenoptera venom anaphylaxis

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Background: Mast cell disorders (MCD) are characterized by inappropriate mast cell activation in different tissues and patients are prone to severe allergic reactions. Among triggers causing anaphylactic reactions in these patients, hymenoptera venom appears to be the most common and have severe clinical outcomes. Currently, life-long venom immunotherapy (VIT) is the only established treatment; however, its use has raised concerns about its efficacy and safety.

The aim of this study was to determine the efficacy and safety of VIT in patients with MCD and hymenoptera venom anaphylaxis (HVA).

Method: Until 2018, 298 consecutive adult patients (≥ 18 yo) were referred to the Mastocytosis Centre Karolinska and investigated due to clinically suspected MCD. All patients underwent complete clinical work-up and the final diagnoses were obtained after a bone marrow investigation following WHO-criteria.

Thirty-two patients with HVA and MCD (16 with indolent systemic mastocytosis, 9 with monoclonal mast cell activation syndrome, 7 with elevated (>11.4 ng/mL) baseline tryptase levels without pathologic bone marrow biopsy findings) received VIT and were enrolled in the study. All patients were allergic to vespula venom. Total IgE, venom-specific IgE, component-specific venom IgE, venom-specific IgG4 and serum baseline tryptase levels (sBT) were routinely assessed. The patients were thoroughly followed up during VIT both clinically and with above mentioned biomarkers.

Results: The patients, 17 male (53%), received VIT for a median of 50.5 months (range 3-136 months). Eleven (34%) experienced adverse reactions during VIT; 6 patients (54.5%) during induction and 5 (45.4%) during maintenance phase. Fifteen (47%) were re-stung while undergoing VIT, of these 6 (40%) presented with local reaction, 6 (40%) with systemic reaction, 3 (20%) did not react at all. Six (40%) of the re-stung patients did not take Epinephrine, of them 5 presented with local reaction and 1 did not develop any symptoms at all. No significant changes were observed regarding levels of sBT, total IgE, venom-specific IgE or venom components during VIT compared to baseline levels. However, we have shown that venom-specific IgG4 levels increased during VIT ($P < 0.01$).

Conclusion: Our preliminary results show that VIT provides effective and safe treatment in most patients with MCD, although the incidence of adverse reactions during VIT is increased. The increased levels of venom-specific IgG4 during VIT may correlate with treatment outcome.

0124 | Does oral immunotherapy improve quality of life in children with severe peanut allergy?

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Background: Desensitization by peanut oral immunotherapy (OIT) has been reported to increase quality of life (QoL) in parental reports.

Objective: Based upon both parental and child reports, we primarily aimed to determine if two years of OIT improved QoL in children with anaphylaxis to peanut. Secondarily, we aimed to determine factors that influenced change in QoL from pre-treatment, including level of desensitization, maintenance dose, perceived treatment burden and ineligibility to participate in OIT due to low reactivity threshold.

Method: In 5-15 year-old children with anaphylaxis to peanut, QoL was assessed in 57 children randomised to OIT and 20 to observation only, and in 19 ineligible for the Take-Away OIT trial. Parents completed the Food Allergy Quality of Life—Parental Burden (FAQL-PB) (1 = not troubled, 7 = extremely troubled) and the Pediatric Quality of Life Inventory Version 4.0 (PedsQL 4.0) (reverse-scored (0 = 100, 1 = 75, 2 = 50, 3 = 25, 4 = 0), and children the PedsQL 4.0 at screening (T_0), after one year (up-dosing) (T_1) and after two years of treatment (T_2). A visual analogue scale (VAS) was completed at T_1 and T_2, reporting perceived burden of adverse events (AEs), the taste and amount of peanut, and the time spent on OIT.

Results: At T_2, 94.6 % (35/37) of the OIT children was desensitized up to a cumulated dose of 7500 mg peanut protein, regardless of

level of maintenance dose (range (mg) 350, 5000). Quality of life improved significantly from T_0 to T_2 by parental assessment only, in children receiving OIT compared to controls (mean change in PedsQL 4.0 (95 % CI) 9.3 (4.3, 14.3) vs 0.4 (−7.1, 8.0) (P = 0.04). No significant improvement or deterioration was observed based upon the FAQL-PB. Neither level of desensitization at T_2, nor maintenance dose or perceived treatment burden influenced change in QoL. Ineligibility of their child to participate in OIT was associated with decreased QoL reported by the parents.

Conclusion: In children with anaphylaxis to peanut, parents but not the children reported improved QoL after two years of OIT. Ineligibility for OIT decreased QoL in parents.

0125 | The SQ tree SLIT-tablet reduces rhinoconjunctivitis symptoms and medication use during the tree pollen season (hazel, alder and birch pollen seasons)—Results from a large multi-centre phase 3 trial

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Background: The SQ tree SLIT-tablet (ALK, Denmark) is in development for once-daily, home-administered treatment of moderate to severe allergic rhinitis and/or conjunctivitis induced by pollen from the birch homologous group. Here, we report the results of a phase 3 trial.

Method: The TT-04 trial (EudraCT 2015-004821-15) was a randomised, DBPC, phase 3 trial. 634 subjects were randomised 1:1 to the SQ tree SLIT-tablet (12 DU dose) or placebo. All subjects received at least 16 weeks of treatment before start of the 2017 tree pollen season, i.e., hazel, alder and birch pollen seasons. The efficacy endpoints assessed daily rhinoconjunctivitis symptom score (DSS), daily rhinoconjunctivitis medication score (DMS) and the sum of these; i.e., total combined score (TCS) during the birch pollen season (BPS) and TPS. The primary endpoint was average TCS during the BPS. Safety endpoints primarily included adverse events.

Results: The treatment effects on the average TCS, DSS and DMS in the BPS and TPS were all statistically significantly greater for the SQ tree SLIT-tablet compared to placebo. The primary endpoint of average TCS during the BPS showed an estimated absolute difference of 3.02 corresponding to a reduction of 39.6% in favour of the SQ tree SLIT-tablet relative to placebo (P < .0001). For the average TCS during the TPS the estimated absolute difference was 2.27, corresponding to a difference of 36.5% relative to placebo (P < .0001). For the average DSS, the estimated absolute differences were 1.32 for the BPS, corresponding to a difference of 36.8% relative to placebo (P < .0001), and 0.99 for the TPS, corresponding to a difference of 32.7% relative to placebo (P < .0001). DSS and DMS contributed almost equally to the observed treatment effect during both the BPS and TPS. The TCS during the alder and hazel season was analysed post-hoc and demonstrated an estimated absolute difference between the SQ tree SLIT-tablet and placebo of 1.21, corresponding to a difference of 29.7% relative to placebo (P = 0.0015). Treatment was well-tolerated with local reactions in the mouth and throat as the most common treatment-related adverse events; the majority were mild or moderate in severity and had onset on day 1-2. No deaths or anaphylactic reactions were reported with the SQ tree SLIT-tablet.

Conclusion: The TT-04 trial demonstrated a positive benefit-risk balance with a clinically relevant treatment effect of the SQ tree SLIT-tablet during the entire TPS.

0126 | A post-hoc analysis of a novel endpoint for asthma exacerbations in a SQ HDM SLIT-tablet phase III allergic asthma trial

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Background: A recent publication (Fuhlbrigge et al, Lancet Respiratory Medicine, 2017) describes a novel asthma exacerbation endpoint (CompEx, based on diary data or severe exacerbations whichever comes first) for evaluation of asthma therapies. CompEx is constructed on data from a large number of patients included across several randomised controlled trials. It is proposed as a surrogate endpoint that can be used to design shorter and smaller trials assessing exacerbations that can mirror the treatment effect on severe exacerbations. CompEx was applied post-hoc to a house dust mite (HDM) SLIT-tablet allergic asthma trial (EudraCT 2010-018621-19; Virchow et al, JAMA, 2016).

Method: The trial included 834 subjects randomised 1:1:1 to once-daily treatment for up to 18 months with placebo, 6 SQ-HDM, or 12 SQ-HDM (ALK, Denmark). Subjects had >1 year history of HDM allergic asthma and associated rhinitis, not well-controlled by inhaled corticosteroid, and positive tests of HDM sensitisation. Diary data on peak expiratory flow, reliever use, and asthma symptoms were recorded each morning and evening, and night-time awakenings each morning during the 6 months efficacy assessment period. The primary outcome was the hazard ratio (HR) for first moderate or severe (ModSev) asthma exacerbation during the ICS reduction period (starting >7 months after randomisation). CompEx was compared with the primary outcome and with severe exacerbations after 3 and 6 months.

Table 1

	Severe exacerbations Event frequency, % (n/N)	HR (95% CI)	*P*-value	Moderate or severe exacerbations Event frequency, % (n/N)	HR (95% CI)	*P*-value	CompEx Event frequency, % (n/N)	HR (95% CI)	*P*-value
3 months	4% (19/505)	0.26 (0.09;0.79)	0.018	19% (97/505)	0.58 (0.39;0.88)	0.010	25% (125/501)	0.56 (0.39;0.81)	0.002
6 months	6% (28/505)	0.53 (0.25;1.15)	0.108	28% (142/505)	0.68 (0.49;0.95)	0.025	34% (169/501)	0.65 (0.48;0.89)	0.006

Results: Results for 12 SQ-HDM vs placebo are presented in Table 1. The CompEx endpoint increases the event frequency from 4% severe events and 19% ModSev events to 25% CompEx events at the 3 months assessment period. Further, the frequency of CompEx events at 3 months (25%) corresponds to ModSev exacerbations events at 6 months (28%). The HRs and *P*-values were comparable within the asthma exacerbation definitions.

Conclusion: The CompEx endpoint behaves as ModSev (cross-validation) with somewhat higher event rate and reduction, giving smaller CI and lower *P*-value. The results substantiate the CompEx definition and its utility as a useful and powerful marker of exacerbations. Further, CompEx is confirming the primary outcome in the SQ HDM SLIT-tablet trial. Future evaluation should include CompEx in a prospective allergy immunotherapy trial.

0127 | Comparison of long term effect of perennial and preseasonal SCIT of seasonal allergic rhinitis adult patients with hypoallergenic grass pollen extracts

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Background: The incidence of allergic rhinitis has increased steadily during the last 20 years. The inability of patients to avoid pollen allergens during the season and the variable efficacy of symptomatic treatments insure that allergen immunotherapy (AIT) retains a place as the only causal method of the treatment. The aim of our study was to evaluate the long term efficacy of perennial immunotherapy in comparison with preseasonal one.

Method: An open comparative study was conducted with a total of 37 hay fever patients. Pre-seasonal subcutaneous immunotherapy (SCIT) was performed in a group of 22 patients (mean age 24.4 ± 4.6 years) and perennial treatment in 15 patients (mean age 23.8 ± 4.3 years). The diagnosis was made on the basis of a detailed clinical history, skin prick tests and as IgE serum level estimation. We used for SCIT hypoallergenic extract of rye and grass pollen (Allergopharma, Reinbek, Germany). Symptom's medication score (sms) was recorded by all patients. The efficacy of AIT was estimated by symptom medication score after completion of therapy and 20 years later.

Results: Significant reduction of symptoms medications score was observed both just after completion the therapy and 20 years later. However long lasting effect was more pronounced in the group of patients treated with the perennial schedule. SMS after 3 years of AIT in perennial AIT group was reduced from 8.6 to 2.6 and in preseasonal AIT group from 8.2 to 3.6 ($P < 0.05$). SMS after 20 years was 3.9 in perennial AIT group and 5.1 in preseasonal one. There were no differences in number of side effects between investigated group of patients during the course of therapy. Significant lower number of new sensitization and new asthma development was recorded 20 years after discontinuation of AIT in patients underwent perennial immunotherapy.

Conclusion: Perennial in comparison to preseasonal AIT showed higher clinical benefit particularly in terms of long-lasting efficacy with the same safety profile.

0128 | Prevention of asthma progression through allergy immunotherapy—results from a large cohort study

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Background: Allergy immunotherapy (AIT) is regarded as the only treatment modality that is able to modify the natural course of the allergic disease. Long-term effects after treatment discontinuation and preventative effects on the development of asthma symptoms and asthma medication have been documented for some products. It has not yet been investigated whether AIT is able to prevent the progression of preexisting asthma compared to routine care.

Method: We undertook a large cohort study utilizing a healthcare insurance database including ICD-10 diagnoses and prescription data for 1.74 million individuals from Germany over 10 years. Internal case validation methods specified that at least two ICD-10 codes J 45 (Asthma) and at least two prescriptions of asthma medication (SABA, ICS, ICS+LABA) were required to classify patients as having asthma. The prescribed asthma medications were grouped according

to the treatment steps recommended by GINA 2006. Subgroups of different age groups in 2005 (B: 12-17 years; C: 18-50 years; D: 50 + years) were evaluated. Transitions between the GINA steps were analyzed using Cox regression models controlling for age and sex.

Results: AIT resulted in a significantly reduced risk to step up with asthma medication from GINA step 1 to GINA step 3 compared to no AIT. This effect was most pronounced in younger patients (Hazard ratio (95% CI): overall: 0.87 (0.80-0.95), group B: 0.72 (0.58-0.88), group C: 0.89 (0.80-0.98), group D: 1.09 (0.87-1.38). AIT also resulted in a decreased risk to step up from GINA step 3 to GINA step 4. The number needed to treat to prevent 1 patient stepping up from GINA 3 to GINA 4 within 5 years was 10.9 (8.2-16.2).

Conclusion: This large prospective cohort study is the first to demonstrate that AIT effectively prevents the progression of asthma defined as a step up in the treatment steps according GINA recommendation. The effect may be limited to younger patients without concomitant COPD. The results of this study should be considered supportive of product specific efficacy documented in randomized clinical trials.

MONDAY, 28 MAY 2018
OAS 23
WHAT'S NEW IN MOLECULAR ALLERGOLOGY?

0129 | CCD interference of purified natural allergens in component-resolved diagnosis (CRD): Differences between singleplex and multiplex testing

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Background: Purified natural allergens used for CRD may carry CCDs (cross-reactive carbohydrate determinants) and thus interfere with proper allergy diagnosis just like allergen extracts. We observed that certain natural allergens from hazelnut (nCor a 9) and soy (nGly m 5 and 6) might behave differently in ImmunoCAP singleplex and ISAC multiplex testing. While 7S globulins (Gly m 5) nearly always carry potential N-glycosylation sites, 11S globulins (Cor a 9, Gly m 6) do not appear to be glycoproteins.

Method: CCD-positive sera were tested on nCor a 9 (11 S globulin), nGly m 5 (7S globulin) and nGly m 6 (11S globulin) using Phadia ImmunoCAP and ISAC microarray. CCD inhibition was carried out using the ProGlycAn® CCD inhibitor.

Results: Among 18 sera with varying IgE levels to bromelain (1.01-44.6 kUa/l), 94% were positive >0.35 kUa/l to nCor a 9 (0.15-16.5), 89% to nGly m 5 (0.15-7.66), and 94% to nGly m 6 (0.23-16.0) in the ImmunoCAP. IgE binding to all three allergens correlated strongly with bromelain and between each other (r = 0.86-0.99) and could be completely blocked by the CCD inhibitor. None of the sera was positive to the same allergens in the ISAC. Among another 68 CCD-positive patients tested routinely with ISAC, only 2 reacted with nCor a 9 and 2 with nGly m 5. Differences in CCD-detection between singleplex and multiplex testing were also observed for the pollen glycoallergens nCup a 1 (cypress) and nSal k a1 (Salsola). While nCup a 1 and nSal k 1 were recognized in the ISAC by only 58% and 50% of 12 selected CCD-positive sera and with throughout low binding intensity, the same allergens were positive in respectively 97% and 100% of sera in the ImmunoCAP with binding scores close to those for bromelain.

Conclusion: Clinicians should be aware that the seed storage proteins nCor a 9, nGly m 5 and nGly m 6 behave CCD-negative on the ISAC microarray platform but show significant CCD interference when tested by ImmunoCAP singleplex. Considering that the 11S globulins Cor a 9 and Gly m 6 are probably not glycosylated, this might indicate contamination of allergen preparations with other glycoproteins.

0130 | IgE –reactive globulins from chickpea with homology to peanut allergens Ara h 1 and Ara h 3

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Background: Sensitivity to legumes is a prevalent food allergy in the Mediterranean area, with lentil and chickpea being the most frequent causes of allergic reactions. Most legume allergens are seed storage proteins, profilins, or pathogenesis-related proteins. However, allergenic proteins from chickpea have not been well defined. This study presents the purification of two allergens from chickpea, an 11S globulin and a 7S vicilin.

Method: Proteins were extracted from chickpea flour using precipitation and centrifugation. Globulin fractions, containing 7S vicilins and 11S legumins, were further purified using ion exchange and/or gel-filtration chromatography. The 7S vicilin and 11S legumin were analyzed by SDS-PAGE and LC-MS/MS. Sera from patients with known peanut, chickpea and/or lentil allergies were used to test for IgE reactivity using a chimeric ELISA. Sequence homology was analyzed using BLASTp. Homology modeling was performed using the Phyre2 web portal for protein modeling, prediction and analysis.

Results: Chickpea 11S globulin (legumin) consists of multiple polypeptides, which present as 20kD, 37kD, and 54kD bands on SDS-PAGE. All three polypeptides were identified as 11S seed storage protein by LC-MS/MS. Eleven of thirty-nine sera from patients allergic to peanut and/or lentils showed IgE reactivity against the purified 11S globulin. The chickpea legumin amino acid sequence shares 51% identity and 62% homology with peanut allergen Ara h 3.

The 7S globulin fraction presented as multiple polypeptides on SDS-PAGE, ranging from 20kD to 70kD. Fifteen of thirty-nine sera from patients allergic to peanut, lentil and/or chickpea showed IgE reactivity against 7S vicilin. The amino acid sequence obtained by LC-MS/MS confirmed identity of 7S vicilin, which shares 49% identity and 71% homology with peanut allergen Ara h 1.

Conclusion: The purified chickpea allergens were confirmed to be 11S legumin and 7S vicilin. Presence of multiple polypeptides indicates proteolytic processing characteristic for both plant storage proteins. Detection of IgE reactivity to chickpea legumin in sera from peanut, lentil and/or chickpea allergic patients suggests that 7S and 11S globulins may be cross-reactive allergens of the legume family. Purified chickpea allergens may be useful for diagnosis of allergy to legumes.

0131 | Cyclophilin: A novel cross-reactive determinant in peanut

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Background: Component-resolved diagnostics is an important tool in the investigation of patients with suspected peanut allergy, in particular for the purpose of distinguishing between primary and cross-reactive peanut sensitization. Known targets of cross-reactive pollen sensitization in peanut are PR-10 (Ara h 8), profilin (Ara h 5) and cross-reactive carbohydrate determinants (CCD). However, in a subset of subjects, we found that pollen-dependent IgE binding to peanut extract could not be explained by either of these known cross-reactivities. The aim of this study was to identify an hitherto unknown determinant of cross-reactivity between pollen and peanut.

Method: Sera of 15 peanut sensitized subjects that tested negative to Ara h 1, 2, 3, 6, 8, 9, profilin and CCD, and whose IgE binding to peanut extract could be inhibited by grass pollen extract, were used in this study. Peanut extract was separated by different chromatographic methods and fractions displaying IgE binding activity were subjected to MS/MS analysis on an Orbitrap Fusion instrument. IgE antibody measurements were performed using ImmunoCAP.

Results: MS/MS analysis of a basic IgE reactive fraction, purified by anion and cation exchange, size exclusion and reversed phase chromatography, produced a convincing match (73% coverage) to a cyclophilin (peptidyl-prolyl *cis-trans*-isomerase)-like amino acid sequence predicted from a peanut EST record (Acc No GO340500). The peanut cyclophilin sequence was 172 amino acid residues long and predicted to have no signal peptide, a molecular weight of 18.3 kDa and an isoelectric point of 8.4. It showed 86% sequence identity with carrot cyclophilin, a previously described IgE reactive protein. Thirteen of the 15 sera studied (87%) displayed IgE antibody binding to carrot cyclophilin, at a level which was on average 9-fold higher than the level of IgE to peanut. Further, the carrot protein could outcompete the IgE binding to the isolated peanut fraction. Taken together, our observations suggest that cyclophilin is a low-abundance protein in peanut and therefore probably not a primary sensitizer.

Conclusion: Cyclophilin was identified as a novel IgE binding protein in peanut and appears to represent an important cross-reactive overlap between pollen and peanut. Alongside PR-10, profilin and CCD, cyclophilin may become a valuable marker of pollen-related food sensitization, representing a lower risk of severe food allergy than primary food sensitization.

0132 | Fluorescent labelling of major honeybee allergens nApi m 1 and rApi m 2 with Quantum dots and its application in multiplex basophil activation test

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Background: Api m 1 and Api m 2 are two major allergens of honeybee venom. Basophil activation test (BAT) is an *in vitro* approach for the evaluation of the clinical relevance of IgE antibodies. Labelling of recombinant allergens with fluorescent probes could represent a new approach for multiplex assessment of allergenic activity with flow cytometry.

Method: nApi m 1 (Latoxan, France) and rApi m 2 (from Medical University of Vienna) were conjugated to Qdot® 705 or 800 ITK™ Amino (PEG) Quantum Dots and Qdot® 705 or 800 ITK™ Carboxyl Quantum Dots. IgE reactivity of Qdot-labelled allergens was assessed with immunodot assay using of rApi m 1 or rApi m 2 sIgE-positive sera of honeybee allergic patients. Allergenic activity was assessed with basophil activation test (BAT) using CD123-PE/HLA-DR-APC/CD63-FITC labelled antibodies. Qdot 705 was measured in PerCP and Qdot 800 was measured in Pe-Cy7. Samples were acquired and analysed on FACS Canto II flow cytometer.

Results: Both Amino and Carboxyl Qdot-labelled nApi m 1 and rApi m2 allergens clearly showed positive and specific IgE reactivity evaluated with immunoblotting. Importantly, there was no non-specific IgE binding to Qdots. We then tested whether Qdot labelled allergens are able to crosslink surface IgEs and induce activation of the basophils of honeybee allergic patients. We demonstrated that only Amino but not Carboxyl Qdot-labelled nApi m 1 and rApi m 2 are able to activate basophils, what obviously suggest that allergenic activity is preserved only in case of Amino Qdot-labelling. Furthermore, we showed that Qdot 705 Amino-labelled Api m 1 and Qdot 800 Amino -labelled Api m 2 could be used in multiplex analysis of basophil activation according to the subpopulation analysis of basophils according to the binding of allergens labelled with different fluorescent probes.

Conclusion: Quantum Dots labelling of allergens does not affect IgE reactivity; however IgE cross-linking and allergenic activity is preserved only in case of Amino (PEG) Qdots labelling. These novel experiments are the basis for development of multiplex basophil activation test and further development and use of fluorescent-labelled allergen components.

0133 | Development of Bet v 1 - specific DNA aptamers for quality control of birch pollen extracts

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Background: Birch pollinosis is a prevalent disease in northern Europe as well as North America and Japan. The main cause of birch pollen allergy is identified as Bet v 1—the major birch pollen allergen—to which more than 95% of patients worldwide are sensitized. Allergen-specific immunotherapy (AIT) is the most effective treatment option carried out using birch pollen extracts however, it has been demonstrated that it can also be achieved using Bet v 1. Current methods for quality control of pollen extracts used in AIT are lacking standardisation and are not addressing individual allergens. Aptamers are short DNA sequences that can bind with a high affinity and specificity to their target molecule due to their unique three-dimensional shape allowing for reproducibility from batch-to-batch production. Therefore, we aimed to produce aptamer sequences against the major birch allergen Bet v 1 to use in a variety of experimental procedures including the quality control of Bet v 1 extracts in ELISA-derived assays called enzyme-linked apta-sorbent assays (ELASA).

Method: Selection of Bet v 1 aptamers was performed *in vitro* by Mag-SELEX (Systemic Evolution of Ligands by EXponential Enrichment). Nine cycles were performed including counter selections against other proteins and negative selection against the magnetic beads used for selection. As the main use of these aptamers will be in ELASA setups, selection steps for PBS and TBST were also included. Following the last cycle, PCR amplified aptamer sequences were ligated into plasmids and cloned into a bacterial strain for sequencing.

Results: Of the 42 plasmids sequenced, 2 duplicate sequences were identified with a 100% similarity, thus a total of 40 different sequences were obtained. Following alignment, they were grouped into families, where possible, and a motif search between sequences of the same family was carried out. These motifs were also compared to the predicted secondary structures to identify similar structural segments regardless of the low primary sequence homology.

Conclusion: DNA sequences able to bind to Bet v 1 were generated by the Mag-SELEX method that can be used in ELASA. Identification of aptamer sequences binding to Bet v 1 will allow the development of standardised techniques regarding the quality control of birch pollen extracts but a possible use in AIT could also be developed. This research was supported by the University of Salzburg´s priority program "Allergy-Cancer-BioNano Research Centre".

0134 | New immunoproteomic approaches to delineate the complete proteomic profile of olive pollen and help solving its complex allergogram

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Background: Olive pollen is one of the main allergenic sources in the Mediterranean countries. To date, thirteen allergens,—Ole e 1 to Ole e 12 and Ole e 14 –, have been identified and characterized from its complex allergogram. However, some olive pollen allergens remain unknown, especially due to technical restrictions and a limited proteomic and genomic information. In this study, we have used two new proteomic and immunological approaches in combination with recently available genomic data to obtain a complete protein profile from olive pollen and its allergenic composition.

Method: The first approach consisted of generating a tryptic digest of the olive pollen extract for LC-MS/MS analysis using a Q-exactive. Thereafter, serum IgG from patients allergic to olive pollen were isolated and used in an immunoprecipitation technique to restrict the LC-MS/MS analysis to the most immunoreactive proteins in the extract. cDNAs from candidate allergens were cloned and expressed as N-terminal His-tagged recombinant proteins in *E. coli* to analyze their IgG and IgE reactivity by ELISA and Western blot.

Results: The obtained proteomic data shows that most of the pollen proteins are related to response to stimuli processes and to metabolic and cellular pathways. More than 20 allergenic protein families not previously described in olive pollen were identified. Three candidate allergens, a cyclophilin, a pectate lyase and a cytosolic malate dehydrogenase were cloned and produced in bacteria or yeast. All of them were recognized by the IgGs from olive pollen allergic patients' sera supporting the immunoproteomic approach and LC-MS/MS results. Moreover, cyclophilin showed IgE reactivity with allergic patients' sera. Accordingly, it has been defined as a new olive pollen allergen with overlapping features with those of the major allergen Ole e 1, highlighting the relevance of these approaches.

Conclusion: We present the most complete proteomic analysis of olive pollen, extending the list of candidate allergens and identifying three new IgG-immunoreactive proteins and a new masked allergen. These findings have significant implications for both, diagnosis and allergen immunotherapy purposes.

TUESDAY, 29 MAY 2018
OAS 24
BIOLOGICS IN ATOPIC DERMATITIS

0135 | Early response to upadacitinib in moderate-to-severe atopic dermatitis: Results from a phase 2b randomized, placebo-controlled trial

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Background: Atopic dermatitis (AD) is a chronic, inflammatory, skin disease characterized by pruritic lesions. Upadacitinib (UPA), a selective JAK-1 inhibitor, is investigated for treatment of patients with AD and other inflammatory diseases. We evaluated early response to UPA treatment from the initial 16-week, double-blind portion of a phase 2b, 88-week, dose-ranging trial.

Method: Adults with moderate-to-severe AD (EASI ≥16, BSA ≥10%, IGA ≥3) not adequately controlled by topical treatment, or for whom topical treatments were not medically advisable, were randomized to once-daily monotherapy with UPA 7.5, 15, or 30 mg, or placebo (pbo). Missing data were handled by last-observation-carried-forward (continuous variables) and non-responder-imputation (categorical variables).

Results: Of the 167 randomized patients; 166 received study drug (42 in each UPA dose-group; 40 in pbo). Mean percent improvement from baseline in EASI score at week 2 was 39.4%/55.9%/59.0% ($P < .001/<.001/<.001$) for UPA 7.5/15/30 mg groups vs 9.1% in pbo; at week 16 (primary endpoint), 39.4%/61.7%/74.4% ($P < .01/<.001/<.001$) for UPA vs 23.0% pbo. Mean percent improvement from baseline in weekly average of daily pruritus Numerical Rating Scale (NRS) at week 1 was 19.0%/28.3%/36.2% ($P < .001/<.001/<.001$) UPA vs −0.8% pbo; at week 16 (secondary endpoint), 39.6%/48.0%/68.9% ($P < .01/<.001/<.001$) UPA vs 9.7% pbo. Achievement of EASI 75 at week 2 was by 14.3%/26.2%/33.3% ($P < .05/<.001/<.001$) UPA patients vs 2.4% pbo; at week 16 (secondary endpoint), 28.6%/52.4%/69.0% ($P < .05/<.001/<.001$) UPA vs 9.8% pbo. Post hoc analysis showed a positive effect on daily pruritus NRS as early as day 2 (mean percent improvement from baseline: 20.8%/19.3%/33.4% for UPA 7.5/15/30 mg vs 1.7% pbo). Using daily assessment, 7.3%/11.8%/28.9% for UPA 7.5/15/30 mg patients vs 2.8% pbo showed improvement ≥4 points in pruritus NRS at day 2. The most common adverse events (AEs), UPA groups vs pbo, were upper respiratory tract infection (16.7%/11.9%/11.9% vs 10.0%) and AD exacerbation (16.7%/7.1%/11.9% vs 7.5%). Serious AEs (n) were atrial fibrillation (1; pbo), appendicitis (1; UPA 15 mg), pericoronitis (1; UPA 7.5 mg), skin infection and AD exacerbation (1; UPA 7.5 mg).

Conclusion: UPA treatment induced skin improvements as early as the first visit at week 2; improvements in pruritus were recorded as early as day 2. The positive benefit/risk profile of UPA supports proceeding to phase 3 trials in AD.

0136 | Dupilumab in patients with moderate-to-severe atopic dermatitis and comorbid asthma requiring treatment: Analysis from two pooled phase 3 trials (liberty ad solo 1&2)

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Background: Dupilumab, a fully human monoclonal antibody against IL-4Ra, inhibits signaling of IL-4 and IL-13, type 2/Th2 cytokines involved in atopic/allergic diseases such as atopic dermatitis (AD), asthma, and allergic rhinitis which often associate as comorbidities. This post hoc subgroup analysis examined if concurrent active asthma requiring treatment impacted the efficacy of dupilumab treatment for AD in two pooled phase 3 monotherapy trials (SOLO 1: NCT02277743; SOLO 2: NCT02277769). We also examined if dupilumab improved asthma control in AD patients (pts) with concurrent active asthma. Dupilumab is approved in the EU, USA, and other countries for the treatment of adults with inadequately controlled moderate-to-severe AD.

Method: Adult pts with moderate to severe AD whose disease is inadequately controlled with topical prescription therapies or when those therapies are not advisable were randomized 1:1:1 to dupilumab 300 mg weekly (qw): every 2 weeks (q2w): placebo (PBO) for 16 weeks. Endpoints included proportion of pts with both Investigator's Global Assessment (IGA) 0/1 and ≥2-point improvement from baseline, ≥75% improvement from baseline in Eczema Area and Severity Index (EASI-75), and ≥4-point improvement in peak pruritus numerical rating scale (NRS). Asthma Control Questionnaire (ACQ-5) outcomes were assessed in pts with active asthma.

Results: The proportion of AD pts with concurrent active asthma requiring treatment was generally balanced across treatment groups (28.0%/29.5%/25.5%; PBO/q2w/qw), and had slightly more severe AD than pts without concurrent asthma. At Wk 16 more pts receiving dupilumab q2w or qw achieved an IGA 0/1 and ≥2 point improvement vs PBO regardless of concurrent asthma (31.9%/32.2% vs 10.9%; q2w/qw vs PBO) or without (39.1%/38.4% vs 8.8%). More

pts receiving dupilumab also achieved EASI-75 and ≥4-point improvement in peak pruritus NRS vs PBO regardless of concurrent asthma (51.1%/47.5% vs 13.2% and 40.8%/42.7% vs 9.7% respectively) or without (46.3%/51.2% vs 13.3% and 37.4%/38.6% vs 11.3%) ($P < 0.0001$ for all comparisons). Dupilumab improved asthma control (ACQ-5) in pts with concurrent asthma (mean change from baseline [SE]; -0.23 [0.08]/-0.32 [0.08] vs 0.00 [0.08]; $P = 0.0136$/ $P = 0.0016$). The most common adverse events (≥10%) attributable to dupilumab were injection site reactions and conjunctivitis.

Conclusion: Dupilumab improves signs and symptoms of AD regardless of asthma comorbidity, and also improves asthma control, as measured by ACQ-5, in pts with active asthma.

0137 | First-in-class phase 2a study of anb020 (anti-il-33) in the treatment of moderate-to-severe atopic dermatitis

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Background: Atopic dermatitis (AD) is a common debilitating pruritic skin condition associated with atopic asthma, rhinitis and food allergy. Moderate-severe disease carries significant morbidity and health economic burden, with major unmet medical need. The alarmin IL-33 is over-expressed in atopic dermatitis lesions and has been implicated in driving a type 2 immune response, including group 2 innate lymphoid cells and Th2 cells. ANB020 is a potent anti-IL-33 cytokine antibody with prior demonstration of safety, pharmacokinetics and pharmacodynamic activity in a healthy volunteer phase 1 study.

Method: We undertook a phase 2a proof of concept study of a single intravenous administration of 300 mg ANB020 (anti-IL-33) in 12 adults with moderate-severe atopic dermatitis. Skin was sampled before and after ANB020 administration at house dust mite-challenged or saline-challenged sites for exploratory mechanistic analyses. The patients were followed clinically for 20 weeks using EASI, 5-D pruritus and other outcome measures.

Results: Rapid clinical response was observed at day 15 post-ANB020 administration with nine of 12 patients (75 percent) achieving EASI-50, of which three patients (25 percent) also achieved EASI score improvement of 75 percent relative to baseline (EASI-75). Sustained clinical response was observed at day 57 post-ANB020 administration, with nine of 12 patients (75 percent) achieving EASI-50, of which five patients (42 percent) also achieved EASI-75.

Conclusion: This first-in-class study of ANB020 (anti-IL-33) potentially represents a significant advance in the treatment of moderate-severe atopic dermatitis, addressing a major unmet medical need.

0138 | Safety of dupilumab in moderate-to-severe atopic dermatitis: Clinical laboratory results from three phase 3 clinical trials (LIBERTY AD: SOLO 1, SOLO 2 and CHRONOS)

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Background: Dupilumab (anti-interleukin-4-receptor-α mAb) is approved in several countries for the treatment of adults with moderate-to-severe atopic dermatitis (AD). Previous phase 3 clinical trials demonstrated the efficacy and safety of 16/52 weeks (weeks) treatment with dupilumab in AD. We report clinical laboratory data from SOLO1&2 and CHRONOS.

Method: SOLO1&2 (16 weeks) and CHRONOS (52 weeks) were 3 randomized, double-blinded, placebo (PBO)-controlled phase 3 trials. Patients (pts) were randomized 1:1:1 (SOLOs) or 3:1:3 (CHRONOS) to dupilumab qw, q2w, or PBO. Pts in CHRONOS received a standardized regimen of concomitant topical corticosteroids that could be tapered/stopped based on response.

Results: Clinical laboratory data were assessed in 1376 pts from SOLOs and in 740 pts from CHRONOS. Baseline (BL) characteristics were balanced across treatment groups. Hematology and serum chemistry values were generally consistent with BL data. Dupilumab-treated pts had greater mean initial increase from BL in eosinophils (eos) vs the PBO group in SOLOs, with the highest increase observed at Wk4 (0.10/0.09/-0.01 $x10^9$/L [qw/q2w/ PBO]). Eos returned to near BL levels by Wk16. The increase in eos was not seen in CHRONOS. The incidence of treatment-emergent (TE) eosinophilia (≥500 cells/mcL) was similar for dupilumab and PBO in all 3 trials. Marked TE eosinophilia (≥5000 cells/mcL) was reported in <1% of dupilumab-treated pts and none in PBO-treated pts. There were no adverse events associated with eosinophilia. In most cases eos returned to near BL levels during study treatment. A greater mean decrease in lactase dehydrogenase (LDH) from BL through Wk16/Wk52 was observed in dupilumab- vs PBO-treated pts (SOLOs Wk16: -76.0/-69.7/-26.2; CHRONOS Wk52: -79.5/-89.8/-45.9 IU/L [qw/q2w/ PBO]). With exception of BL values in CHRONOS, mean LDH values for each treatment group were within the normal range throughout the study period. No clinically meaningful changes between the treatment groups were observed for other hematological, chemistry or urinalysis parameters; vital signs; electrocardiogram and physical examination parameters.

Conclusion: There were no untoward clinically important changes in laboratory parameters that could be attributed to the study treatment. Consistent with data from clinical trials, EU/US prescribing information do not require laboratory tests before initiation/during

treatment with dupilumab; however, laboratory monitoring may be required in some regions based on local guidelines.

0139 | Baseline disease signs and symptoms and systemic treatment of patients with atopic dermatitis included in the German AD registry treatgermany

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Background: Clinical patient registries can provide valuable information on medical care under real life conditions relevant for evidence-based clinical decisions.

Method: Adults with moderate-to-severe AD [current/previous anti-inflammatory systemic treatment and/or objective Scoring of AD (SCORAD) ≥20] are included in the multi-centered German AD Registry *TREATgermany*. Patients are prospectively followed over a period of at least 24 months with assessment of demographic data, the objective disease severity [EASI, objective SCORAD, IGA] and the patients' disease rating [PGA, patient oriented eczema measure (POEM)] as well as assessment of pruritus, pain and sleeping problems on 10-point VAS. The patients' treatment satisfaction is documented using validated questionnaires.

Results: From June 2016 until December 2017, 243 patients (mean age of 42.8 ± 14.6 years, 38.6% females) were included into the registry. In the minority of patients (22.4%) the onset of AD was during adulthood. According to inclusion criteria, patients show a moderate-to-severe disease at baseline (mean objective SCORAD 39.1 ± 14.4; mean EASI 13.3 ± 10.9), but there is a difference between physician and patient assessment (P = 0.05, Wilcoxon test). In the vast majority an involvement of the face and hands is reported (77.4% and 80%, respectively).

Assessment of subjective disease symptoms revealed moderate itching (5.2 ± 2.6 points) and less pain (3.1 ± 2.6) and sleep disorders (3.9 ± 3.3) and mean POEM scores of 15.3 ± 7.3 points (maximum 28 points). A percentage of 88.5% of patients reports an oral intake of antihistamines. Systemic treatment with glucocorticosteroids is documented in 60.5% of patients followed by cyclosporine (42%). However, 65.4% of patients suffered from AD all over the last 12 months before enrolment (mean 10.2 ± 3.1 months). Only 17.1% of patients feel their AD well controlled (≥ 10 weeks) resuming the past 12 weeks, and the patient's satisfaction with medical treatment is 6.4 ± 2.8 points (scale 0-10).

Conclusion: This baseline data analysis of 243 patients included into the *TREATgermany* registry provides an insight into clinical characteristics and current treatment modalities of patients with moderate-to-severe AD. Despite systemic treatment in the majority of patients, data reflect a high burden of disease and the need for further safe treatment options effective for long term control.

0140 | Dupilumab in moderate-to-severe atopic dermatitis by history of asthma and allergic rhinitis: Analysis from a 52 week randomized phase 3 trial (liberty ad chronos)

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Background: Dupilumab, a fully human monoclonal antibody against interleukin (IL)-4 receptor-a, inhibits signaling of IL-4 and IL-13, type 2/Th2 cytokines involved in atopic/allergic diseases such as atopic dermatitis (AD), asthma, and allergic rhinitis (AR). This post hoc subgroup analysis was intended to determine if a history of asthma or AR impacts the efficacy of dupilumab treatment for AD in the long-term phase 3 CHRONOS trial (NCT02260986). Dupilumab is approved in the EU, USA, and other countries for the treatment of adults with inadequately controlled moderate-to-severe AD.

Method: Adult patients (pts) were randomized 3:1:3 to dupilumab 300 mg weekly (qw): every 2 weeks (q2w): placebo (PBO) for 52 weeks with topical corticosteroids or calcineurin inhibitors. Endpoints included proportion of pts with Investigator's Global Assessment (IGA) 0/1 and ≥2-point improvement from baseline (primary), and a ≥ 75% improvement from baseline in Eczema Area and Severity Index (EASI-75).

Results: Baseline characteristics were generally balanced between the treatment arms in each of the subgroups; with a history of asthma: PBO (n = 162), q2w (n = 51), and qw (n = 151); and without asthma history: PBO (n = 153), q2w (n = 55), and qw (n = 168); with a history of AR: PBO (n = 142), q2w (n = 54), and qw (n = 142), and without AR history: PBO (n = 173), q2w (n = 52), and qw (n = 177).

At Week 52 more pts receiving dupilumab q2w or qw achieved an IGA 0/1 and ≥2-point improvement vs PBO regardless of asthma comorbidity; with asthma: 32.6%/36.4% vs 12.1% (q2w/qw vs placebo; $P = 0.0008$/$P < 0.0001$); without asthma: 39.5%/43.5% vs 12.9% ($P = 0.0001$/$P < 0.0001$). Similar results were observed for pts with AR comorbidity; with AR: 31.8%/45.3% vs 16.1% ($P = 0.0723$/$P < 0.0001$); without AR: 40.0%/35.9% vs 9.9% ($P = 0.0001$ both). Additionally, more pts receiving dupilumab achieved EASI-75 vs PBO regardless of asthma comorbidity; with asthma: 65.2%/63.6% vs 20.7%; without asthma: 65.1%/64.5% vs 22.6%. Similar results were observed for pts with AR comorbidity; with AR: 65.9%/71.8% vs 25.0%; without AR: 64.4%/58.2% vs 19.1% ($P < 0.0001$ for all AR comparisons). The most common adverse events (≥10%) attributable to dupilumab were injection site reactions and conjunctivitis.

Conclusion: Dupilumab improves signs of AD regardless of a history of asthma or AR, implying that a potentially increased type 2/Th2 burden does not impair dupilumab efficacy in AD.

TUESDAY, 29 MAY 2018
OAS 25
PEDIATRIC FOOD ALLERGY

0141 | Food introduction styles in the first year of life and risk of allergic diseases in the PASTURE birth cohort

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Background: There is growing evidence that specific feeding practices and dietary habits early in life (e. g. farm milk consumption) have protective effects on allergic diseases like asthma. Feeding styles and their evolvement in early infancy have not been explored by hypothesis-free clustering approaches yet. So we aimed to determine food introduction patterns monitored by a monthly Food Frequency Questionnaire in the first year of life and analyzed by means of Latent Class Analysis (LCA) and to relate these to the lifetime prevalence of asthma at age 6 years.

Method: PASTURE is a prospective birth cohort study involving children from rural areas in 5 European countries (Austria, Finland, France, Germany and Switzerland) designed to evaluate risk- and preventive factors for atopic diseases, including the potential effects of dietary patterns of the children over time. Feeding practices were reported by parents in monthly diaries between the 3rd and 12th month of life. Parents indicated for each of 17 most common food items whether it was given to the child in the last 4 weeks and, if so, how often in 4 categories (never/less than once a week/1-6 times a week/daily). The resulting 153 4-staged ordinal variables were entered in a LCA. Asthma (lifetime prevalence) and potential confounders were assessed by a parental questionnaire at age 6.

Results: Data with at least one reported feeding practice over time was available for 1042 of the 1133 recruited children. Best LCA model fit was achieved by the 4-class solution. Latent Class (LC) 1 (N = 165) included mostly French and LC 2 (N = 173) mostly Finnish children. The other LCs, containing subjects mostly from the other centers, clearly distinguished between children whose parents preferred an early (LC 3, N = 414) vs a late (LC 4, N = 290) food introduction. The difference in asthma prevalence between these LCs (7.6% vs 4.3%) was fully explained by parental history of atopic diseases and a resulting avoidance strategy. In contrast, the elevated risk in LC 2 vs all other LCs (aOR = 8.83; P = 0.0006) was genuinely attributable to this particular feeding style irrespectively of country. It was explained by meat- without concomitant milk (products)-consumption on a daily basis (change in estimate = 81.3%), involving over a third of the asthma cases in the cohort.

Conclusion: The imbalance of overly frequent meat- and low milk- and milk products intake in infancy may induce physiological processes strongly increasing subsequent asthma risk.

0142 | Longitudinal S. aureus colonization in the LEAP Study: Relationship with eczema severity, resolution, IgE production and food allergy

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Background: Eczema has been shown to be a risk factor for food sensitisation/allergy. Patients with eczema are more likely to be colonized with *S. aureus*. The association between *S. aureus* colonization and food sensitisation/allergy has not been described in a RCT. The aim of our study was to investigate the relationship of skin and nasal *S. aureus* colonization with A) sIgE production to peanut and hen's egg allergens, B) peanut and egg allergy, C) eczema severity and persistence in LEAP and LEAP-On study participants.

Method: Outcomes were assessed at screening, 12-30-60-72 months of age; eczema severity was determined using the modified SCORAD and sIgE to peanut and hen's egg white were measured using ImmunoCAP. Skin and nasal swabs were obtained at all assessments except 72 months of age and were cultured the same day. Persisting egg allergy at 60 and 72 months of age was defined as SPT ≥6 mm to raw or pasteurized hen's egg in the participants diagnosed as egg allergic at screening. Peanut allergy was determined by oral food challenge. The associations between *S. aureus* colonization and sIgE production as well as peanut and egg allergy were corrected for eczema severity.

Results: Participants with skin *S. aureus* had higher SCORAD throughout LEAP. A significant, but less strong, association was noted for nasal *S. aureus* and SCORAD. Participants with immediately preceding skin *S. aureus* had different eczema resolution

patterns observed between 12 and 30, and 60 and 72 months; skin *S. aureus* predicted increasing eczema severity and persistent eczema. High levels of peanut and hen's egg white sIgE production at each follow-up were associated with skin *S. aureus* positivity at any time-point. Participants with skin or nasal *S. aureus* at any study-point were more likely to have persistent egg allergy [at 60 (P = 0.042) and 72 (P = 0.055) months]. Similar results were found for peanut allergy [at 60 (P = 0.055) and 72 (P = 0.031) months]. Five of the six participants to develop peanut allergy in the consumption group had skin *S. aureus* at at least one time point during LEAP.

Conclusion: *S. aureus* had an immune and disease-modulating effect in LEAP and LEAP-On participants. *S. aureus* may influence the development of tolerance to foods. These findings are clinically relevant because *S. aureus* was shown to be a risk factor for the development or persistence of allergic disease. Therefore, there may be a role for the treatment of S. aureus colonized eczema.

0143 | The changing face of allergen challenges since their introduction to Ireland: 12 years of single centre experience in Cork

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Background: Food allergy management has changed dramatically since Ireland's first allergy clinic opened in 2006. Egg and milk are now often introduced at home in baked form but peanut and other foods and drugs are still mainly introduced in the setting of a formal oral food challenge (OFC) in hospital.

Method: We performed a retrospective review of OFCs performed on 1837 children at Cork University Hospital (CUH), Cork, using a rolling series format. Repeated tests on previously challenged children were excluded. The allergens investigated were peanut, unadulterated/pasteurised egg, baked egg, tree nuts, unadulterated liquid milk and drugs. A severe reaction was defined by the presence of shortness of breath, dyspnea, desaturation, throat involvement, wheeze, or IM adrenaline use.

Results: A total of 1644 oral challenges were included, peanut (n = 593, positive 304 -51%). Peanut OFCs increased from 20 to 68 per 100 OFCs over the course of the study. Tree nut OFCs increased from 5 to 22 per 100 OFCs, predominantly due to an increase in hazelnut (n = 52) and cashew (n = 23) challenges. Milk, unadulterated and baked egg decreased from 11%, 22% and 24% to 0%, 0% and 6% respectively. The severity of reaction and increasing peanut eliciting dose had a statistically significant positive linear relationship. Milk and straight egg had no statistical significant relationship between dosing and severity.

Conclusion: A change in OFC profile was observed for peanuts and tree nuts (both relatively increasing) and for milk and eggs (both effectively eliminated, outside research protocols) over the first 12 years of OFC in Ireland, reflecting the dynamic adoption of evolving home-based treatment strategies for milk and egg allergies and the persisting need for hospital based OFC for peanut and tree nuts.

0144 | Eosinophilic esophagitis in children with a history of esophageal atresia

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Background: Patients with congenital esophageal atresia/tracheoesophageal fistula (EA-TEF), repaired soon after birth may suffer from respiratory and gastrointestinal complications for a long after, due to tracheomalacia, esophageal dysmotility or even esophagitis with a variety of presenting symptoms. In the last years there is growing evidence that eosinophilic esophagitis (EoE) is more prevalent in patients with EA-TEF, because of obvious risk factors (severe reflux, altered microbiome, barrier dysfunction) and probably others. The role of allergy is also strongly discussed. The value of timely intervention has been shown. However, for early diagnosis of EoE, high suspicion index is needed. The purpose of this report was therefore, to draw physicians' attention to the multiple faces of the disease in this group of patients and the particularities in its management.

Method: The experience on the EoE in children with congenital EA-TEF of three centers in Athens, Greece, during the last five years is reported.

Results: Eight patients (age 4-12 years) with a history of EA-TEF operated soon after birth, with persistent dysphagia refractory to antacid medications with proton pump inhibitors (PPIs) were diagnosed with EoE. The diagnosis was made after endoscopy with typical endoscopic macroscopic findings of the disease (5/8 patients have strictures) and prominent eosinophilia histologically (30-120 Eos pHPF) for all of them, despite the long term use of PPIs. A positive atopic history and positive patch-tests were found in 5/8 and 7/8 respectively. They all were treated with topical steroids (swallowed budesonide) with histologically and clinical improvement in 7/8. Five patients needed elimination diet (cow's milk avoidance). EoE symptoms improved beyond the reduction of eosinophilia and more over the strictures and the need for interventions such as dilatations.

Conclusion: EoE should be considered in patients with EA, especially when there is dysphagia refractory to standard treatment even more, when a need for dilatations. In most of them elimination diet should be considered.

0145 | Food protein induced enterocolitis syndrome, the experience from a Greek multicentric case series

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Background: Food protein–induced enterocolitis syndrome (FPIES) is a non–IgE-mediated food allergy which is often misdiagnosed due to its non-specific presenting symptoms. The most common triggers are reported cow's milk, rise, fish, and soy but a regional variation in common triggering foods might exist. The aim of our study was to describe demographic features, causative agents and outcomes of children suffering from acute FPIES at six Greek Pediatric Allergy Centers.

Method: A retrospective study was performed over a 4-year period (2013-2017). Hospital medical record databases and hospital outpatient charts were screened for the diagnosis of FPIES. Information on the outcome was collected.

Results: Eighty seven (87) children with FPIES were diagnosed. Fish was the most common trigger food (48.2%), followed by cow's milk, rice, egg, poultry, beef and wheat. Eighty two (92.3%) children reacted to a single food. The number of diagnoses significantly increased between 2013 and 2017 ($P < 0.001$). Vomiting was the most common symptom (94.6%). The mean age at the first episode was 9 months [2 month-2 years] and the first visit to Pediatric Allergy Clinic was 19 months [2 months-6 years]. To make diagnosis OFC was performed on 14.7% children.

22/87 children (25.3%) achieved tolerance at a mean age of 31 months [17 months-6 years].

Conclusion: FPIES is a not rare type of food allergy in children. Fish is the most common food trigger in Greece. Most of the children do not achieve tolerance in pre-school age.

0146 | Up-dosing challenge in children with food protein enterocolitis syndrome: Timing and threshold dose

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Background: Food Protein Induced Enterocolitis Syndrome (FPIES) is a non-IgE-mediated food allergy characterized by repeated and projectile vomiting, pallor and lethargy, occurring 1-4 hours after food ingestion. Sometimes diarrhea may follow the initial signs. Diagnosis of FPIES is based on clinical history and, if necessary, on the result of the oral food challenge (OFC) with the trigger food. The latest International Guidelines recommends administering the challenge food at a dose of 0.06 to 0.6 grams, usually 0.3 grams of the food protein per kilogram of body weight in three equal doses over 30 minutes.

As FPIES symptoms are delayed, we think that giving several doses within one hour, does not leave enough time to see if even the first dose would have caused reactions.

We usually adopted a different OFC protocol, which provides the assumption of 25% of the full dose and after 4 hours, in the absence of adverse reactions, the remaining dose. The aim of our study is to observe if the initial dose is sufficient to elicit symptoms, making reliable longer intervals between doses.

Method: A retrospective study was undertaken. All children diagnosed with FPIES were enrolled from January 2016 to December 2017. The diagnosis of FPIES was based on the International Guidelines. Children with FPIES underwent OFC with the trigger food after 12 to 18 months from the latest FPIES episode and with foods considered at risk to test their tolerance.

Results: 50 children were enrolled (25 males, 25 females). Mean age at the first FPIES episode was 7.6 months ±4.6 standard deviation (SD); mean age at diagnosis was 14.6 months ±23.3 SD. Milk represents the most common trigger food (52% of patients) followed by fish (30% of patients). The majority of patients have FPIES to a single food (80%).

Two-hundred and twenty-one OFCs were performed: 7.7% (17/221) failed; the symptoms started after a mean of 2.5 hours ± 1.1 DS from the initial dose assumption.

Conclusion: All the 17 failed OFCs (7.7%) showed the occurrence of symptoms after the 25% of the full dose with a mean time of 2.5 ± 1.1 SD, showing that even lowest doses are sufficient to elicit the reactions. According to our results, this OFC protocol is a valid alternative to the one currently used and it should be validated in a higher number of children.

TUESDAY, 29 MAY 2018
OAS 26
ASTHMA FROM EPIDEMIOLOGY TO DIAGNOSIS

0147 | Longitudinal patterns of exacerbations from infancy to school age

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Background: Previous studies which used data-driven methodologies have reported the existence of an exacerbation-prone asthma phenosubtype, which is independent of asthma severity. However, longitudinal patterns of asthma exacerbations during childhood have not been studied. We sought to investigate whether there are distinct longitudinal trajectories of asthma exacerbations from infancy to school-age that could facilitate better understanding of the heterogeneity of asthma syndrome.

Method: We used longitudinal k-means modelling (an unsupervised data-driven method), to analyse linked primary care data from 916 participants in a population-based birth cohort study (Manchester Asthma and Allergy Study), to ascertain clusters of children with similar trajectories of asthma exacerbations during childhood (n = 160). We tested the validity of these clusters in relation to lung function, airway hyperreactivity and inflammation, allergic sensitisation, and the use of asthma medication.

Results: A two-cluster model provided the optimal solution for our data set. Based on the pattern of exacerbations from infancy to age 8 years, we assigned the clusters as: "Early-onset frequent exacerbations (FE)" (n = 10) and "Infrequent exacerbations (IE)" (n = 150). Shorter duration of breastfeeding was the strongest risk factor for FE (median weeks 0 (IQR: 0-1.75) vs IE, median weeks 6 (IQR: 0-20), $P < 0.001$). Children in the FE cluster were more likely to exhibit persistent wheeze (90% vs 47%, $P = 0.03$) and have poorer lung function, more airway hyperreactivity, and more airway inflammation throughout childhood (Table 1). In a post-hoc analysis, when we compared children in the exacerbation clusters with those who have wheezed only (n = 389), and those that wheezed but had no exacerbations (n = 338), other early life risk factors such as atopic sensitisation (IE - RR: 3.2 (95% CI: 2.1-5.1), $P < 0.001$) (FE - RR: 10.9 (95% CI: 2.1-57.7), $P = 0.004$), exposure to tobacco smoke at birth (FE-RR: 2.8 (95% CI: 1.3-6.3) , $P = 0.02$), position in sibship (IE - RR: 1.5 (95% CI: 1.0-2.3), $P = 0.03$), and day care attendance (IE - RR:0.6 (95% CI: 0.4-0.9), $P = 0.01$) were significantly associated with exacerbations.

Table 1

	Infrequent Exacerbations, n = 150 RR (95% CI)	Frequent exacerbations, n = 10 RR (95% CI)	*P*-value
FEV1, mean (95% CI), age 5	93.8 (86.1-103.6)	101.6 (101.1-105.1)	<0.001
FEV1, mean (95% CI), age 8	95.6 (88.5-104.4)	92.3 (87.9-100.7)	<0.001
sRAW, mean (95% CI), age 3	1.2 (1.0-1.4)	1.5 (1.3-1.6)	<0.001
sRAW, mean (95% CI), age 5	1.2 (1.1-1.4)	1.3 (1.2-1.7)	<0.001
sRAW, mean (95% CI), age 8	1.2 (1.1-1.4)	1.8 (1.2-1.9)	<0.001
FEV1/FVC, mean (95% CI), age 8	86.1 (81.6-90.0)	78.8 (75.9-83.9)	<0.001
FeNO, mean (95% CI), age 8	11.5 (7.7-23.1)	58.5 (46.9-76.9)	<0.001

Conclusion: We have identified two distinct patterns of asthma exacerbations during childhood with different outcomes, early-life risk factors, and lung function when compared to children who wheeze, but have no exacerbations. These results indicate that exacerbations represent an independent susceptibility phenotype.

0148 | The GA2LEN-Score: Development and validation of a new approach to identify adult asthma in epidemiological studies

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Background: One of the questions in epidemiology is the identification of adult asthma in studies. We aim to 1) develop and validate a multivariate prediction model to identify adult asthma in epidemiological studies; 2) explore cut-offs to rule-in/rule-out asthma, compared to physician-diagnosed asthma after a structured clinical interview and diagnostic tests, blinded to the self-administered questions.

Method: We've included 711 adults from the Control and Burden of Asthma and Rhinitis study (PTDC/SAU-SAP/ 119192/2010), a nationwide population-based study conducted in Portugal. All participants signed the consent form. The predictors were self-administered questions from the GA2LEN (Global Allergy and Asthma European Network) questionnaire. The GA2LEN-score was

developed using exploratory factor analysis. The model's performance was tested in both the derivation (about 80% of the participants) and validation cohorts. Internal consistency, discriminative power and diagnostic accuracy were assessed. The cut-off to rule-in asthma was defined as the minimum number of positive answers to obtain a PPV ≥ 85% simultaneously in both cohorts. The cut-off to rule-out asthma was defined as the maximum number of positive answers to obtain an NPV ≥ 95% or more simultaneously in both cohorts.

Results: The resulting GA2LEN-score includes 6 questions: self-reported ever asthma, asthma attack in the last 12 months, current asthma medication, wheezing, wheezing without a cold and wheezing with breathlessness; it has a high Cronbach's alpha (0.85, 95% CI 0.83-0.87) and good discriminative properties (AUC 89.0%, 95% CI 85.4-92.5). The scoring is the sum of positive answers. Asthma is present (ruled-in) for scores ≥ 4 (specificity 99.2%, 95% CI 95.5-100.0; PPVs 91.7%, 95% CI 59.7-98.8; accuracy 87.4%). Asthma is excluded (ruled-out) for a score of 0 (sensitivity 93.1%, 95% CI 77.2-99.1; NPV 98.0%, 95% CI 92.8-99.5; accuracy: 82.8%).

Conclusion: The GA2LEN-score is based on self-administered questions and was validated compared to the physician-led asthma diagnosis. It is short, easy to use, and can be applied to identify the presence or to screen for asthma in epidemiological studies.

0149 | Comorbidities of allergic rhinitis and allergic asthma

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Background: Allergic rhinitis (AR) and allergic asthma (AA) are closely related diseases. It is well known that AR is the main risk factor for the development of AA. The aim of the present study was to investigate associations between AR and AA and other comorbidities in an age dependent manner.

Method: A German insurance database including ICD-10 diagnoses for 1.74 million subjects was used. For these subjects the 100 most common ICD-10 diagnoses in 2014 were selected. Diagnoses were evaluated in a network graph stratified by sex/age for AR and AA. The strength of the association between different diagnoses was evaluated by Cramer's V correlation. Subgroups looking at different age groups in 2005 (A: 0-11 years 122 714 subjects; B: 12-17 years; 79 785 subjects; C: 18-50 years; 637 411 subjects; D: 50 + years 899 530 subjects) were evaluated.

Results: The number of comorbidities increased with the age groups. In the 0-11 age group, both AR and AA were mostly correlated to atopic diseases like atopic dermatitis. In the 12-17 age group comorbidities like depression, somatoform disorders and anxiety came in addition as being predominantly associated to AA. For the 18-50 year age group, associations to depression, somatoform disorders and anxiety diagnoses increased. Correlations to musculoskeletal diagnosis were also identified, whereas the associations to atopic diseases were smaller then in younger age groups. Many associations have also been enhanced by the presence of multimorbid patients, especially in age group D.

Conclusion: In addition to the known association of AR and AA with other atopic diseases a clear correlation of AR and especially AA with mental disorders was documented. The number of comorbidities increased with age. Costs of these diseases should be considered in cost calculations for AR and AA. This analysis also helps to understand which comorbidities impact costs of patients with AA and AR in different age groups.

0150 | Electronic nose analysis of exhaled breath condensate is able to distinguish children with asthma and to identify those in need of corticosteroid therapy

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Background: The diagnosis and phenotyping of childhood asthma is particularly complex due to concomitant virus-induced and/or transient wheezing which may lead to misdiagnosis and inappropriate treatment. The present study aimed to assess how volatile organic compounds (VOC) in exhaled breath condensate (EBC) may assist in paediatric asthma diagnosis and treatment decision.

Method: Participants, aged 6 to 18 years, were recruited on a random basis during visits to an outpatient allergology clinic and to a juvenile football team training session. After obtaining the legal guardians' informed consent, lung function, airway reversibility and skin-prick tests were performed. An EBC sample was collected from each patient. After processing, EBC breathprints were measured using an electronic nose (eNose) system. Information on medical diagnosis of asthma, rhinitis and atopic dermatitis were retrieved for each participant. The reference standard was the medical diagnosis of asthma, based on the objective testing as well as symptoms history and

Table 1

	Method	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC (CI 95%)	*P*-value
Asthma diagnosis	BD	61.3	44.4	100	0.72 (0.60-0.84)	0.005
	eNose	79.7	77.8	84.2	0.81 (0.69-0.93)	<0.001
Intermittent asthma	BD	59.4	31.3	68.8	0.50 (0.34-0.67)	1.000
	eNose	40.6	50	37.5	0.56 (0.40-0.73)	0.457
Persistent asthma	BD	70.3	51.7	85.7	0.69 (0.55-0.82)	0.010
	eNose	79.7	93.1	68.6	0.81 (0.70-0.92)	<0.001

physical examination, according to the Global Initiative for Asthma guidelines. A total of 51 participants were included. A hierarchical cluster model based on the eNose sensor resistances was then created.

Results: The two-cluster exhaled VOC-based hierarchical model was able to significantly discriminate individuals with asthma from those without the disease ($P < 0.001$). Individuals who had persistent asthma and were under inhaled corticosteroid therapy were also significantly distinguished in the model ($P < 0.001$). The method showed higher overall accuracy, sensitivity and AUC values when compared to spirometry with bronchodilation (Table 1).

Conclusion: The developed hierarchical model based on exhaled breath condensate VOC analysis by eNose was able to distinguish individuals with a medical diagnosis of paediatric asthma. In addition, patients with persistent asthma were significantly discernible from those with intermittent asthma. This methodology may prove to be a practical asset in assisting the physician's decision to administer corticosteroid therapy in paediatric patients with asthma.

0151 | Asthma diagnosis using statistical models of serum micrornas expression

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Background: Asthma is an immunological based disease of the airways, characterized by inflammation and airflow obstruction. The complexity of asthma makes it a heterogeneous disease that presents diverse phenotypes. Our present study shows a set of miRNAs for asthma diagnosis, where miR-185-5p stands out as a possible biomarker associated with asthma severity.

Method: A set of 14 miRNAs differentially expressed in eosinophils from asthmatic patients compared to controls were studied in serum samples from 138 asthmatic patients and 39 controls by qPCR. The asthmatics group was subdivided according to the severity phenotype following the GEMA guidelines in intermittent asthmatics, mild, moderate and severe persistent asthmatics. ROC curves, logistic regression model and a Random Forest model were performed to determine the possible role of these miRNAs as asthma biomarkers.

Results: From the 14 studied miRNAs in serum, only miR-1246, miR-144-5p, miR-320a, miR-185-5p, and miR-21-5p were differentially expressed in asthmatics ($P < 0.01$). MiR-185-5p expression is higher in intermittent and mild asthmatics compared to controls, and even higher in moderate and severe persistent asthmatics ($P < 0.01$), showing that its expression rises with severity. MiR-185-5p expression is also higher in patients that presented adult onset asthma (> 18 years old) compared to young onset asthma and in patients who were admitted at least once in the intensive care unit (ICU) ($P < 0.05$). Of the 5 deregulated miRNAs in asthmatics, miR-1246, miR-320a and miR-185-5p present an AUC over 0.7 and can be asthma biomarkers, being the best miR-185-5p (AUC = 0.78). We also developed a logistic regression model that classifies patients in healthy or asthmatics with an AUC of 0.86 using the expression of only 3 miRNAs (miR-185-5p, miR-1246, and miR-144-5p), and a Random Forest model with miR-320a, miR-185-5p, and miR-144-5p that classifies subjects into healthy, intermittent asthmatics, mild, moderate and severe persistent asthmatics (AUC = 0.75).

Conclusion: Here we show that miRNAs measured in serum can be used for creating statistical models that may be applied for asthma diagnosis and severity discrimination.

0152 | Patients with bronchiectasis and asthma: A new phenotype/endotype?

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Background: Bronchiectasis is emerging in the last few years as a relevant comorbidity of asthma. The complex relation between inflammatory mechanisms of asthma and of bronchiectasis may explain why patients with the two concomitant diseases have been described as the most severe ones.

The aim of this study was to characterize patients with clinically relevant bronchiectasis under clinical, functional, inflammatory and immunitary points of view, pointing a specific focus on those with associated asthma.

Method: All consecutive patients with confirmed and clinically relevant bronchiectasis who came to our attention in a 6-month period have been included into the study. All patients have been evaluated for: demographic data, clinical history, lung function, high-resolution chest CT scan, inflammatory biomarkers (i.e.: exhaled nitric oxide - FENO, blood and sputum eosinophils, exhaled breath condensate - EBC concentration of Cysteinyl-Leukotrienes - EBC CysLTs, serum periostin…), immunitary biomarkers (i.e.: serum concentration of IgG, IgM, IgA, IgE, IgG1, IgG2, IgG3, IgG4), and markers for oxidative stress (8-isoprostane EBC concentration - EBC-8IP).

Results: Sixty-eight patients have been consecutively enrolled (24% affected also by asthma). Patients with concomitant asthma had lower prevalence of cystic (12.5% vs 38.5%, $P = 0.04$) and higher cylindric forms of bronchiectasis (100% vs 78.8%, $P = 0.04$), reduced annual rate of bronchiectasis exacerbation ($P = 0.04$). FENO was higher in asthmatics ($P = 0.001$) and positively correlated with the annual exacerbation rate ($R^2 = 0.35$, $P = 0.026$). EBC-8IP concentration was lower in asthmatics ($P = 0.04$), but no difference was found in EBC-CysLts. Asthmatics were also characterized by higher number of eosinophils in both blood ($P = 0.002$) and sputum ($P = 0.007$) and reduced concentration of serum IgG1 ($P = 0.015$) and IgG3 ($P = 0.008$).

Conclusion: Patients with concomitant bronchiectasis and asthma seem to define a novel disease phenotype/endotype with lower extension of bronchiectasis, reduced oxidative stress biomarkers, increased Th2-like inflammation and an altered function of immune system. This may lead patients to a vicious circle of inflammation, predisposing to infections, predisposing to bronchiectasis and infections again, leading the patient to have more frequent asthma exacerbations.

TUESDAY, 29 MAY 2018
OAS 27
IMPACT OF THE MICROBIOME ON ALLERGIC DISEASES

0153 | Composition of gut microbiota in infancy and its protective effect on asthma at school age

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Background: Though asthma is a disease of the airways, the involvement of the gut microbiome in disease evolution has been discussed in the context of the so-called gut-lung axis paradigm for a polygenetic disease with a strong environmental component. While the environment supposedly directly acts on the airways, it is postulated that the gut-lung axis describes the influence of the gut microbiome on asthma, which already has been observed in murine models of allergic airway disease and was clinically substantiated in asthma patients.

Aims: To determine whether gut bacterial microbiota at age 12 months is associated with the development of asthma until school age and which factors predispose for the respective microorganisms in 867 farm and non-farm children of the PASTURE study (Protection against Allergy–Study in Rural Environments).

Method: The V4 region of 16S rRNA genes of stool samples from infants of the PASTURE birth cohort was analyzed by using the Illumina MiSEQ platform. Sequences were classified in 1578 OTUs with QIIME and further analyzed on the family level with R. Principal component analysis (PCA) based on centered log ratio transformed relative abundance of all bacterial families was performed. Associations of PCA axes with asthma or determinants were calculated by regression models. Odds Ratios (OR) and 95%-confidence-intervals (CI) are reported for the z-standardized microbial effects on doctor diagnosed asthma and the respective phenotypes atopic (any specific inhalant IgE $\geq$ 0.7kU/L) and non-atopic asthma.

Results: The first seven PCA axes explained 50% of the variance; two of them were significantly associated with asthma, one with asthma in general and more specifically with non-atopic asthma (OR and 95%-CI: 0.65 (0.45-0.93), P = 0.017, the other only with atopic asthma (OR and 95%-CI: 0.61 (0.41-0.93), P = 0.020). Prominent bacterial families loading positively on these axes were *Ruminococcaceae* and *Clostridiaceae*, whereas *Enterococcaceae* and *Streptococcaceae* loaded negatively.

Environmental variables including siblings and farm milk consumption were positively whereas clinical variables including use of antibiotics and cesarean section were inversely associated with these PCA axes. A mediation of the effect of environmental or clinical variables on asthma via the microbiome as assessed by PCA was only minimal.

Conclusion: Gut bacteria composition was associated with childhood asthma, supporting the gut microbiota as a modifiable target in asthma protection.

0154 | Microbiome differences in saliva are associated with childhood asthma among African Americans

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Background: Asthma is a common chronic airways disease that results from a combination of genetic and environmental factors, including the exposure to microbes. Recent studies have sampled the lower airways with invasive methods and have demonstrated that the airways of asthma patients show a high diversity of bacteria, and are enriched in certain pathogenic species. However, considering that this disease has a high prevalence during childhood, more accessible and non-invasive sampling procedures of bacterial composition would be clinically relevant. Here we aimed to evaluate this possibility assessing the bacterial composition of saliva of children with and without asthma.

Method: A total of 114 saliva samples from African Americans (57 asthma cases and 57 controls) collected as part of Study of African

Americans, Asthma, Genes, & Environments (SAGE II) study were analyzed. The V4 16S rRNA region was sequenced in a MiSeq platform (Illumina) with 250 bp paired-end reads. Data processing was performed using QIIME. Differences in the normalized Shannon diversity index and relative genera abundance among cases and controls were assessed using non-parametrical tests with R. Statistical significance was declared using a Bonferroni correction. Furthermore, the association of multivariate genera abundance with asthma was analyzed by means of logistic regression models including age, gender, and genetic ancestry as covariates.

Results: A total of 15 genera from 5 phyla were detected, being *Prevotella*, *Streptococcus*, *Veillonella*, and *Haemophilus* the most abundant genera. Asthma cases showed higher diversity than controls (*P*-value = 0.008). Additionally, statistically significant differences in the mean relative abundance between cases and controls were found for two genera: *Streptococus* and *Veillonella*. This result was confirmed in multivariate models, where higher abundance of *Streptococus* was associated with protection for asthma (OR = 0.92; 95% CI = 0.87-0.97; *P*-value = 0.004) and *Veillonella* was associated with asthma risk (OR = 1.12; 95% CI = 1.02-1.22; *P*-value = 0.015).

Conclusion: We identified changes in the salivary microbiome composition associated with asthma susceptibility in African American children.

0155 | The effect of probiotic and nasal microbiota on inflammatory response, viral load, and symptom severity in experimental rhinovirus challenge

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Background: The role of nasal microbiota, or its modulation by probiotics, in viral respiratory infections has not been established. Meta-analyses suggest that probiotics could be beneficial on reducing the risk of respiratory infections in humans, however, the mechanisms of action have not been studied in controlled clinical trial setting.

Method: We collected nasal swabs and washes, and fecal samples over time, in a randomized double-blind placebo controlled clinical study assessing the effect of prophylactic probiotic *Bifidobacterium animalis* subsp. *lactis* BI-04 (BI-04) supplementation on experimental rhinovirus infection in 115 healthy adults (NCT01669603). The nasal and fecal microbiota were characterized by 16S rRNA gene sequencing and the resulting data were compared with nasal inflammatory marker concentrations, viral load, and clinical symptoms during the infection.

Results: Probiotic BI-04 supplementation influenced nasal wash inflammatory response and reduced the viral load during the infection. The sequencing results showed that the nasal microbiota clustered into six types. The clusters predominant of *Staphylococcus*, *Corynebacterium/Alloiococcus*, *Moraxella*, *and Pseudomonadaceae/* Mixed had characteristic inflammatory marker and viral load profiles in nasal washes. The nasal microbiota types of subjects also influenced the severity of clinical cold symptoms during rhinovirus infection. Rhinovirus infection and probiotic intervention did not significantly alter the composition of nasal or fecal microbiota.

Conclusion: Our results suggest that probiotic BI-04 supplementation influences innate inflammatory response and viral load in nasal washes, and that the nasal microbiota type influences the virus load, host innate immune response, and clinical symptoms during rhinovirus infection. The effect of probiotic supplementation or modulation of airway microbiota should be further investigated in the context of allergic airway diseases.

0156 | The role of Staphylococcus spp. and antimicrobial peptides in atopic dermatitis

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Background: Staphylococcus aureus (SA) frequently infects skin lesions in patients with atopic dermatitis (AD). Humoral factors of innate immunity are designed to control the microbial balance. Secretion of antimicrobial peptides (defensins particularly) by keratinocytes forms the basis of humoral response.

This study aims to investigate the interaction between the intensity of staphylococcus skin colonization and the expression level of defensins in AD.

Method: 34 children (boys and girls in roughly equal proportions) with acute AD (13.46%) and active chronicle forms (15.54%) were involved in our study before the external therapy. The average age of patients was 5.1 ± 1.4. The data of SCORAD varied from 10 to 96. Skin smears were taken simultaneously from healthy and affected areas before the initiation of treatment. Seeding of obtained samples was performed on blood agar. Bacterial strains were identified according to conventional microbiological tests and their types were determined by means of the automated microbiology analyzer. In the skin samples for detection of expression of HBD-1, HBD-2 and HBD-3 genes we used the methods of RNA extraction, reverse transcription reaction and real time PCR technique. Statistical evaluation methods were also performed.

Results: The defensin expression rates did not have any significant differences neither in AD lesions nor in healthy skin keratinocytes in case of SA absence in lesional skin. In the event of Staphylococcus epidermidis detection the HBD-1 expression level was comparable

to control figures (188.5 to control 214 relatively) in 57% of cases; there was a four times suppression in 28% of cases. The expression of inducible HBD-2 and HBD-3 was reliably higher in lesional and non-lesional skin keratinocytes in 37-40% and in 60-100% of patients respectively. The presence of SA resulted in the suppression of HBD-1 (40%) and HBD-2 (42%) expression with a cocurrent induction of HBD-3 expression in AD lesions in 50% of patients (2894 to control 478 relatively). There was a twofold increase in the induction of HBD-2 in unaffected skin areas.

Conclusion: Not only SA but also other staphylococci can both induce and suppress defensins rates thus adversely affecting clinical course and therapy efficacy.

0157 | Short chain fatty acids (SCFA) activate the intrinsic apoptosis pathway in eosinophils from atopic individuals

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Background: Lung eosinophilia is a hallmark of asthma and is believed to play a crucial role in the pathogenesis of allergic inflammatory diseases. In the last decades, a dramatic increase of asthma and allergies has been observed in Western countries, which was linked to gut microbiome composition. SCFA, e.g. acetate, propionate and butyrate are produced in high concentration in the gastro-intestinal tract by commensal bacteria and are readily secreted into the blood stream and thereby show various biological functions. Prompted by the observation that propionate hampers lung eosinophilia in models of allergic inflammatory diseases we hypothesize that SCFA modulate the survival of eosinophils.

Method: Induction of apoptosis was detected using annexin V/propidium iodide (PI) double staining, JC-1 staining and caspase 3/7 activation assay. mRNA expression was detected via real-time RT-PCR.

Results: We found that both propionate and butyrate induce apoptosis in human peripheral blood eosinophils from atopic donors, starting 18 hour after the initial treatment. This result was confirmed via the reduction of the mitochondrial membrane potential, as detected with JC-1 staining and caspase 3/7 activation assay. These findings suggest an involvement of the intrinsic apoptotic pathway in eosinophils. Additionally, IL-5 pretreatment could not prevent the activation of caspase 3/7 as induced by propionate or butyrate. Furthermore, IL-5RA as well as anti-apoptotic transcripts BCL-XL and MCL-1 were downregulated after incubation with propionate or butyrate for 3 h.

Conclusion: We could show for the first time that the SCFA propionate and butyrate are able to interfere with survival pathways in eosinophils from allergic donors, in terms of induction of apoptosis, mitochondrial depolarization, modulation of expression patterns of survival promoting factors and activation of effector caspases. Crucially, this effect could not be prevented by IL-5 pretreatment. Therefore, we propose that propionate and butyrate could serve as potential therapeutic agents in allergic inflammatory diseases.

0158 | Gut Microbiota from infant with Cow's Milk Allergy Promotes Th-2 immunity and allergic response in a murine model of sensitisation and challenge

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Background: Cow's milk allergy (CMA) is a significant health burden affecting up to 5% of infants. Emerging data raises the hypothesis that an altered gut microbiota in infants with CMA contributes to disease onset, persistence and extra-intestinal manifestations [1]. Herein, we report the effects of faecal microbiota transfer (FMT) of healthy control (HC) and CMA infants in a murine model.

Method: Infants (6 HC, 5 CMA; 5-16 months of age) were recruited from the community and Great Ormond Street Hospital (London, UK) respectively (Research Ethics Committee ref: 14/LO/0364). Initial stool characterization included quantification of bifidobacteria and *Eubacterium rectale/Clostridium coccoides* group (ER/CC) by FISH. Three-week old germ-free mice (C3H/HeN) were inoculated with stool from a HC and CMA infants, matched for age (9 months), sex (female) and birth mode (C-section). After 12 days, the mice were sensitised for 5 weeks with whole whey protein (WP) and cholera toxin (CT) (as adjuvant) or received CT only (non-sensitised control). Following an oral challenge with β-lactoglobulin (BLG), allergic responses and sensitisation markers were measured (clinical scores, mMCP1, allergen-specific and total IgE, IgG1, IgG2a).

Results: Decreased levels of bifidobacteria and increased levels of ER/CC characterised CMA infant faecal microbiota, as previously observed [1]. No significant differences were observed in allergen-specific sensitisation markers (BLG-specific IgE, IgG1 and IgG2a) in mice colonized with CMA or HC infant microbiota. However, mMCP1 levels were significantly increased in WP+CT compared to CT for both CMA-FMT ($P < 0.05$) and HC-FMT ($P < 0.01$). Interestingly, mice colonized with the CMA microbiota had increased IgE-levels ($P < 0.001$ and $P < 0.005$) and IgG1/IgG2a ratio ($P < 0.05$ and $P < 0.005$) in both the WP+CT and CT-groups when compared with mice colonized with HC microbiota. Moreover, the clinical score was only significantly enhanced in sensitised (WP+CT) mice with CMA-FMT ($P < 0.01$) as compared to CT, and significantly higher compared to sensitised mice with HC-FMT ($P < 0.05$).

Conclusion: CMA-associated infant gut microbiota induced systemic Th-2 immunity and increased IgE levels and resulted in an enhanced responsiveness to cow's milk allergen in a murine model of sensitisation and challenge.

1) Candy, D. C A., et al. (2017). Pediatr Res. https://doi.org/10.1038/pr.2017.270.

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TUESDAY, 29 MAY 2018

OAS 28

MANAGEMENT OF ASTHMA FROM BENCH TO BEDSIDE

0159 | CCR10 + ILC2s with ILC1-like properties exhibit a protective function in severe asthma

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Background: In line with evidence for a role for type 2 innate lymphoid cells (ILC2s) in respiratory allergies, we previously showed that patients with severe allergic asthma have high numbers of circulating ILC2s expressing the CCR10 chemokine receptor.

Method: CCR10$^+$ ILC2s were analyzed in the blood of allergic and non-allergic severe asthmatic patients, as well as healthy controls. Phenotypic and functional properties of human CCR10$^+$ and CCR10$^-$ ILC2s were assessed by multiparametric flow cytometry and intracellular stainings. The role of CCR10$^+$ ILC2s in asthma pathophysiology was studied in a mouse model of birch pollen-induced allergic asthma.

Results: When compared to healthy controls, CCR10$^+$ ILC2s are enriched in the blood of both allergic and non-allergic severe asthmatic patients, and further, can also be detected in the lungs. Plasma concentrations of the CCR10 ligand CCL27 are also significantly increased in severe asthmatics when compared to non-asthmatic individuals. Surprisingly, CCR10$^+$ ILC2s secrete little IL-4 and IL-13, but rather exhibit ILC1-like properties, including a capacity to produce IFN-g. CCR10$^+$ ILC2s depletion as well as blocking of IFN-g activity exacerbates airway hyperreactivity in a mouse model of allergic asthma, providing evidence for a protective role for these cells in Th2-mediated inflammation.

Conclusion: Frequencies of CCR10$^+$ ILC2s and CCL27 concentrations are increased in the blood in relationship with asthma severity, irrespective of the allergic status of the patients. The functional characterization of CCR10$^+$ ILC2s in human samples and in mouse asthma models suggests that these cells can downregulate Th2-mediated airway inflammation through IFN-y production.

0160 | Differential expression of airway epithelial tight junctions, mucins and inflammasome-related molecules in different asthma phenotypes

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Background: Asthma is a chronic respiratory disease with marked clinical and pathophysiological heterogeneity. Specific pathways are thought to be involved in the pathomechanisms of different inflammatory phenotypes of asthma, however direct *in vivo* comparison has not been performed. In this study, we sought to perform an *in vivo* unbiased investigation of the molecular pathomechanisms of different inflammatory phenotypes of asthma. Based on the differentially expressed gene families, we studied in detail the mRNA, protein expression and localisation of tight junctions (TJs), mucins and inflammasome-related molecules in order to define signatures of each phenotype. To translate our findings into human settings, we analysed the role of IL-1β and IL-17 on the barrier function and mucins in human primary differentiated bronchial epithelial cells (HBECs) from healthy and asthmatic donors.

Method: We developed mouse models representing three different phenotypes of airway inflammation- eosinophilic, mixed, and neutrophilic asthma, via different methods of house dust mite sensitisation and challenge. Transcriptome analysis was performed using whole lung tissues, followed by quantitative RT-PCR, western blot analysis and confocal microscopy. HBECs were cultured in air-liquid interface conditions.

Results: By whole genome transcriptome profiling, we found that airway TJ, mucin and inflammasome-related genes are differentially expressed in these distinct phenotypes. Detailed analysis of molecules from these families revealed that Zo-1 and Cldn18 were downregulated in all phenotypes, while increased Cldn4 expression was characteristic for neutrophilic inflammation. Mucins Clca1 (Gob5) and Muc5ac were upregulated in eosinophilic and even more in neutrophilic asthma. Increased expression of inflammasome-related molecules such as Nlrp3, Nlrc4, Casp-1 and IL-1β was characteristic for neutrophilic asthma. In addition, we showed that inflammasome/Th17/neutrophilic axis cytokines- IL-1β and IL-17 impaired epithelial

barrier function and increased mucins expressions in HBECs from controls and asthmatic patients.

Conclusion: Our findings suggest that differential expression of TJs, mucins and inflammasome-related molecules in distinct asthma phenotypes are linked to pathophysiology and might reflect the differences observed in the clinic. These data also link cellular phenotypes with molecular endotypes and open a new window for more precision approaches to asthma patients.

0161 | Reslizumab decreases nasal adverse events and upper respiratory-associated concomitant medication use in patients with eosinophilic asthma and nasal polyps

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Background: Patients with eosinophilic asthma have an increased risk of nasal polyps (NP). Intravenous (IV) reslizumab (RES) is a humanised anti-interleukin-5 monoclonal antibody which significantly reduces blood eosinophils and clinical asthma exacerbation risk, and improves lung function, asthma control and quality of life in patients with inadequately controlled eosinophilic asthma (ICEA). This analysis examined whether RES treatment reduced nasal and upper respiratory disorders recorded during the treatment period as adverse events (NAEs) and use of upper respiratory-associated concomitant medications (URACMs) in patients with asthma and self-reported NP.

Method: Data were pooled from two 52-week Phase 3 trials of IV reslizumab 3 mg/kg vs placebo q4 wks in patients with ICEA. In this post-hoc analysis of pooled data from patients with self-reported NP, the incidence of the following NAEs was recorded during the treatment period: nasal congestion, worsening of nasal polyps, sinus congestion, increased upper airway secretion, rhinorrhea, and upper respiratory tract infections (nasopharyngitis, sinusitis, upper respiratory tract infection [URTI], acute sinusitis, pharyngitis, rhinitis, chronic sinusitis, acute tonsillitis, and tracheitis). Patients' use of URACMs, including antibacterials, antihistamines, systemic corticosteroids (SCS), cough and cold preparations, and nasal preparations including nasal corticosteroids, nasal antihistamines and nasal decongestants, was also recorded.

Results: At baseline, 245/953 patients across the two trials (pooled data) reported NP (PBO: 124, RES: 121). NAEs occurred in more PBO patients vs RES patients: 58 PBO (47%) vs 41 RES (34%), RR 0.72 (95% CI: 0.53, 0.99). The majority of NAEs were URTIs (54 [44%] PBO vs 40 [33%] RES), followed by worsening of nasal polyps (4 [3%] PBO vs 1 [<1%] RES). URACMs were used by 45 (36%) PBO patients and 30 (25%) RES patients, RR 0.68 (95% CI: 0.46, 1.01). There were numerically more PBO patients than RES patients who received prescriptions for antibacterials 32 (26%) vs 23 (19%), antihistamines 5 (4%) vs 1 (<1%), SCS 25 (20%) vs 10 (8%), and nasal preparations 7 (6%) vs 5 (4%), with 8 patients in each group receiving cough and cold preparations.

Conclusion: Patients with ICEA and comorbid NP at baseline experienced fewer NAEs when treated with RES vs PBO. Additionally, fewer RES-treated patients received several classes of upper-respiratory associated concomitant medications.

0162 | Dupilumab reduces percentage volume of sinuses occupied by disease in patients with CRSwNP

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Background: Dupilumab, a fully human anti-interleukin (IL)-4Rα monoclonal antibody, inhibits signaling of IL-4 and IL-13, which are key drivers of type 2-mediated inflammation, and is approved in the EU, USA, and other countries for treatment of adults with inadequately controlled moderate-to-severe atopic dermatitis. In a phase 2a study (NCT01920893), dupilumab improved endoscopic, radiographic, and clinical endpoints in patients with chronic rhinosinusitis with nasal polyposis (CRSwNP) refractory to intranasal corticosteroids. This post hoc analysis evaluates the effect of dupilumab on the percentage disease occupation of the ethmoid, frontal, sphenoid, and maxillary sinuses and the ostiomeatal complex (OMC) in patients with CRSwNP using volumetric assessments.

Method: Sixty adults with CRSwNP were assigned (1:1) to 16 weeks of weekly subcutaneous 300 mg dupilumab (initial 600 mg loading dose) or placebo, plus daily mometasone furoate nasal spray twice daily (total daily dose 400 μg). Percentage volume occupied by disease of the sinuses was monitored by 3D volumetric computed tomography (CT) scanning of the sino-nasal cavities comparing the results at baseline and at Week 16.

Results: Mean (SD) percentage disease occupation of the sinuses at baseline ranged from 67.69 (30.35) in the right sphenoid sinus to 93.28 (10.09) in the left ostiomeatal complex. Patients treated with 300 mg of dupilumab showed significant reductions ($P < 0.001$) in percentage volume occupied by disease in the left and right ethmoid, frontal, sphenoid, and maxillary sinuses and the OMC. Least-squares mean difference (95% CI) vs placebo (for left/right sinus, respectively) were −21.31 (−27.29, −15.33)/−22.40 (−29.58, −15.22) for the ethmoid sinuses, −34.79 (−47.74, −21.85)/−32.59 (−44.31, −20.88) for the frontal sinus, −33.79 (−45.98, −21.60)/−25.37 (−36.91, −13.82) for the sphenoid sinus, −29.54 (−42.06, −17.01)/

−35.05 (−48.10, −21.99) for the maxillary sinus, and −17.71 (−28.33, −7.09)/−23.04 (−34.18, −11.90) for the OMC. These improvements reflect the significant increase in 3D volumetric measurement of air volume (mL) across all sinuses and OMC (5.59 [2.68, 8.51]/7.06 [3.80, 10.31] vs placebo, $P < 0.0001$).

Conclusion: Dupilumab significantly reduced percentage volume occupied by disease in the left and right sino-nasal cavities and increased air volume in CRSwNP patients.

0163 | Asthma with multiple allergies, is associated with early and total response to omalizumab

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Background: Omalizumab is a humanized anti-IgE monoclonal antibody used in children with severe allergic asthma. Due to the cost of this biotherapy it is important to clearly define the target population who will benefit the most from treatment.

Objective: The aim of this study was to define predictive markers of a total response to omalizumab at 4 months of treatment.

Method: We conducted a retrospective study, including children, aged from 6 to ≤18 years, with severe or refractory asthma treated by omalizumab. The response to omalizumab was evaluated at 4 months during a multidisciplinary discussion, (taking into account, ACT score, the number of severe exacerbations, and lung function).

Results: 45 children with average age of 12.6 (±2.7) years were evaluated as complete responders (22 patients (49%)) , partial responders (20 (44%)), and non-responder (3 (7%)). On statistical logistic regression eczema and a high FEV_1were predictive markers of complete response at 4 months (compared to partial and no response) with an OR 1.50 (CI 95% [1.09; 1.9], $P = 0.005$), and 1.02 (95% CI [1.01; 1.20], $P = 0.001$), respectively. On a cluster analysis with 15 variables, we identified 3 independent clusters: Cluster 1, consisted in 16 children with eosinophilic asthma (eosinophilia > 300/mm3 n = 15 (94%), $P = 0.001$), abnormal lung function (mean FEV1 65.4 ± 10.2, $P = 0.006$) and poor response to omalizumab (n = 1 (6%), $P < 0.001$ with a complete response). Cluster 2 included 11 patients with uncontrolled asthma, with tobacco smoke exposure (n = 6 (54.5%), $P = 0.016$), normal lung function and inconstant response to omalizumab (4 (37%) with a complete response). Cluster 3 was composed of 18 severe asthma children with multiple allergies (n = 15 (83.3%), $P = 0.001$), and eczema (n = 13 (72%), $P < 0.001$) mostly normal lung function and constant response to omalizumab (n = 16 (89%), $P < 0.001$ with a complete response).

Conclusion: Two statistical approach confirm that multiple allergic co-morbidities, specially eczema with multiple sensitizations and normal lung function, are associated with early response to omalizumab.

0164 | Extended dose intervals vs dose reduction of omalizumab for controlled allergic asthma

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Background: Omalizumab (anti-IgE) has been used as a treatment for severe asthma for more than ten years. However, no consensus exists regarding the treatment duration considering not only the incurred costs, but also the dependence on patients' compliance for the rest of their lives. Currently, common practice is life-long therapy without adjustment of the dose or the treatment intervals. This study evaluated asthma control after either dosage interval extension or dose reduction in patients with asthma controlled by omalizumab.

Method: Thirty-seven patients were recruited from the outpatient clinic of the Allergy Centre Charité Berlin, Germany. Initially all had uncontrolled severe allergic asthma (FEV_1< 80 %) despite therapy with high dose inhaled corticosteroids. Also, all were sensitized to at least one perennial allergen. Following institution of omalizumab, dosed individually according to the European prescribing information, the mean time to reach a stable state (asthma control test ≥ 20 points, FEV_1 ≥ 70 % predicted, no exacerbations in the last 4 months) was 81 weeks (SD ± 52). Patients were then assigned to receive either extended treatment interval or a reduction in omalizumab dosage.

For the 26 patients in the extended interval group, dose intervals were extended by either one week (n = 1), two weeks (n = 13), three weeks (n = 6), even four weeks (n = 6). For the 11 patients in the dose reduction group, the omalizumab dose was reduced to two thirds of their initial dose. The primary outcome was time until loss of asthma control.

Results: In the extended interval group 19 patients (73 %) maintained good asthma control for at least 30-166 weeks. Of the remaining 7 patients, the median time to loss of asthma control was 35 weeks [95 % CI 30; 40 weeks]. In contrast, all patients in the dose reduction group lost asthma control. The median time of loss of control was 10 weeks [95 % CI 8; 13 weeks]. However, one patient experienced deterioration only after 187 weeks. Thus, extension of dose interval led to a significantly ($P < 0.001$) longer period of good asthma control than dose reduction.

Conclusion: If patients or physicians wish to reduce the cumulative dose of omalizumab after achieving good asthma control, extension of the intervals seems to be the better approach compared with dose reduction.

TUESDAY, 29 MAY 2018

OAS 29

NOVEL APPROACHES IN OCCUPATIONAL ALLERGY

0165 | Investigating discordance between diagnostic tests for laboratory animal allergy

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Background: The major risk factor for developing laboratory animal allergy (LAA) is exposure to laboratory animal proteins. Workers can develop specific-IgE to these proteins ('sensitization') and subsequently symptoms such as rhinitis and occupational asthma. To prevent disease progression, sensitive diagnostic tests are needed to identify sensitized workers and protect them from further, harmful exposure. Currently, there is no gold-standard diagnostic test to detect sensitization to mice and recent work has shown discordance between tests that are available. Additionally, new research has identified putative uncharacterised allergens within mouse urine and mouse epithelium.

Aim: To investigate LAA diagnostic test discordance by examining allergens in mouse urine and mouse epithelium.

Method: Laboratory animal workers exposed to mice (n = 743) were recruited to the SPIRAL (Safe Practice in Reducing Allergy in Laboratories) study. Sensitization was determined through commercial skin prick test (SPT) to mouse epithelium and measurement of specific-IgE to mouse epithelium and urine (ImmunoCAP 100e). Workers with discordant results between tests were selected and their sera run on western blots; proteins from in-house mouse urine and epithelium extracts were separated using SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were incubated with workers' serum and enhanced-chemiluminescence used to visualize specific IgE protein binding.

Results: Mouse urine and epithelium extracts; IgE binding was detected to proteins of 14-18 kDa (Major Mouse Urinary Protein Complex; includes Mus m 1 and Mus m 2). Binding was also observed to proteins of ~65 kDa, correlating with the known molecular weight of mouse serum albumin. Further IgE binding was detected to 5 other uncharacterized proteins.

2/4 workers who were negative to mouse urine on the ImmunoCAP and mouse epithelium on SPT, showed high IgE binding to mouse urine extract proteins on western blots. 6/8 workers had who positive IgE binding to proteins in mouse urine and/or epithelium on western blots, had negative SPT to mouse epithelium.

Conclusion: Current individual diagnostic tests for LAA do not identify all cases of sensitization to mouse proteins. The clinical implications of a false negative result are significant. The uncharacterised proteins found in this work need to be identified and used to create a panel of mouse allergens to form the basis of a sensitive gold-standard diagnostic test.

0166 | From workplace to home environment: Spreading of mouse allergens by laboratory animal workers

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Background: Laboratory animal workers (LAW) working with mice are exposed to mouse allergens (MA). Exposure to MA can lead to occupational allergies and asthma. If MA are spread to home environments, the longer duration of exposure might increase the risk for allergic symptoms. Little is known about the spreading of MA. This study aimed to assess: 1. whether spreading of MA from workplace to home environment takes place, 2. which factors increase spreading of MA.

Method: In a cross-sectional study we took dust samples from the homes of 107 LAW and 13 controls. From 90 LAW we took additional dust samples from their working place. Samples were analysed using mus m 1 ELISA kits. Through a questionnaire we assessed socio-demographic data, allergies and cleaning habits. In LAW we also assessed types of cages used, work tasks and protective clothing.

Results: MA concentration was higher in home environments of LAW (median (ng mus m1)=11.3) than in controls (median = 1.1; $P = 0.016$; Kruskal-Wallis test). The highest workplace MA concentration was found in the scullery (median = 145000.0), followed by the changing rooms (median = 10.2) and staffrooms (median = 7.5). MA concentration was higher in homes of LAW who fulfilled cleaning tasks (cleaning of cages, floors, etc.) ($P = 0.034$) and who changed their linen at home less than once a month ($P = 0.024$). MA concentration at home was not associated with duration of mouse contact ($P = 0.909$) and age of sleeping mattress at home ($P = 0.649$).

Conclusion: Spreading of MA from workplace to home environment takes place. LAW who fulfilled cleaning tasks were found to have higher MA concentration at home. Special focus should be given to reduce MA concentration during cleaning in laboratory animal facilities.

0167 | Application of a mass-spectrometry based method to identify novel occupational allergens

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Background: Occupational allergic asthma is caused by exposure to airborne workplace agents, which are often high molecular weight (HMW) proteins derived from plant or animal species. Those working in animal testing laboratories may be particularly at risk of exposure to animal allergens, e.g.: from mice or fruit flies. We aimed to identify allergens in mice and fruit flies using a novel method that exploits advances in proteomic technology.

Method: Samples of mouse urine, mouse epithelium and fruit fly were collected from animal research facilities, extracted in 0.01 mol/L ammonium carbonate at 4°C overnight, then dialysed and lyophilised. Protein from the extracts was separated by SDS-PAGE and stained with Coomassie blue. Protein was then transferred to nitrocellulose membranes and incubated with pooled sera from patients with high levels of allergen-specific IgE. The IgE-binding proteins were observed using a sensitive chemiluminescence method. These allergenic proteins were excised and digested with trypsin and the resulting peptides were analysed by high performance liquid chromatography/tandem mass spectrometry.

Results: In mouse urine, IgE-binding bands were observed at 19 kDa and 62 kDa and as expected, identified as Mus m 1 and Mus m 4. For a ~35 kDa band, an uncharacterised protein (Prot. Acc. Q3UN62) was the most abundant protein identified.

In mouse epithelium, Mus m 4 was the most abundant protein in 5 IgE-binding bands, ranging 25-62 kDa. In a 150 kDa band in mouse epithelium the most abundant protein was titin (Prot. Acc. A2ASS6), a putative allergen found in black tiger prawn.

In fruit fly, IgE-binding bands were observed at ~10 kDa and 100 kDa, and the most abundant protein identifications through mass spectrometry were fructose-bisphosphate aldolase (Prot. Acc. C8VV14) which is a known allergen found in salmon and tuna, and alpha-mannosidase (Prot. Acc. Q9VKV1), respectively.

Conclusion: We identified specific IgE-binding proteins from mouse urine, epithelium and fruit fly extracts, including some that are not widely recognised as allergens in current databases. Combining standard immunological techniques with mass spectrometry allows us to identify previously unknown allergenic proteins, using relevant environmental samples and serum from individuals with allergic symptoms in that environment. This is of particular value in the occupational setting.

0168 | High prevalence of seasonal pollinosis among the farmers due to Kans grass and its IgE mediated cross reactivity patterns with other grasses: An immunochemical approach

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Background: An increasing number of allergic complaints occurred among farmers during the blooming season of Kash grass, *Saccharum spontaneum* (SSo). SSo pollen were reported to be airborne but no attempts were made to characterize the immunoreactive proteins present in it. The present study aimed to profile the sensitization of allergic farmers to SSo pollen and to characterize the IgE binding proteins of SSo and their cross-reactivity patterns with other grasses.

Method: The UPPA precipitated antigenic profile of Kans Grass pollen extract along with other grass pollen extracts originated from *Cynodon dactylon* (Cd), *Chloris barbata* (Cb), *Digitaria ciliaris* (Dc), *Eragrostis tenella* (Et), *Oryza sativa* (Os), *Imperata cylindrica* (Ic), *Saccharum officinarum* (So) and *Zea mays* (Zm) were analyzed by SDS-PAGE followed by Periodic Acid-Schiff staining. 148 farmers with naso-bronchial allergy were diagnosed allergic both by ISAAC questionnaire survey and SPT using SSo antigen. The allergenic extract was used to set up *in vitro* immunoenzymatic tests such as ELISA and IgE specific immunoblotting to identify the immunoreactive proteins in a panel of sera collected from 12 immunotherapy-free polysensitized subjects. Dotblot inhibition was employed to determine the allergenic relationship of eight common grasses whereas immunoblot-inhibition demonstrated a component-based cross-reactivity.

Results: Though Kans grass (SSo) flowers from July to October, grass pollen were trapped in Burkard sampler round the year. SSo pollen was found to evoke about 71.24% sensitivity among atopic farmers (OR = 2.00, $P < 0.0001$) causing early spring hay fever, allergic rhinitis and seasonal allergic conjunctivitis (SAC). Four allergens of 38 kDa, 53.8 kDa, 62.2 kDa and 97.2 kDa were showed their intensity and frequency of recognition by human IgE antibodies. These were identified as new and major allergens of *S. spontaenum* pollen which were detected to be glycosylated by PAS staining. Ic, Cd and Cb showed 50% inhibition with 5, 10 and 20 μg of antigen followed by the other grass pollen such as Zm, Os, Dc, Et. Allergenic protein components of SSo pollen of 19.6, 33.8, 38, 53.8 and 97.2 kDa showed some sequence homology to those of Ic, Cd, So, Cb, Os, Zm, Et and Dc grass pollen types confirming their cross-reactivity with SSo pollen.

Conclusion: Identification of four new major glycoprotein allergens from Kans grass pollen causing seasonal pollinosis among Indian farmers and its cross-reactivity with other grass pollen have been delineated in this study.

0169 | Does icam-1 play a role in work-related asthma and rhinitis?

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Background: Intercellular adhesion molecules type 1 (ICAM-1 or CD 54) are proteins exposed on activated cells participating in allergic inflammatory reactions. High levels of soluble ICAM-1 (sICAM-1) have been observed in serum blood of patients with asthma aggravation. However, the essential role in assessment of local airways inflammation in asthma plays the evaluation of induced sputum (IS) and nasal lavage (NL) supernatants. The aim of this study was to measure sICAM-1 concentrations (Co) in NL and IS supernatants (SN) in patients with different types of work-related asthma (WRA) occupationally exposed to high (HMW) and low molecular weight (LMW) agents. Hypothetically, indication on the significance of sICAM-1 in asthmatic subjects may allow to consider this factor as a new target for inhalant therapy.

Method: In 60 patients with WRA-like symptoms a specific inhalant challenges (SIC) with occupational allergens were carried out. In 240 samples of NL and IS supernatants, collected before and 24 hour after the SIC, the level of sICAM-1 was assayed by using Human Adhesion 6-plex FLOWCytomix Multiplex Kid (Bender Medsystems, Austria). The detection scope ranged 0.5-4.000 ng/mL.

Results: Among patients with WRA the level of sICAM-1 was significantly higher in IS in comparison with NL SN before ($P < 0.01$) as well as after the SIC ($P = 0.01$). The significant increase in sICAM-1 Co after SIC was observed in WRA only in NL ($P < 0.01$), but not in IS ($P = 0.73$). Spearman's rank correlation coefficient revealed that higher Co of sICAM-1 in NL were positively associated with higher Co IS before SIC ($P = 0.02$, $r = 0.3$) and after, however in the 2ndcase it was not significant ($P = 0.54$, $r = 0.08$). Higher percentages of eosinophils in NL were associated with lower levels of sICAM-1 in IS before [$P < 0.01$, $r = (-0.39)$] and after the SIC [$P < 0.01$, $r = (-0.45)$]. The Co of sICAM-1 were higher in NL before SIC among patients with occupational asthma (OA) due to LMW in comparison to HMW. Contrarily, in the group with work-exacerbated asthma (WEA) occupationally exposed to HMW-A, the level of sICAM-1 was higher in NL and IS before and after the SIC in comparison with LMW-A. Among patients with OA due to LMW-A Co of sICAM-1 in IS before SIC was higher than in WEA exposed to LMW-A.

Conclusion: Adhesion molecule ICAM-1 seems to be involved in airway inflammation in work-related allergic diseases, especially occupational asthma and rhinitis related to LMW exposure.

0170 | Multiple chemical sensitivity: Allergy or psychiatric disorder? Analysis of clinical parameters in hospitalised patients between 2013 and 2017

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Background: Multiple Chemical Sensitivity (MCS) has long been debated because of difficulty in recognizing it as a defined nosographic entity, mostly considered a border line syndrome between organic and psychiatric disorders.

Method: 21 cases were studied through specialist examinations (allergy, otorhinolaryngology and psychiatric), instrumental and laboratory tests (nasal and laryngeal endoscopy, methacholine test, skin prick test, total IgE and specific IgE for food and environmental allergens, ESR, CRP, tryptase, ECP, homocysteine, vitamins B12 and D, folate and oxidative status: TAC, ROS, LDL), validated questionnaires on quality of life and psychological well-being, QEESI questionnaire and neuropsychological tests.

Results: 4 cases were excluded because diagnosed as diseases that could justify symptoms (2 were related to allergy, 1 with indolent systemic mastocytosis, 1 with gastric neuroendocrine tumor). Out of 17 patients, 14 were women (82%), the average age of onset was 44 years and most of them belonged to medium-high occupational classes. The prevalence of unemployed was 23.5%. 47% of subjects had familial anamnesis positive for mental disorders, while 35% for allergic and autoimmune diseases. Gastro-esophageal reflux and functional somatic syndromes (fibromyalgia, chronic fatigue and irritable bowel syndromes) prevailed among comorbidities. Neither positive methacholine nor clinically relevant allergy were observed. Auditory and visual reaction times were frequently slower and more variable. Indeed, 82% of cases had psychological distress. The psychopathological spectrum was characterized by symptoms of somatization, depression and anxiety. In most cases laboratory tests proved a slight increase in inflammatory parameters, particularly ESR, tryptase, ECP and homocysteine, and the oxidative status showed a normal total antioxidant capacity against increased oxidative stress.

Conclusion: In MCS cases psychiatric symptoms and psychological distress are major causes of impaired quality of life and work performance, and translate into a high prevalence of unemployment. From the standpoint of occupational medicine such patients should be considered as fragile and worthy of specific support. No relevance was observed for allergic disease, but allergy tests as well as psychiatric examination are fundamental to define differential diagnosis. Intriguingly, oxidative status and inflammatory parameters might suggest the presence of low grade chronic inflammation.

TUESDAY, 29 MAY 2018
OAS 30
NOVEL APPROACHES TO DIAGNOSIS OF FOOD ALLERGY

0171 | A dosing interval of 40 minutes is favorable in comparison to 30 minutes for a safe oral food challenges with hen's egg and wheat

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Background: The interval between ingestion may influence the safety of oral food challenge (OFC) tests, especially in patients with severe food allergies.

Method: We conducted a retrospective case-control review of the results of OFCs with boiled egg white, udon noodle (containing 2.6% wheat protein) and cow's milk performed from April 2012 to March 2016. The objective cases were positive OFCs in which allergic symptoms were provoked in 4 increasing doses every 40 minutes (40-min method; 0.2-0.5-1-2 g or 0.5-1-2-5 g). An equivalent number of control OFCs were selected from positive OFCs performed with 5 increasing doses every 30 minutes (30-min method; 0.2-0.5-1-2-5 g). To avoid the influence of the potential severity of the patients, we matched the prediction scores (reported by Sugiura) of cases and controls. The prediction score was determined based on the specific IgE levels of ovomucoid (Allergol Int 2016), milk (Allergol Int 2017) and wheat (PAI 2017 in press) in addition to some background factors (*i.e.*, age, status of food avoidance, history of anaphylaxis). The severity of the provoked symptoms was quantified based on the total score (TS; 0-240 points) of anaphylaxis scoring Aichi (ASCA).

Results: A total of 337 OFCs performed using the 40-min method (egg, n = 198; wheat, n = 56; milk, n = 83) were compared to an equivalent number performed using the 30-min method. The total ingested dose before the appearances of symptoms in the 40-min method was significantly lower in comparison to that in the 30-min method for egg (median 3.5 g vs 3.7 g, $P < 0.01$) and wheat (median 3.5 g vs 3.7 g, $P < 0.01$). Furthermore, the TS in the 40-min method was significantly lower in comparison to that in the 30-min method for egg (median 15 vs 20 points; $P < 0.05$) and wheat (median 11 vs 20 points; $P < 0.01$). The rate of severe reaction (TS≥40) in the 40-min method was also significantly lower in comparison to that in the 30-min method for the egg OFCs (10% vs 17%, $P < 0.05$); however, there were no differences in the total ingestion dose for milk OFCs (median 1.7 vs 1.7 mL, $P = 0.67$) or the TS (median 15 points vs 15 points, $P = 0.81$) of the 40- and 30-min methods. No difference in the rate of adrenaline injection was observed between the 40- and 30-min methods in the egg, wheat and milk OFCs.

Conclusion: The results suggest that a 40-min interval is safer than a 30-min interval in hen's egg and wheat OFCs, especially in the patients with a low threshold dose.

0172 | Homologous tropomyosins from vertebrate and invertebrate: Purification and allergenicity assessment

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Background: Seafood is one of the most common elicitors for food-allergic reactions while, among crustacean species, ingestion of shrimp (*Penaeus monodon*) is considered as pre-dominant cause of adverse reactions. Tropomyosin, a muscle protein, is the major allergen in invertebrates such as crustaceans. Vertebrate tropomyosins are non-allergenic proteins, an observation which is not well understood so far. The aim of this study was first to isolate pairs of allergenic (native, recombinant) and non-allergenic tropomyosins and following, to compare those proteins at the biomolecular level and as to their allergenicity.

Method: Homologue muscle tropomyosins from shrimp (*Penaeus monodon*) and chicken (*Gallus gallus*; breast, leg) were purified by column chromatography. Recombinant tropomyosins were expressed in *E. coli*, followed by chromatographic protein isolation. Purified proteins were compared by Edman degradation, mass spectrometry (MS), antibody-binding studies (immunoblot, ELISA) and circular dichroism analysis. Allergenicity was assessed by IgE-ELISA and skin testing using shrimp-allergic patients. Biological activity was determined in basophil activation tests (BAT) in comparison with mediator release experiments using rat basophil leukemia (RBL) cells.

Results: Tropomyosins were purified to homogeneity by column chromatography at a milligram scale. MS and Edman analysis revealed the identity of all isolated proteins as muscle tropomyosins. Circular dichroism analysis showed characteristic alpha-helical structures as well as high protein stability towards thermal treatment for the tropomyosins. Specific IgE sera titer were up to 9-times higher to shrimp than to chicken tropomyosin. BAT was positive with shrimp allergens at up to 100-times lower allergen concentrations than with chicken homologs RBL assays were also positive with the target molecules. Biomolecular assays on allergen characterization as well as IgE- and BAT-assays gave similar results for both native and recombinant proteins. In addition, skin reactivity of shrimp-allergic patients was positive with both shrimp and chicken tropomyosins but at up to 100-times lower concentrations with the shrimp allergen.

Conclusion: Tropomyosins from vertebrate and invertebrate exhibit similar biomolecular characteristics while they vary by their allergenic potency. Both tropomyosins might be used as standard proteins, representing high and low allergenic molecules, in future experimental set-ups for the risk assessment of novel food sources

0173 | Investigation of reduced ELISA recovery of almond and hazelnut traces from roasted nut samples by SDS-PAGE and mass spectrometry

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Background: Unintended traces of tree nut allergens in food pose a high risk factor for allergic consumers. Almonds (*Prunus dulcis*) and European hazelnut (*Corylus avellana*) are among the most commonly used tree nuts and pose a strong allergenic potential. The most commonly used method to check for potential cross contamination is an Enzyme-linked Immunosorbent Assay (ELISA). Unfortunately, available tests have limited sensitivity in detecting traces of processed nuts (e.g. roasted or baked). Improvements in ELISA detection of roasted/baked nuts are therefore of interest. To achieve this, an understanding of processes leading to loss of sensitivity is required.

Method: Almonds and hazelnuts were roasted under controlled conditions in a drum roaster at varying temperature and time profiles. Cookie dough was spiked with known amount of nut samples obtained from roasting experiments prior to baking. Proteins from raw nuts, roasted nuts and baked cookies were extracted and analysed with ELISA, SDS-PAGE, MALDI-TOF-MS and LC-MS/MS to identify the extracted proteins and to investigate effect of roasting / baking on protein detection.

Results: Analysis of the nut extracts using SDS-PAGE showed a broad range of protein bands for both nut species. In accordance to the ELISA results, the bands became less prominent with rising roasting times and temperatures. This was a first indicator, that the thermal processing has either a detrimental effect on the extractability or leads to the degradation of the proteins. All bands were subjected to a tryptic digest followed by MALDI-TOF and LCMSMS analysis, which resulted in identification of Cor a 9 and prunin as the most prevalent proteins in the extract of hazelnut and almond, respectively. Both proteins present highly abundant storage proteins in the respective tree nut. Detection of these proteins by mass spectrometric methods showed reduced sensitivity with increasing roasting temperature and times which correlated with data from ELISA. Further data on optimisation of extraction conditions will be presented.

Conclusion: The study showed that the major part of the proteins extracted from hazelnuts and almonds consists of mainly allergenic proteins (Cor a9 and prunin). Additionally, the results indicate that the loss of ELISA recovery is accounted for by inefficiencies in extraction of the processed nuts and/or a possible degradation of the proteins during processing.

0174 | Identifying suitable children for abbreviated oral food challenges to cow's milk or egg

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Background: Oral Food Challenges (OFCs) are expensive and time consuming. Guidelines recommend OFCs (as opposed to home introduction) in children who are likely to tolerate the food but have a history of sensitization or allergic reaction(s) to the food tested. Implementing abbreviated protocols ('supervised feeds') might increase efficiency and, potentially, patient satisfaction. However, as starting doses are higher than in OFCs, and this might lead to more severe reactions, selecting children at Very Low Risk (VLR) of reacting is paramount if supervised feeds are to be implemented. Such VLR selection criteria have not been developed so far.

Aim: To evaluate the suitability of set criteria to identify children at VLR of reacting at OFCs to milk or egg.

Method: 'VRL criteria' for fresh milk, cooked and raw egg OFCs were agreed as follows: (a) children with history of milk or egg allergy based on sensitization and/or on previous (non-anaphylactic) reaction plus; (b) no accidental reaction in the previous 12 months plus; (c) currently negative SPT (<3 mm) to fresh milk, egg extract and raw egg, respectively. We retrospectively reviewed medical records of children fulfilling the VLR criteria who underwent an OFC to fresh milk, cooked egg or raw egg, respectively, in our tertiary service in London between January 2016 and April 2017. OFC positive rate and safety data were recorded.

Results: 949 OFCs were conducted (40 OFCs to fresh milk, 58 to cooked egg and 38 to raw egg). 507 were OFCs to nuts. Mean age was 8 years (SD: 4.8). The overall positive OFC rate was 15% (143/806), whereas for fresh milk 25% (10/40), cooked egg 19% (11/58) and raw egg 15.8% (6/38). Anaphylaxis occurred in 3.3% of overall OFCs (31/949), 10% (4/40) for milk, 3.4% (2/58) for cooked egg, 2.6% (1/38) for raw egg. 42 children met VLR criteria and none experienced anaphylaxis at OFC. Positive OFC rates in them were 12.5% (2/16) for fresh milk, 7.1% (1/14) for cooked egg and 8.3% (1/12) for raw egg. This positive OFC rate did not differ from the rate in milk/egg allergic children who did not fulfil the VLR criteria or that in the overall population undergoing OFCs ($P < 0.05$).

Conclusion: 10% of children who fulfilled the VLR criteria reacted at OFC. It remains arguable whether this is acceptable for supervised

feeds, as these might involve a higher risk for severe reactions due to the larger doses administered. Future initiatives on supervised feeds should focus on nuts, as these account for 53% of our OFCs.

0175 | Diagnosing red-meat allergy; What to learn from skin and blood testing

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Background: Patients with red-meat allergy are sensitized to the carbohydrate galactose-alpha-1,3-galactose (gal-alpha-gal) and have delayed type 1 allergic reaction after ingestion of mammalian meat or innards. Diagnosing these patients is difficult due to the delayed reaction, and is often based on case history and serology. We aimed to characterize sensitization pattern to different sources of allergen in patients suspected with red meat allergy.

Method: All patients referred to the Allergy Center at Odense University Hospital from 2009-2017 suspected of red-meat allergy (n = 123) were evaluated with thorough case history, skin testing and serology; 45 patients had their diagnose confirmed, whereas 78 were ruled out. Patients were evaluated based on specific IgE to gal-alpha-gal, beef and pork, Skin Prick Test (SPT) with beef (cooked and raw), pork (cooked and raw), pork kidney (cooked and raw), lamb (cooked and raw), deer (cooked and raw), and Gelofusine™ (colloid plasma expander, B. Braun Medical, Germany). Diagnostic values were calculated using ROC curves. Twelve patients with confirmed red-meat allergy had additionally Histamine Release (HR) from basophil leucocytes measurements with the same allergens as used in SPT together with bovine thyroglobulin and porcine gelatin (Sigma Aldrich, USA).

Results: IgE against Gal Alpha Gal > 2.70 kU/l showed the best diagnostic capacity (sens/spec 0.91/0.96) followed by SPT with raw pork kidney (0.89/0.89) and IgE to raw pork and raw beef. The remaining SPT behaved poorly. Two patients with red-meat allergy had s-IgE to gal-alpha-gal < 0.35 kU/l. Heat treatment reduced sensitivity compared to raw material of all SPT. This was confirmed by HR where the dose-response curves for cooked beef, pork and pork kidney all were shifted to the right. Bovine thyroglobulin, porcine gelatin and pork kidney elicited the highest levels of histamine. Lamb, deer and Gelofusine™ did not provide any additional value.

Conclusion: IgE to gal-alpha-gal and in-vivo test with pork kidney are the most important test, whereas bovine thyroglobulin (the source of gal-alpha-gal in IgE test) shows potential in histamine release. In contrast to previous reports from in-vitro experiments, the allergens in meat seem heat labile in when used in SPT and in HR.

0176 | Preliminary evaluation of technical and predictive value of a simplified basophil activation test for food allergy characterization in children

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Background: The need for an oral food challenge (OFC) surrogate sparing unpleasant or even dangerous allergic reactions is growing in correspondence to the continuous increase in prevalence and severity of paediatric food allergy. Basophil activation test (BAT), while initially proposed more than 20 years ago, has been recently reported as a promising tool for predicting outcome of OFC suggesting that BAT screening might help diminish the need for OFC. To test this hypothesis, a collaboration was started between (i) the APHM Paediatric Allergology Department, where specialized paediatric allergists perform OFCs on a routine basis, (ii) the APHM Immuno-Allergology Laboratory where BATs have been routinely performed since 2007, and (iii) the Beckman Coulter R&D unit striving to streamline cumbersome flow cytometry procedures. A clinical research study has been started in 2017 in Marseille and a first set of preliminary results, upon recruitment of one third of the expected cohort, is presented here.

Method: BAT was performed in 31 paediatric subjects prior to OFC in context of diagnostic or follow-up procedures.

OFC: Incremental doses were given every 20 minutes until tolerance of final dose (EAACI guidelines, Bindslev-Jensen C, 2004). OFC was stopped and considered positive if objective signs were noted by the attending physician.

BAT: A simplified and optimized BAT protocol based on ready-to-use dry and room temperature stable reagents was applied. For each subject, the occurrence of *ex vivo* reactivity was verified and a dose-response curve approach was adopted to assess patient blood reactivity toward the considered extract. BATs were performed on whole blood, within 24 hour of collection and prior to any *in vivo* test.

Results: Among the 31 patients recruited so far allergic responses were tested as follows: 15 /peanut, 5 /egg, 4 /nuts different from peanuts, 4 /bovine milk, 1/peas, 1/kiwi and 1/shrimp. While 3 patients presented significantly lower *in vitro* reactivity than the bulk of the cohort, only 1 patient had to be excluded from the data treatment because of total unresponsiveness. Considering the positive or negative outcome of the OFC, the maximum ingested dose that significantly varied from patient to patient, a high correlation was observed between OFCs and BATs.

Conclusion: While this needs to be further verified and characterized, this preliminary dataset confirms the potential of BAT in clinical allergology laboratories.

TUESDAY, 29 MAY 2018
OAS 31
IMMUNOTHERAPY VACCINES: NEW CONCEPTS

0177 | Vaccination of grass pollen allergic patients with recombinant B cell epitope-based grass pollen vaccine BM32 induces a peptide-specific de novo immune response against major grass pollen allergens

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Background: We have treated patients suffering from grass pollen-induced allergic rhinitis with a recombinant hypoallergenic vaccine comprising peptides from 4 major timothy grass pollen allergens (Phl p 1, 2, 5 and 6), fused to the PreS protein domain of hepatitis B virus (BM32) for two years in a double-blind, placebo-controlled, field study. Here we compared epitope-specificities, levels and kinetics of the allergen-specific IgG_1 and IgG_4 antibody induced by AIT with the recombinant grass pollen allergy vaccine BM32.

Method: Patients were immunized with three monthly injections of two different BM32 doses (pre-seasonal), boosted with one injection (post-seasonal) in the treatment year one and followed by another pre-seasonal three-injection course in year two. The concentrations and kinetics of grass pollen allergen-specific and peptide-specific IgG_1 and IgG_4 responses were measured by quantitative ELISA.

Results: AIT with BM32 induced grass-pollen allergen-specific and peptide-specific protective IgG responses in active but not placebo-treated patients. According to quantification of specific IgG_1 and IgG_4 levels the majority of BM32-induced IgG was a *de novo* immune response directed against peptide epitopes which at the same time reacted with the complete allergens. The allergen-specific IgG_1 response showed a rapid rise and decline whereas the IgG_4 response started later and remained sustained.

Conclusion: Our data indicate that the majority of grass pollen allergen-specific IgG results from a *de novo* immune response, is peptide specific and reacts with the complete allergens in patients immunized with the recombinant B cell epitope-based grass pollen vaccine BM32.

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0178 | Evaluation of a treatment scheme with the recombinant grass pollen allergy vaccine BM32 yielding high allergen-specific IgG responses associated with clinical efficacy

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Background: BM32 is a recombinant hypoallergenic grass pollen allergy vaccine based on four Aluminum hydroxide-adsorbed fusion proteins consisting of B cell epitope-derived peptides from the four major timothy grass pollen allergens, Phl p 1, 2, 5 and 6 fused to the hepatitis B-derived coat protein preS. Treatment with BM32 has been shown to reduce symptoms of grass pollen allergy in patients suffering from allergic rhinitis.

Aim: To determine a pre-seasonal treatment schedule giving highest grass pollen allergen-specific IgG responses.

Method: In a prospective, double-blind, placebo-controlled, monocentric phase IIb combined field and exposure chamber study, development of IgG_4 and IgG_1 antibodies against Phl p 1 and Phl p 5 (primary endpoint) was evaluated in subjects receiving either placebo (n = 32), 3 (n = 32), 4 (n = 30) or 5 (n = 30) monthly pre-seasonal injections of 80 mg of BM32 (i.e., 20 mg of each fusion protein). Clinical response before and after treatment was determined by total nasal symptom score in the allergen exposure chamber and by mean daily combined SMS, SS, and MS during peak of the grass pollen season.

Results: A significant induction of allergen specific IgG_1 and IgG_4 compared to placebo was achieved with all active dosing regimens in the full analysis set (n = 124). The group receiving 5 pre-seasonal injections of BM32 developed the highest and most sustained allergen-specific IgG_1 and IgG_4 responses as compared to the other groups. A dose-dependent reduction in TNSS of 42.9% (BM32 5x), 42.1% (BM32 4x) and 7.3% (BM32 3x) vs placebo was observed after the grass pollen season. The group receiving 5 pre-seasonal injections of BM32 showed the best clinical effect yielding a 23.8% lower daily SMS compared to placebo. Treatment with BM32 was safe and well tolerated with mainly local injection site reactions.

Conclusion: Pre-seasonal treatment with five injections containing 20 mg of each of the four BM32 fusion proteins induced the highest allergen-specific IgG responses, was clinically effective, safe and well-tolerated.

0179 | Deep immunophenotyping shows tolerance induction by successful CpG/Fel d 1-based immunotherapy in a murine asthma model

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Background: Allergen-specific immunotherapy (SIT) is the only disease-modifying treatment for perennial allergic rhinitis/asthma which restores immune tolerance against allergens. Adjuvants are potential tools to improve SIT effects. CpG oligodeoxynucleotide (CpG-ODN) is a promising adjuvant previously used at low doses in clinical trials. Recent evidence shows that high doses of CpG-ODN can induce immune tolerance. A successful high dose CpG-based SIT has been developed by us in a murine asthma model to the major cat allergen Fel d 1. The objective of this study is to elucidate immunophenotypical changes occurring after a full course of high dose CpG-based SIT.

Method: BALB/c mice were sensitized by three i.p. injections containing a mixture of recombinant Fel d 1 (rFel d 1) and alum. Subsequently the mice received three courses of immunotherapy i.p. using a solution of rFel d 1 and CpG-ODN (1 mM). Finally, allergen challenge was performed through nasal instillation of rFel d 1 to trigger the allergic response. Lungs (effector organ), mediastinal lymph nodes (MLN, draining LN) and spleen (general immune response) were immunophenotyped by mass cytometry 18 hour after the final challenge using a panel of 34 extracellular and intracellular markers. Three animal groups were analyzed: i) allergic, without SIT; ii) allergic, SIT treated; and iii) untreated control.

Results: In lungs, a clear improvement of allergic parameters was found after the CpG SIT, such as a 20 fold reduction of eosinophil and 10 fold reduction of mast cell number, as well as a 50% reduction of IL-13 production by Th2 cells. The results in the effector organ matched with those in secondary immune organs. In MLN, B cell number was lower by 20% and CD69 expression in B cells by 50% in the CpG SIT group. In addition, CpG SIT let to a significant decrease of Gata3 expression in Th2 cells. In the spleen, the number of Treg was increased by 25% after CpG SIT. This was paralleled, by a 15% higher expression of transcription factor FoxP3 in Tregs.

Conclusion: Using mass cytometry, a single cell high throughput immunophenotyping technology, we analyzed the immune cell changes in a high dose CpG/Fel d 1 SIT model. The analyses of lungs and secondary immune organs showed an induction of tolerance by high CpG SIT. These results will help to further understand how high CpG/allergen SIT modulates the immune system towards tolerance and to support validations of novel SIT approaches using CpG as adjuvant for patients with perennial rhinitis/asthma.

0180 | Preventive sublingual allergen immunotherapy with house dust mite extract modulates epitope recognition in pre-school children

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Background: The preventive effect of allergen immunotherapy (AIT) on allergy and asthma development is currently assessed using primary and secondary AIT approaches. Knowledge of the immunological effects of these interventions is limited and the impact on epitope diversity remains to be defined.

Method: We used high-density peptide arrays that included all known *Dermatophagoides pteronyssinus* (Der p)and *Dermatophagoides farinae* (Der f) allergens and the whole proteome of Der f to study changes in House Dust Mite (HDM) linear peptide recognition during a 2-year preventive double-blind placebo-controlled sublingual HDM AIT pilot study in 2-5-year-old children with sensitization to HDM but without symptoms.

Results: AIT-treated patients showed significantly less *de novo* IgE-binding peptides and a significantly higher number of IgG binding peptides compared to placebo-treated individuals ($P < 0.05$). HDM-IgG4 diversity did not differ between the two treatment groups. However, increased HDM-specific IgG4 diversity correlated positively with increased IgE peptide diversity in the placebo group, whereas it inversely correlated in the treatment group. Thus, changes in IgG4 epitope diversity may reflect different mechanisms depending on the treatment: while increased specific IgG4 epitope diversity reflects *de novo* IgE generation in the placebo group, it reflects the induction of a regulatory response in the pAIT group.

Conclusion: These data suggest a protective modulation of atopic disease progression at a molecular level by preventive sublingual immunotherapy.

0181 | Novel insights into the immunological mechanisms of action of allergoids coupled to mannan as next generation vaccines for allergen-specific immunotherapy

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Background: Glutaraldehyde-polymerized grass pollen allergoids coupled to nonoxidized mannan (PM) represent novel suitable vaccines for allergen-specific immunotherapy (AIT). PM promotes Th1/Treg cell responses by targeting human dendritic cells (DCs) and upon subcutaneous injection in mice. Aluminium hydroxide (alum) is the most widely used adjuvant in human vaccines but its way of action is not fully understood.

The aim of this work is to study the immunogenicity of PM after *in vivo* sublingual administration in mice as well as the immunological mechanisms by which alum condition the capacity of PM to promote healthy immune responses to allergens.

Method: BALB/c mice were sublingually or subcutaneously immunized and cell responses were assayed by flow cytometry. Allogeneic cocultures of PM-activated human monocyte-derived DCs (hmoDCs) or total DCs and naïve $CD4^+$ T cells in the presence or absence of alum were performed to analyse T cells polarization. $FOXP3^+$ Treg cells were quantified, purified by cell sorting as $CD4^+CD25^{high}CD127^-$ and mixed with CFSE-labeled autologous PBMCs to study the suppression capacity.

Results: *In vivo* sublingual immunizations of mice with nonoxidized PM resulted in an increase of $CD4^+CD25^{high}FOXP3^+$ Treg and Th1 cells in submandibular lymph nodes and spleen. Those effects were abolished when using oxidized PM. Importantly, subcutaneous immunization in the presence of alum reduced the number of $FOXP3^+$ Tregs induced by PM and increased IgE production and IgG1/IgG2a ratio. Immunizations with alum also increased IL-4, IL-5 and IFN-γ and reduced IL-10 production induced by PM. *In vitro* experiments with hmoDCs demonstrated that alum decreased the number of Tregs induced by PM. Accordingly, alum significantly decreased IL-10-producing T cells generated by PM-treated hmoDCs, while increasing IL-5-, IFN-γ- and IL-17-producing T cells. The same results were obtained when using an enriched fraction of total DCs including mDCs and pDCs. The Treg cells generated by PM-treated hmoDCs inhibited the proliferation of autologous PBMCs in a dose-dependent manner, showing a more potent suppression capacity than Treg cells generated by hmoDCs treated with PM in the presence of alum.

Conclusion: We provide novel insights into the effects of sublingual administration of PM and the influence of aluminium hydroxide in the immunomodulatory properties imprinted by PM in DCs, which might have important implications for future development of novel AIT protocols.

0182 | Development of a recombinant vaccine based on virus-like particles for the treatment of peanut allergy

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Background: Peanuts harbour at least 12 different antigens responsible for allergy induction in humans. The major allergens Ara h 1 and Ara h 2 are recognised by IgE from more than 95% of peanut-sensitive patients. To treat peanut allergy we displayed Ara h 1 and Ara h 2 on virus-like particles (VLPs) derived from plant viruses and tested their safety and efficacy pre-clinically.

Method: Ara h 1 was purified from extracts while Ara h 2 was produced recombinantly. Both allergens were separately coupled to VLPs and used to immunise mice previously sensitised to peanut-extract. Allergic responses were measured by skin prick tests, intestinal inflammation upon oral and temperature drop upon intravenous challenge.

Results: We demonstrated that both allergens induce strong and protective IgG responses in peanut allergic mice and ameliorate local allergic symptoms in skin-prick-tests and allergen induced gut-inflammation. Systemic symptoms upon intravenous challenge with allergen-extract were also strongly suppressed. Despite strongly enhanced immune responses induced by allergens displayed on VLPs, the allergens were fully detoxified as they failed to trigger an allergic response in sensitised mice and failed to activate basophils of allergic individuals.

Conclusion: Vaccination against either Ara h 1 or Ara h 2 alone was sufficient to induce protection against the whole extract consisting of multiple allergens. We will elucidate the mechanism of this clinically highly relevant phenomenon.

TUESDAY, 29 MAY 2018
OAS 32
AEROBIOLOGY AND ALLERGY

0183 | Age-related association of environmental changes with asthma exacerbations: A time-series analysis in Seoul Metropolitan City, Korea

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Background: Asthma exacerbation is known to be affected by respiratory virus infection (e.g. rhinovirus), air pollutants, aeroallergen sensitization, and/or weather change. This study aims to compare effects of comprehensive risk factors including air pollutants, weather changes, aeroallergens, and viral epidemics for AE among different age groups in Seoul, Korea

Method: we obtained number of ED visits for AE, pollutant concentrations [particulate matter of ≤10 μm in size (PM_{10}), nitrogen dioxide (NO_2), ozone (O_3), carbon oxide (CO), and sulfur dioxide (SO_2)], weather variables [humidity, solar sunshine, and diurnal temperature range (DTR)], aeroallergens (tree, grass, and weed), and respiratory viruses (human rhinovirus, influenza virus, and respiratory syncytial virus) in Seoul. Subjects were classified into five groups based on age: <2, 2-5, 6-17, 18-59, and ≥60 years. Then we used the Poisson generalized linear regression model combined with a distributed lag non-linear model (DLNM) for the lagged and non-linear effects.

Results: A total of 28 824 ED visits for asthma exacerbation were identified during the study period. Cumulative risk of AE increases without a threshold in all age groups as pollutants concentration increases. Pollutants showed delayed effect of few days except ozone which had immediate effect. PM_{10} and NO_2 showed highest relative risk (RR) in subjects aged <2 years (PM_{10}, RR 1.37; NO_2, RR 1.43). O_3 had a significant effect on school-aged subjects (RR 1.56). Elderly were at the highest risk of being affected by CO (RR 1.16) and SO_2 (RR 1.19). DTR expressed a significant effect in infants (RR 1.36), preschoolers (RR 1.26), and elderly (RR 1.10). Tree allergens had highest effect in school children (RR 1.25) and adults (RR 1.55). Weed was most important with regard to school-aged subjects (RR 2.08). Humidity, solar sunshine, grass allergen, and respiratory syncytial virus infection lost their statistical significance after adjustment for confounders. Maximum lagged effect of each risk factor mostly occurred between lag day 0 and 4.

Conclusion: Asthma exacerbation leading to ED visits had different risk factors in relation to age. Our results showed that different environmental modification strategies with regard to age are required to prevent asthma aggravation.

0184 | From pollen to fungal spore allergy: Alternaria spores under differing environmental regimes and the need for an electronic Spore Information Network (eSPIN) in Bavaria, Germany

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Background: Airborne pollen and fungal spores are major causes of respiratory allergy worldwide. Although pollen has been extensively studied, still little is known about fungi. What are the environmental factors affecting fungal abundance? Is there a 'safe' place or time-period that we can 'switch off' fungal exposure and allergies? To answer these, we investigated the spatiotemporal abundance of airborne *Alternaria* spores in a variety of climatic and pollution regimes, in both field and laboratory conditions.

Method: *Alternaria* is the most allergenic and one of the most representative in the atmosphere across the globe. The abundance of airborne *Alternaria* spores has been examined in 24 sites in Bavaria, Germany, in 2015. Monitoring took place using Hirst-type volumetric traps and counts were performed on a 2-hourly basis. Differences among bioclimatic zones across Bavaria were investigated. Airborne *Alternaria* spores were also monitored in Augsburg (in 2017) using a portable Hirst-type volumetric trap. Sampling was conducted 3-4 times a week and twice per day, early in the morning and evening, in four different sites across the city varying in vegetation, urbanisation levels and temperature. Finally, *A. alternata* was experimentally grown under a variety of temperatures and different nutrient availability. Spore production was examined in variable climatic scenarios, along with an IPCC climate change scenario for 2100.

Results: Fungal spores of *Alternaria* seem to be more abundant when temperature is lower, both in field measurements and experimental conditions. Spores showed their peak concentrations mostly in the evening and at night. This pattern was consistent regardless of the bioclimatic zone, air pollution or urbanisation level involved. This was more intense in extreme locations like the Alpine region. In laboratory conditions, *A. alternata* produced more spores with increased nutrient availability but less with elevated temperature.

Conclusion: *Alternaria* spores are inversely correlated with temperature. This might be good news as lower spore production means less exposure and fewer fungal allergies. However, we have to keep in mind that *Alternaria* is an endophytic fungus, thus being influenced by the plants hosting it. So, it is also suggested that *Alternaria*exhibits a delayed response to environmental stress, which would mean abrupt changes in the future. Unpredictable *Alternaria* spore abundance highlights the need for a spore information network to warn of high-risk exposure.

0185 | Mapping plant-derived panallergen sensitization in Southern France

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Background: Sensitization to plant panallergens affects the symptoms, the diagnostic and the management of food and airborne allergy. Large-scale epidemiological data are lacking in France.

Method: Retrospective analysis of allergen microarray data (ImmunoCAP ISAC® 112, Thermo Fisher Scientific, Sweden). Microarrays were performed in the Immunology Department of the Marseille University Hospitals, 2011-2017. Patients were from Southern France, French Riviera to the Atlantic Ocean below the 45th parallel North. IgE responses were studied for PR-10, profilins, LTP and polcalcins, counting 10, 4, 8 and 2 homologues on the ISAC platform.

Results: 1004 patients, median age 14 (0.1-83), sex ratio 1. sensitization to the LTP family was the most prevalent: 27%, median age 11. Pru p 3 (70%) and Jug r 3 (64%) were the most frequent, Tri a 14 the less frequent (25%). LTP sensitization profiles were well correlated to Pru p 3 (rho $\geq$ 0.6), except for Par j 2 and to a less extent Tri a 14 and Cor a 8. A 22% prevalence of PR-10 sensitization, median age 13, was found. Bet v 1 (84%) and Cor a 1 (78%) were the most frequent. 35 patients (16%) were Bet v 1-negative but Cor a 1 (46%), Gly m 4 or Mal d 1 positive. The mean complexity of the PR-10 profile varied as a function of age: 4.7/10 homologues (0-5 years), 5.8 (6-12), 6 (13-17), 6.8 (18-39), 5.5 ($\geq$40). Sensitization to profilins was 15%, median age 12. Hev b 8 was the most frequent (93%), Phl p 12 the less (62%). There was a strong correlation between sensitization to Hev b 8, Mer a 1 and Bet v 2 ($\geq$0.9). Sensitization to polcalcins was rare: 4%, median age 13 and highly correlated for the two homologues ($\approx$1).

Conclusion: Sensitization to plant panallergens from the LTP (27%), PR-10 (22%) and profilin (15%) but not polcalcin (4%) families was a common finding. There was a strong correlation among most members of each family, supporting molecular spreading as a sensitization mechanism and suggesting the potential value of a global profile analysis. Age-dependent variations of the PR-10 profile complexity suggests its involvement in the atopic march.

Our study was performed in patients referred for suspected or confirmed allergic diseases and therefore it cannot be held as representative of the general population. However, pollen exposure, which is generally seen as the starting point for plant panallergen sensitization, is the same all over a given region. Therefore we believe our results provide indirect data for the general population.

0186 | Predictors of successful mouse allergen reduction in inner-city homes of children with asthma

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Background: Exposure to mouse allergen in inner-city environments has been associated with asthma morbidity. Interventions to reduce these exposures can have beneficial health effects; however, it is important to choose homes where the intervention will be successful. This study aims to identify home characteristics that predict successful reduction of mouse allergen levels after intervention.

Method: This was a randomized clinical trial conducted in two cities in the United States: Baltimore, Maryland and Boston, Massachusetts. Participants (aged 5-17 years) with asthma, mouse sensitization, and mouse exposure in the home were randomized to receive professionally delivered integrated pest management (IPM) with education or pest management education alone. A successful reduction was defined as a 90% reduction in the level of mouse

allergen measured in vacuumed floor dust from the bedroom. Home characteristics were then evaluated as possible predictors associated with successful reduction of mouse allergen.

Results: Overall, 44% (151 / 346) of the homes had a successful reduction in the level of mouse allergen during the study period. There was not a significant difference in the rate of successful reduction in the IPM group vs the education alone group (46% vs 41%, $P = 0.38$). Regardless of intervention group, there was a significant difference in rates of successful reduction favoring those homes with a higher baseline mouse allergen level (57% vs 31%, $P < 0.01$). After adjusting for possible confounders, the following home characteristics were also associated with successful reduction: Boston location (OR = 2.83, 95% CI = 1.51-5.30, $P < 0.01$), percentage of walkable floor in the bedroom at baseline (OR = 2.53, 95% CI = 1.24-5.15, $P = 0.01$), and allergen proof covers on the participants' beds at baseline (OR = 2.04, 95% CI = 1.00-4.17, $P = 0.05$).

Conclusion: This study demonstrated certain predictive factors associated with successful reduction of home mouse allergen levels including baseline allergen levels, city location, and factors possibly representative of inhabitants with higher motivations at baseline including the presence of allergen proof covers and higher percentage of walkable floors within the home. In future intervention studies, these factors could be helpful markers to preselect homes with the best chance of an efficient and successful allergen reduction.

0187 | Phl p 5 major allergen and air pollution in the atmosphere of Córdoba (Spain)

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Background: In Córdoba, Spain, grass aeroallergens usually follow similar progression that pollen season. However, in some sporadic days, aeroallergens were detected in the absence of pollen grains, probably due to different external events: high humidity before storm episodes, long-distance transport and/or pollutant exposition.

Pollutants can influence the percentage of allergen release and therefore, the sensitivity of allergic subjects. In addition, particulate air pollutants can act as carriers of allergens, helping their dispersion and access to the respiratory tract.

Air pollution can also cause stress to plants and reduce the flowering intensity, so that they can produce fewer pollen grains. However, other studies indicate that in these cases the aeroallergens increased.

The purpose of the study is to verify the possible influence of pollutants on grass pollen and Phl p 5 into the atmosphere.

Method: The major allergen Phl p 5 was detected using the Burkard multi-vial Cyclone sampler over a 3-year period (2012-2014) and were quantified by ELISA Double Sandwich. Airborne grass pollen was collected using a Hirst-type volumetric spore trap, following the methodology recommended by European Aerobiology Society (EAS). The air quality data were provided by the Junta de Andalucía. Statistical comparative studies among the three different particles have been carried out.

Results: Our results show that the main atmospheric pollutants that could affect Phl p 5 allergen concentrations are particles matter (PM_{10}), ozone and nitrogen dioxide. It has been observed higher Pollen Allergen Potency during the exposition to high concentrations of these pollutants. This fact occurred in most of the sporadic days with discrepancies between aeroallergen and airborne pollen concentrations. On the other hand, days with higher CO_2 and temperatures showed a higher pollen concentration.

Conclusion: Pollution in cities triggers the chances of suffering from allergies. Ozone, nitrogen dioxide and particulate matter seem to be the three main pollutants responsible for intensifying the aeroallergen concentration in the city of Córdoba. However, the air pollution is not the unique cause of this increment, since other factors like main meteorological parameters have also an important role.

0188 | Relevant pollen sensitization in the same geographical area at different age ranges

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Background: The prevalence of atopy and sensitization to inhalant and food allergens is increasing, especially in developed countries. Sensitization depends on the level of allergen exposure and the age of the subjects.

The aim of this work was to study the most prevalent pollen sensitization in children and adolescents living in a well-defined biosystem: Blanca- Murcia (South- East of Spain).

Method: We included children and adolescents (3 -19 y.o.) born in Blanca (Murcia). For enrolment, speeches were delivered at Nurseries, Primary and Secondary schools and in their health care centers as well as information leaflets were given to their parents. Prior to the inclusion in the study, a written informed consent was signed by their parents. All the subjects received a population-adapted questionnaire to fill in and skin prick tests to most prevalent inhalant allergens were performed. Further an oral interview was carried out in each case. We compared the sensitization to the most relevant pollen in the area.

Results: A total of 600 children and adolescents were included: 307 aged from 3 to 9 y.o. and 293 from 10 to 19 y.o. The 53% of children and 59% of adolescents were female, with a significance of $P < 0.01$ when comparing both groups.

The three most relevant pollen were olive tree, grass and *Salsola Kali*, with the following percentage of sensitization: olive tree, 22% of

children vs 36% of adolescents ($P < 0.001$); grass pollen, 8.5% vs 25% ($P < 0.0001$); and *Salsola kali*, 10% vs 21% ($P < 0.001$) respectively.

Conclusion: We have found a highly significant increase in pollen sensitization with groups of different ages. When comparing children vs adolescents, all living in the same community with equal pollen exposure and same environmental conditions, sensitization was higher in adolescents, being three times higher for *Salsola Kali* and double for grass pollen.

TUESDAY, 29 MAY 2018
OAS 33
RISK AND PROTECTIVE FACTORS IN EARLY LIFE

0189 | M. vaccae-based formulation for the primary prevention of atopic dermatitis

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Background: Several studies have reported differences in occurrence of asthma and atopic diseases between rural and urban areas. People living in a farm environment are exposed to microbes like mycobacteria that immunologically favour the development of a TH1-response. Mycobacterium vaccae infection was recently shown to suppress serum IgE and production of typical TH2-cytokine, interleukin (IL) -5 in ovalbumin-sensitized mice. We have clinically tested the possibility of tolerance induction by M. vaccae lysate skin application in newborn children.

Method: A cosmetic formulation - emollient cream containing M. vaccae lysate (0.1% w/w of sonicated 10^8 CFU/mL lysate) - was prepared; vehicle (same formulation without M. vaccae lysate) was used in the control group. 100 newborns at high risk of AD, which was defined as having a parent or full sibling who has (or had) AD, asthma, or allergic rhinitis, were enrolled (under the approval of Local Ethics Committee) into the randomized (1:1) double-blinded controlled trial of daily skin application of the formulation or vehicle. Sample size was calculated to detect 50% reduction of the relative risk, given the 60% risk of AD in the study population. The intervention started within 4 weeks of birth. Primary endpoint was the proportion of newborns having AD symptoms in the first 6 months of life.

Results: 93 participants were included into the data analysis. Daily use of the formulation significantly reduced the cumulative incidence of AD at 6 months (49% in the control group vs 31% in the study group), resulting in relative risk reduction (RR, 0.63; 95% CI, 0.41-0.84; $P = .023$). RR reduction of cow milk sensitization was also detected. No serious adverse events were recorded during the study. The rate of adverse events (skin redness, urticaria, etc) did not differ between groups.

Conclusion: The use of M. vaccae-based formulation on the skin during the neonatal period is a novel approach to the primary prevention of atopic dermatitis. M. vaccae lysate-containing formulation offers benefits compared to emollient use. The trial is ongoing to estimate the rate of eczema at 12-month time point.

0190 | Farming, cytokines, 17q21 and wheeze in the first year of life

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Background: Specific cytokines are associated with environmental factors as well as with asthma in childhood. Furthermore, 17q21 variants that are associated with increased asthma in childhood have been shown to interact with the protective effect of farming on wheeze in early life. The structural patterns underlying these complex associations, however, are unclear. Our aim was to define cytokine patterns in early life and to analyze their associations with early farming environment and wheeze in the 1. year of life stratifying for 17q21 variants.

Method: In 460 children of the rural PASTURE birth cohort we analyzed the production of IL-1β, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IFN-γ and TNF-α released by innate lipopolysaccharide (LPS)-stimulated PBMCs at the age of 1 year. All cytokines measurements were dichotomized into above or below detection level and included in a latent class analysis. Single-nucleotide polymorphisms related to GSDMB at 17q21 were genotyped. Farming environment was assessed in yearly questionnaires and wheeze was assessed in diaries on a weekly basis.

Results: Latent class analysis revealed 3 patterns with a low, intermediate and high ability to produce cytokines after LPS stimulation. In the latter group all cytokines showed the highest proportion of detectables, i.e. independent of TH1 and TH2 mechanisms. Children in the highest class were more likely to have grown up in a farming environment: Maternal contact with different farm animal species in pregnancy, regular stay in stables and farm milk consumption both of the mother in pregnancy and of the child in the 1. year were most prevalent in the highest class as compared to the intermediate and lowest class (stay in stable in 1. year: 29.7%, 27.6%, 17.9%, P (trend) =0.012; farm milk consumption in 1. year: 44.0%, 37.9%, 26.8%, P (trend)=0.002). When analyzing the overall effect of cytokine classes on wheeze in the 1. year no effect was observed. However, in the subgroup of children carrying the asthma risk allele rs2290400-T, a

significant inverse association of a high ability to produce cytokines after LPS stimulation and wheeze was observed (adjusted odds ratio compared to low class 0.45; 95% confidence interval 0.21-0.96, $P = 0.040$).

Conclusion: Children with early wheeze and at high risk of going on to asthma development (carriers of 17q21 risk alleles) show weak innate immune responses in the first year of life. These data suggest that stimulation of the innate immune response may prevent the onset of asthma.

0191 | Early exposure to environmental peanut in dust is associated with the development of peanut allergy but this effect is abrogated by early peanut consumption

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Background: The Dual Allergen Exposure Hypothesis describes a window of opportunity during infancy, where depending on whether exposure to peanut allergen is through the skin or gut, allergy or tolerance develops respectively. A dose-response relationship between environmental peanut exposure (EPE) in dust and the development of peanut allergy has been reported in both high-risk and population-based studies in children with a disrupted skin barrier (as measured by filaggrin loss-of-function mutations or a history of eczema). To date, no study has assessed whether early peanut consumption mitigates the impact of EPE on the development of peanut allergy.

Method: Early environmental peanut exposure in dust was quantified in the Enquiring About Tolerance (EAT) study using a polyclonal ELISA directed against whole peanut protein. Infant bed-sheet dust samples were obtained at 3 months of age prior to being randomised to either early introduction of allergenic foods (including peanut) or exclusive breastfeeding until 6 months. Peanut allergy was determined between 1-3 years. Penalized logistic regression was used to assess the relationship between EPE and peanut allergy.

Results: Environmental peanut exposure significantly increased the odds of developing peanut allergy; for each natural log (ln) unit increase in peanut dust exposure, peanut allergy increased by OR 1.57 (95% CI: 1.09-2.28, n = 515) adjusting for family history of food allergy, maternal peanut consumption during breastfeeding and study randomisation group. In children randomised to early peanut introduction, EPE was no longer associated with peanut allergy (OR 1.24, 95% CI: 0.70-2.22, n = 246, $P = 0.695$). In children randomised to the standard introduction, early EPE conferred an even greater risk of developing peanut allergy (OR 1.99, 95% CI: 1.20-3.32, n = 269, $P = 0.008$). At average levels of EPE there was an interaction between eczema and eczema severity on the development of peanut allergy.

Conclusion: This is the first population-based study to show a positive association between environmental peanut exposure and the development of peanut allergy overall. The study supports the Dual Allergen Exposure Hypothesis in that early peanut consumption prevented the development of peanut allergy even in children with high environmental peanut exposure. Furthermore, eczema and eczema severity increased the impact of environmental peanut exposure on the development of peanut allergy, analogous to the findings in the high-risk CoFAR study.

0192 | Defining childhood eczema: The variety of operational definition causes divergence in its prevalence estimate and model performance

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Background: Atopic dermatitis [AD] is one of the most common skin diseases, but there is no objective test that can unequivocally confirm the diagnosis, and no uniform clinical definition, resulting in numerous case definitions in epidemiological and genetic studies. The lack of the standard operational definition may impact on the generalizability and consistency of results, and hamper comparisons across different studies.

We propose that research findings may differ substantially if different AD definitions are used. We therefore proceeded to clarify to what extent the operational definitions cause fluctuation in the result of AD studies, in relation to the prevalence estimates and the association with risk factors.

Method: As a first step, we reviewed the operational definitions used in all studies contributing to the recent meta-analyses and systematic reviews of the genome-wide-association studies, and studies on AD persistence. To include recent articles, we searched for further studies in PubMed (2015-17). We then tested the impact of the choice of the "case" and "control" definitions on AD prevalence estimates, and associated risk factors (including *filaggrin-FLG* mutations), among children aged 5 years in two UK birth cohort studies (MAAS and Ashford).

Results: We identified 65 different definitions of AD across 44 reviewed studies. Of those, we chose 6 most common "case" definitions, and 2 common definitions of "controls". The prevalence estimates from different operational definitions ranged between 5% and 32% in MAAS, and 2% and 27% in Ashford. The strength of the

association with *FLG* loss-of-function mutations differed substantially (OR [95% CI] 1.6 [0.9-2.7] - 2.8 [1.2-6.3]; and 1.7 [0.8-3.7]-3.4[0.9-13]), MAAS and Ashford respectively. The percentage of children whose posterior AD probability was in the area of clinical indecision ranged between 2% and 47% in MAAS, and 1% and 29% in Ashford. Using the same "case" definition, but different definition of "controls", the strength of the association between AD and *FLG* loss-of-function mutations differed considerably (RRR [95% CI] 2.3 [1.3-4] vs 1.7 [1.0-2.8]; and 2.2 [1.1-4.5] vs 1.9 [0.99-3.8], MAAS and Ashford respectively.

Conclusion: Use of different operational definitions of AD results in the substantial difference in prevalence estimates, the performance of prediction models, and the association with risk factors. Better uniform definitions are needed to avoid biases due to the definitions.

0193 | Usage of an air purifier to reduce dust and allergen exposure: Results of a pilot study

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Background: Exposure to airborne allergens is an important risk factor for allergic asthma and rhinitis. The aim of this study is to proof if the use of an air purifier (AP) is able to reduce indoor allergen exposure, e.g. exposure to domestic mite and pet allergens.

Method: In a living room, bed room and study of one household with pets, inhalable dust was collected during 1-2 hours of typical household chores on 15 days, either in the morning without or in the afternoon with usage of an AP run on maximal mode (Philips AC3256, The Netherlands). In addition to sampling with stationary and personally carried pumps with a flow rate of 10 l/min on Teflon filters, nasal filters (RhinixPro) were used. After weighing of Teflon filters, all samples (n = 90) were extracted and allergen content was quantified with fluorescence enzyme immunoassays for domestic mites (DM), the cat allergen Fel d 1, and dog allergen Can f 1. The results obtained during sampling with (+AP) or without air purifier (-AP) were log-transformed and compared with the paired t-test (GraphPad Prism 7). *P*-values below 0.05 were considered significant.

Results: Inhalable dust was reduced significantly by AP (median personal dust: 1.16 mg/m³ -AP, 0.76 mg/m³ +AP, median stationary dust: 0.63 mg/m³ -AP, 0.4 mg/m³ +AP). In most cases allergen exposure was also reduced; median allergen concentrations in the air were up to 60% lower with than without AP. However, allergen exposure varied greatly (DM: 1.4-238 ng/m³, Fel d 1: 0.2-65 ng/m³, Can f 1: 1.2-57 ng/m³), and with the exception of dog allergens collected with personally carried pumps, the results with or without AP did not differ significantly. Similar results were obtained by using nasal filters. Median allergen amounts on nasal filters were up to 60% lower with AP than without AP, but only DM values differed significantly.

Conclusion: Typical household chores involve high levels of exposure to indoor dust and allergens. By using an air purifier the inhalable dust concentrations could be reduced. However, although median allergen exposure decreased, the effect was in most cases not great enough to achieve statistical significance.

0194 | Increasing aeroallergen sensitisation: Evidence from three decades of population based studies

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Background: Objective measurements of allergic sensitisation are valuable when assessing time trends, as they are not affected by changes in awareness of allergic diseases. However, a limited number of studies have investigated prevalence of IgE sensitisation among adults over time. Thus, our aim was to investigate time trends in aeroallergen sensitisation in a general adult population.

Method: Three health examination studies of random samples of individuals aged 18–72 years resident in the Western part of the Copenhagen region were conducted in 1990–1991 (n = 567), 2006–2008 (n = 3443), and 2013–2015 (n = 7408). Aeroallergen sensitisation was defined as serum specific IgE $\geq$ 0.35 kU/l to at least one of the allergens; birch (*Betula verrucosa*), grass (*Phleum pratense*), house dust mite (*Dermatophagoides pteronyssinus*), or cat. We used logistic regression to analyse changes in aeroallergen sensitisation between the studies, adjusted for age, sex, and season of examination.

Results: In total, 11 418 individuals were included from the three studies. The prevalence of aeroallergen sensitisation was 26 % in 2013–2015 compared to 23 % and 16 % in 2006–2008 and 1990–1991, respectively. We found a statistically significant increase from 1990–1991 to 2006–2008 (odds ratio 1.91; 95 % CI 1.50–2.43) and from 1990–1991 to 2013-2015 (odds ratio 2.42; 95 % CI 1.91–3.06). Additionally, we found a statistically significant increase from 2006–2008 to 2013-2015 (odds ratio 1.27; 95 % CI 1.15–1.40).

Conclusion: We found that the prevalence of aeroallergen sensitisation increased in an adult Danish general population over three decades.

TUESDAY, 29 MAY 2018
OAS 34
MECHANISMS IN IN VITRO TESTS

0195 | Clinical diagnostic values of drug-specific IFN-gamma releasing cells measurement confirmed by drug provocation tests

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Background: Interferon (IFN)-γ enzyme-linked immunospot (ELISpot) assay is a novel in vitro test for the diagnosis of drug-induced non-immediate hypersensitivity. However, clinical diagnostic values of this test has not been established. The aim of our study was to evaluate the diagnostic values of IFN-γ ELISpot in patients with a history of drug-induced nonimmediate hypersensitivity.

Method: The measurement of drug-specific IFN-γ releasing cells by IFN-γ ELISpot was comparatively analysed with the results of drug provocation test and/or positive patch test with delayed reading, which were considered as confirmatory tests in 41 subjects diagnosed with drug-induced non-immediate hypersensitivity.

Results: Twenty (48.8%) had a history of severe cutaneous adverse reaction including drug reaction with eosinophilia and systemic symptoms, Stevens-Johnson syndrome /toxic epidermal necrolysis, and acute generalised exanthematous pustulosis. The remaining 21 patients had a history of non-severe cutaneous adverse reactions including fixed drug eruption (FDE), maculopapular eruption, and late-onset angioedema. The most common culprit drugs in this study were antibiotics (78.0%), followed by antiepileptics, chemotherapeutic agents, allopurinol, nonsteroidal anti-inflammatory drugs, and miscellaneous drugs. One-fifth (8/41) of patients had positive IFN-γ ELISpot tests based on the cut-off value of 20 spot-forming units per one million PBMCs. All patients with positive IFNγ ELISpot results had either positive drug provocation test or positive patch test. Among 33 patients with negative IFN-γ ELISpot results, six patients later developed positive drug provocation test. Half of patients with false negative IFN-γ ELISpot results had FDE phenotype. The calculated positive and negative predictive values of IFN-γ ELISpot test in this study were 100.0% and 81.8%, respectively.

Conclusion: The result of our study indicated that IFN-γ ELISpot test had a good negative predictive value and an excellent positive predictive value to confirm the diagnosis of drug-induced non-immediate hypersensitivity. However, the capability of this test to confirm the diagnosis of drug-induced FDE is still limited.

0196 | Polyethylene glycol (PEG), an allergen not to be missed

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Background: Polyethylene glycol (PEG) consists of a family of polymers (MW 200 - >20.000) which are used as excipients in medicines. The evidence, that PEG can act as an allergen has only recently emerged and its mechanism of action is still under investigation.

Method: We present a cohort of 11 patients (7 female, 4 male), aged 22 - 87, who were referred to allergy clinics having suffered severe allergic reactions to medications. 9 patients had suffered anaphylaxis, 2 patients generalized urticaria and angioedema.

4 patients reported a reaction to macrogol laxatives (PEG 3350), one of the patients had also reacted to the depot contraceptive medroxyprogesterone injection (PEG 3350). 3 patients to sodium alginate (PEG 20.000), 1 patient to the depot contraceptive medroxyprogesterone injection (PEG 3350) and 3 patients to depot methylprednisolone injection (PEG 3350); one of those patients had also suffered milder symptoms to the anti malarial atovaquone/proguanil (PEG 8000).

Results: Allergy was confirmed by positive skin prick test (SPT) to either the drug itself or PEG (MW 3350 or 20.000) in 5 patients; with positive intradermal test (IDT) to PEG 20.000 in 1 patient and positive challenge to the index drug in 5 patients. Macrogol 3350 was the index drug in 2 patients challenged, depot methylprednisolone in 2 patients, one of whom also had a positive macrogol 3350 challenge.

2 patients with anaphylaxis after sodium alginate and depot medroxyprogesterone but negative skin tests, refused challenges.

3 patients suffered reactions after PEG skin testing.

Conclusion: PEG is poorly absorbed, but exposure to high doses (13 g) of PEG 3350 given as laxatives are the most frequently reported cause of allergic reactions to PEG. When the same MW is given parenteral, a much smaller dose (29 - 87 mg) elicits severe reactions. High MW (20.000) PEG given orally causes reactions despite the small dose ingested (estimated 50 mg). The combination of MW, dose administered and route of administration therefore seem critical in triggering a systemic reaction. This might explain the limited number of drugs that seem implicated in PEG allergy.

We confirm the observation made in the review of E. Wenande and L. Garve (Clinical & Experimental Allergy,46, 907-922, 2016) that skin testing carries the risk of a systemic reaction. Currently neither a commercial test nor a standardised protocol are available. This poses difficulties for investigation of suspected PEG allergy and may cause the diagnosis to be missed.

0197 | Basophil activation test in the diagnostic algorithm of immediate reactions to iodinated contrast media: A pilot study

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Background: Iodinated contrast media (ICM) can cause hypersensitivity reactions (HSR) ranging from mild to life-threatening. Basophil activation tests (BAT) has been proposed as a useful and safe in vitro diagnostic method in immediate-type allergy reactions. The aim of this study was to evaluate the utility and safety of BAT in the diagnosis of HSR to ICM.

Method: Adult patients with a history of HSR to ICM referred to the Allergy Department of Hospital La Paz between 2012 to 2017, and who underwent skin tests (prick and/or intradermal) and BAT were included. Skin tests results were considered as the gold standard. When results were positive, controlled challenge tests (CCT) with an alternative ICM were performed. We considered severe HSR when skin, respiratory or gastrointestinal symptoms and/or anaphylaxis were observed.

Results: A total of 48 patients were included, 25 were males, mean age 53.08 ± 17 years. Iomeprol was the most used ICM (32 patients). Cutaneous symptoms were the most common (96%). Seventeen patients had a history of severe HSR. We performed 603 skin tests with different ICMs (308 prick and 295 intradermal tests), and 36 of them were positive (6 prick and 30 intradermal tests). Four patients had positive skin test to 2 ICMs (iomeprol and iohexol). A total of 194 BATs were done, and 10 positive results were observed. We found that 4 patients had a positive BAT with 2 ICMs (3 with iomeprol and iohexol and 1 with iodixanol and ioversol). All patients with positive BAT had received ICM several times before (mean 6 ± 3). Two patients with severe HSR had negative BAT and underwent skin test by intradermal dilution (1/10), showing a positive skin test and no adverse reactions. BAT showed a sensitivity of 40%, specificity and positive predictive value (PPV) of 100%, and negative predictive value (NPV) 73%. In severe HSR BAT proved a higher sensitivity (60%) and NPV (75%), and same specificity and VPP. Twenty-three patients underwent CCT with an alternative ICM based in negative skin tests and BAT and showed no severe adverse reactions.

Conclusion: BAT is a helpful diagnosis tool in HSR with ICM especially in severe HSR, allowing to perform the allergy work-up with safe alternatives. We propose a diagnostic algorithm in severe HSR with ICM: First BAT, then skin test according to previous results, and if positive, then CCT with an alternative.

0198 | Vemurafenib acts as an aryl hydrocarbon receptor antagonist

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Background: In recent years, the BRAF-inhibitor vemurafenib has been successfully established in the therapy of advanced melanoma. Despite its superior efficacy, the use of vemurafenib is limited by frequent inflammatory cutaneous adverse events that affect patients' quality of life and may lead to dose reduction or even cessation of anti-tumor therapy. To date, the molecular and cellular mechanisms of vemurafenib-induced rashes have remained largely elusive.

Method: In this study we deployed immunohistochemistry, RT-qPCR, flow cytometry, lymphocyte activation tests and different cell-free protein-interaction assays.

Results: We here demonstrate that vemurafenib inhibits the downstream signaling of the canonical pathway of aryl hydrocarbon receptor (AhR) *in vitro*, thereby inducing the expression of proinflammatory cytokines (e.g. *IL1B*, *TNF*) and chemokines (e.g. *CCL5*). In line with these results we observed an impaired expression of AhR regulated genes (e.g. *CYP1A1*) and an upregulation of the corresponding proinflammatory genes *in vivo*. Moreover, results of lymphocyte activation tests showed the absence of drug-specific T cells in respective patients.

Conclusion: Taken together, we obtained no hint of an underlying sensitization against vemurafenib but found evidence suggesting that vemurafenib enhances proinflammatory responses by inhibition of AhR signaling. Our findings contribute to our understanding of the central role of the AhR in skin inflammation and may point towards a potential role for topical AhR agonists in supportive cancer care.

0199 | Regulation of ikzf1 inducible genes in epidermis

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Background: Our previous genome-wide association study documented an association between cold medicine related Stevens-Johnson syndrome / toxic epidermal necrolysis (CM-SJS/TEN) and Ikaros Family Zinc Finger 1 (IKZF1). We also reported that human epidermis and conjunctival epithelium expressed IKZF1, and in primary human conjunctival epithelial cells and adult human epidermal keratinocytes, the expression of IKZF1 mRNA was up-regulated by stimulation with polyI:C, a TLR3 ligand, 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 found that Ikzf1 Tg mice have dermatitis and mucosal inflammation including the ocular surface. In this study, we investigated the regulation of the Ikzf1 inducible genes by TLR3 and IPS-1, which are receptors for polyI:C.

Method: Skin was collected from the mice which are within 1 week after their birth, and epidermis was harvested from the skin using dispase. We compared the gene expression of the epidermis between Ikzf1 Tg mice and WT mice using microarray. Moreover, using TLR3KO, Ikzf1Tg TLR3KO, IPS-1KO, and Ikzf1Tg IPS-1KO mice, we investigated the regulation of Ikzf1 inducible genes by TLR3 and IPS-1 with quantitative RT-PCR.

Results: Microarray analysis showed that Lcn2, Adh7, Epgn, Ifi202b, Cdo1, Gpr37, Duoxa1, Tnfrsf4, and Enpp5 genes were significantly up-regulated in the epidermis of Ikzf1 Tg compared with wild-type. *Ikzf1* gene expression *in the* Ikzf1 Tg was significantly downregulated in Ikzf1Tg TLR3KO mice. Ifi202b, Gpr37 and Tnfrsf4-gene expression *in the* Ikzf1 Tg were significantly down-regulated in both Ikzf1Tg TLR3KO and Ikzf1Tg IPS-1KO mice. Adh7 gene expression *in the* Ikzf1 Tg was significantly up-regulated in Ikzf1Tg TLR3KO but not in Ikzf1Tg IPS-1KO mice.

Conclusion: These findings suggest that *Ikzf1 inducible* genes could be partially controlled by TLR3 and IPS-1.

0200 | Timing of lymphocyte transformation test in children with amoxicillin clavulanic acid confirmed hypersensitivity

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Background: Lymphocyte transformation test (LTT) detects a memory T-cell response in case of delayed reactions to drugs. LTT sensitivity has been reported in the range of 39.9%-65.1% (mean value of 56.1%). At present, the persistence of drug-specific T-cell response over the time cannot be predicted. Consequently it is not known if the timing may influence the LTT sensitivity.

Aim: To detect the persistence of LTT reactivity after the reported reaction in children with amoxicillin clavulanic acid (AMX-CLV) confirmed hypersensitivity.

Method: From 2014 to 2018, children with history of AMX-CLV reactions underwent a complete drug allergy work-up according to the European Network of Drug Allergy (ENDA) guidelines.

In previous mild/moderate reactions LTT and in vivo tests (skin prick test and intradermal tests with AMX-CLV) were performed at first followed by drug provocation test (DPT) with the culprit drug only in case of skin tests negativity. In case of severe reactions or positive in vivo tests the DPT was not performed.

In vitro lymphocyte proliferative response towards AMX and AMX-CLV was detected by LTT measuring the radioactivity of tritiated thymidine (^{3}H) incorporation into the genome vs unstimulated cultures (Stimulation Index >3).

Results: Forty children (20 males and 20 females; mean age 8 years) were studied: 5 out of 40 (12.5%) had history of Steven Johnson syndrome (SJS); 1 out of 40 (2.5%) had history of drug reaction with eosinophilia and systemic symptoms (DRESS) and 3 out of 40 (7.5%) had a long lasting maculopapular exanthema (MPE). Twenty-seven out of 40 (67.5%) had a positive DPT; 4 out of 40 (10%) had positive in vivo tests. Twenty out of 40 (50%) showed positive LTT towards AMX and/or AMX-CLV (group A), the remaining 20 children were negative (group B).

Group A and group B were LTT re-tested after 2.47 ± 1.87 and 2.96 ± 2.92 years, after the reported reaction, respectively, with no significant differences in comparison with the previous test (P = 0.5). Eight out of 20 patients (40%) with positive LTT were further retested after 3.12 ± 1.3 years after the reaction and still exhibited a positive LTT.

Conclusion: According to our results and in agreement with the literature, sensitivity of LTT is of 50% in case of delayed reactions to AMX-CLV and the timing of the adverse reaction when assessed within 2-3 years does not influence it. More patients for a more prolonged time need to be investigated to observe the persistence of T cell specific reactivity.

WEDNESDAY, 30 MAY 2018
OAS 35
PSYCHOLOGICAL IMPACT OF ALLERGIES

0201 | Do the allergic children's parents need psychological support?

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Background: Allergic diseases can affect children's and their parent's social life and school life as well as health related impairments. In this study, we aimed to investigate the parents' view to allergic diseases (atopic dermatitis, allergic rhinitis, asthma, food allergy, drug allergy) in Turkish population and how their children's diseases affect their life.

Method: The study includes one hundred and two children, who were followed by our allergy clinic team for more than 6 months. Children's parents (either mother or father) filled a questionnaire form. This form consisted of demographic questions and 20 multiple-choice questions aimed at understanding parents' view to allergic diseases and how their children's diseases affect their life.

Results: Most of the parents (40%) reported that their children had an allergic disease because of a weak habit of body and 34% thought it was genetic. Six percent of the patients said that their quality of life wasn't affected from their children's allergic disease whereas 16% was highly affected. Parents who had a child with more than one allergic disease were significantly affected. Symptoms in children, and allergen avoidance affected families 42%, 32%, respectively. Half of the parents (48%) had work absenteeism. Forty-six percent of the parent's expectation from their physicians was to cure the disease.

Conclusion: Parents who have a child with more than one allergic disease are significantly more affected and they must have a psychological support.

0202 | Evaluation of quality of life in children aged between 2-7 years with atopic dermatitis

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Background: Atopic dermatitis (AD) affects quality of life (QoL) of the patients and their families by both the symptoms and associated secondary infections. The aim of this study was to evaluate the quality of life in 2-7 year old children with atopic dermatitis and their families and to investigate the relationship between disease severity and the QoL of patients and their families.

Method: The study group included 83 children with AD and 83 normal children aged between 2 and 7 years old and their families. The Turkish version of the Pediatric Quality of Life Inventory (PedsQL) were applied to the parents of the children with AD and the control group according to the age group of the children. The Dermatological Diseases Family Impact Scale (DeFIS) was administered to the same parents. The Patient Oriented Severity Scoring of Atopic Dermatitis (PO-SCORAD) was used to determine disease severity.

Results: While there was no significant relationship between PO-SCORAD index and PedsQL's scores in 2 - 4 years ($P > 0.05$), a negative correlation was found only between PO-SCORAD index and PedsQL's physical scores in 5-7 years ($r = -0.380$ $P = 0.022$). When the PO-SCORAD index scores of the patients were compared with the quality of life of their families, there was a positive correlation only in the 5-7 age group ($r = 0.424$, $P = 0.011$). The PedsQL total scores of children with AD were not significantly different when compared with being atopy or not. The patient's families in the 5-7 age group had worse quality of life than those who were not atopic ($P = 0.034$). In the presence of concomitant allergic disease, the quality of life of the children with AD was not affected. The quality of life of the families of patients with additional allergic diseases in the 2-4 year old group was worse than those without the additional allergic disease ($P = 0.007$). As the quality of life of children with AD worsened, the quality of life of their families deteriorated in both age groups ($r = -0.607$, $P = 0.000$, $r = -0.571$, $P = 0.000$, respectively).

Conclusion: Atopic dermatitis, especially with atopy affects the QoL of children aged 5-7 years and their families. It is important that the QoL questionnaire can be used as complementary instrument for management of atopic dermatitis.

0203 | Effect of intranasal phototherapy on quality-of-life of patients with allergic rhinitis

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Background: Allergic rhinitis affects significantly the quality of patient's life. Recently, it was proven that intranasal phototherapy has a beneficial effect on immunological processes in the nose. The aim of our study was to investigate the effect of intranasal phototherapy on quality-of-life of patients with persistent allergic rhinitis.

Method: A prospective study of patients with confirmed persistent moderate-severe allergic rhinitis was carried-out. Allergic sensitization was evaluated by skin prick and/or measurement of specific IgE

to perennial aeroallergens. Intranasal phototherapy, which directs a combination of UV-A (25%), UV-B (5%) and visible light (70%), was applied three times a week for two weeks continuously. All subjects had to avoid any topical nasal steroid or any antihistamine at least 4 weeks before and during the study. Assessment with rhinoconjunctivitis quality of life questionnaire (RQLQ), total nasal symptom score (TNSS), visual analogue scale (VAS) was performed before and after the treatment.

Results: Quality-of-life of 15 studied subjects (mean age: 30.9 ± 11.7 years) improved significantly after the treatment with intranasal phototherapy (sleep: 2.37 ± 1.93 vs 1.15 ± 0.72, $P = 0.023$; activity: 2.89 ± 1.38 vs 1.31 ± 0.73, $P = 0.003$; nasal symptoms: 2.78 ± 1.54 vs 1.38 ± 0.92, $P = 0.013$; eye symptoms: 1.98 ± 1.80 vs 0.88 ± 0.91, $P = 0.005$; other symptoms: 1.99 ± 1.25 vs 1.16 ± 1.04, $P = 0.010$; practical problems: 3.62 ± 1.91 vs 1.84 ± 1.32, $P = 0.014$; emotions: 2.03 ± 1.45 vs 1.16 ± 1.18, $P = 0.047$). The treatment reduced all symptoms evaluated by TNNS: nasal congestion (2.00 ± 1.00 vs 1.13 ± 0.64; $P = 0.015$), rhinorrhea (1.87 ± 0.99 vs 0.94 ± 0.80; $P = 0.013$), sneezing (1.53 ± 1.06 vs 0.67 ± 0.61; $P = 0.026$), difficulty to sleep (1.27 ± 1.01 vs 0.20 ± 0.56; $P = 0.080$), and total score (8.67 ± 4.01 vs 4.07 ± 2.12; $P = 0.020$). VAS was also changed significantly after the treatment (4.00 ± 1.51 vs 1.60 ± 1.24; $P = 0.003$).

Conclusion: The present data suggest that intranasal phototherapy may relieve nasal symptoms and improve quality-of-life of patients with persistent moderate-severe allergic rhinitis.

0204 | APPEAL (allergy to peanuts impacting emotions and life): Results on the impact of peanut allergy on allergic individuals, parents and caregivers

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Background: Peanut allergy (PA) is usually lifelong and it is known to affect quality of life (QoL) of individuals and parents/caregivers. APPEAL is the first European multi-country survey to evaluate psychosocial burden and PA management in (1) adults with PA; (2) children with PA assessed by parents/caregivers; (3) parents/caregivers of a PA child reporting on their own behalf.

Method: Part one of APPEAL was a quantitative online 30-minute survey, developed by an expert panel and conducted in eight European countries (Denmark, France, Germany, Ireland, Italy, Netherlands, Spain, UK). APPEAL covers clinical and psychosocial variables, including management of PA. Participants were recruited through Patient Advocacy Groups and a specialist recruitment panel.

Results: In total, N = 1846 responses: 419 adults with PA [self-report] and 881 parents/carers of a PA child, who answered for themselves [self-report]; and 546 parents/carers who also answered for the PA child [parent/carer proxy]. The majority of participants (63.8%) were recruited through Patient Advocacy Groups. 81% self-reporting and 44% proxy-reporting were female. Other food allergies were common (72% reported other food allergies) as were asthma (43%), allergic rhinitis (41%) and skin disorders (39%). 47% were diagnosed after the age of four. The survey indicates that PA impacts daily living in >80% of participants, with 40% of participants stating that they live with a high level of uncertainty due to their PA. High levels of frustration, stress and anxiety due to PA were reported (Table 1). Due to PA, 77% of all participants report being made to feel different in a negative way and 43% report bullying on at least one occasion. Almost 18% were not at all reassured by the advice given at first diagnosis. Differences were found across participant groups, age, and country.

Conclusion: This is the first multi-country survey to evaluate the impact of living with PA from multiple perspectives and across multiple European countries. PA has significant negative effects on activities of daily living and leads to high levels of stress, anxiety and frustration.

Table 1 Percentage of participants reporting extremely or very high levels of frustration, stress or anxiety

Participant Group	Frustration	Stress	Anxiety[a]	Anxiety[b]
Adults with PA [self-report]	37	31	56	12
Children with PA [parent/carer proxy]	37	33	60	15
Adult caregivers of a PA child [self-report]	39	48	71	11

[a]Social occasions associated with food.
[b]Social occasions with no food.

0205 | Quality-of-life in Portuguese hymenoptera venom allergic patients on venom immunotherapy

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Background: An anaphylactic reaction to hymenoptera venom is an acute event with negative effects on emotional, social, and

professional functioning. Venom immunotherapy (VIT) has an established efficacy in the prevention of anaphylaxis after stings. However, even those individuals that are on VIT still present psychological impact of a threatening systemic reaction. The aim of the study was to evaluate the Health-Related Quality of Life (HRQoL) of hymenoptera venom allergic patients being on or after finished the treatment with VIT.

Method: Patients over the age of 17 years, with or after finished wasp, bee or *Polistes* VIT were included. We excluded beekeepers and their relatives. The study was carried out in the period of insect flight, using the translated and validated Portuguese version of The Vespid Allergy Quality of Life Questionnaire (VQLQ-P). The VQLQ-P has 14 questions, classified from 1 to 7 (1 the lowest and 7 the highest quality of life score). Statistical analysis was performed with SPSS version 24.0 using student t test, ANOVA and Pearson coefficient.

Results: 38 patients answered the questionnaire (31 men), of whom 19, 12 and 7 presented allergy to wasp, bee and *Polistes*, respectively. The mean score of VQLQ-P was 4.06. We report stronger impact in HRQoL in women vs men ($P = 0.049$). There were no significant differences in HRQoL between the different groups of hymenoptera, the grades of reaction to the hymenoptera sting or being on venom-specific immunotherapy (33 patients). No significant correlation was found with age and duration of treatment. Carrying adrenaline auto-injectors (all patients had a prescription) had a significant impairment of HRQoL ($P = 0.003$).

Conclusion: Systemic reaction to hymenoptera venom has a significant impact on patient HRQoL, even after VIT, which demonstrates a lasting psychological impact of a potentially life-threatening systemic reaction, that validate the importance of the follow-up. As described in the some literature, quality of life is worse in women and in those what carrying adrenaline auto-injectors. This study applies VQLQ-P for the first time in patients with *Polistes* allergy in Portugal, with no difference between wasp, bee and *Polistes* allergic patients.