Epithelial science congress highlights: The European Respiratory Society (ERS) International Congress

4–6 September 2022 | Barcelona, Spain

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UNDERSTANDING THE CENTRAL ROLE OF THE EPITHELIUM IN SEVERE ASTHMA AND BEYOND

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Contents

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Aims

- These slides cover **congress highlights** from abstracts that were presented at ERS 2022
- The abstracts were carefully selected to include data that further the understanding of **epithelial science**; this is not an exhaustive list of all abstracts

Permissions

- Authors of each abstract were notified that their data will be included in this report
- Please note that **the key takeaways** are not corroborated by these authors, but **were developed** based on the data presented within the abstracts **for the purposes of this report**

Conference details

- Please note that this report was **developed specifically for EpiCentral** and is independent of the congress
- The ERS Hybrid Congress was held on 4–6 July 2022 in Barcelona, Spain

Report sections

ERS, European Respiratory Society

Key takeaways

- The airway epithelial cell landscape is altered in severe asthma; for example, there are increased levels of secretory cells in patients with T2 biology compared with non-T2 biology.[1] Moreover, in paediatric patients, ILC2 presence was associated with T2 inflammation and later development of atopic asthma[2]
- In allergic asthma, higher levels of alarmin-expressing cells in the lower airway compared with upper airways may reflect a heightened sensitivity to stimuli in the lungs,[3] and exposure to the common allergen HDM can cause release of epithelial cytokines[4]
- Bronchial thermoplasty has been shown to reduce alarmins S100A7/A8/A9, which otherwise are associated with poor asthma control in patients with severe asthma[5]
- Markers of airway remodelling may correlate with severity of disease in patients with asthma; in particular, sputum *CTSK* expression may be a useful marker of airway remodelling in patients with asthma[6]

CTSK, cathepsin K; HDM, house dust mite; IL, interleukin; ILC2, type 2 innate lymphoid cell; T2, type 2; TSLP, thymic stromal lymphopoietin

1. Shi Y, et al. Poster PA351 presented at ERS 2022; 2. Bonato M, et al. Poster PA2814 presented at ERS 2022; 3. Whetstone CE, et al. Poster PA933 presented at ERS 2022; 4. Ramu S, et al. Poster PA3226 presented at ERS 2022; 5. Gagnon PA, et al. Poster PA3583 presented at ERS 2022; 6. Qin L, et al. Poster PA916 presented at ERS 2022

Role of the epithelium in asthma

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A primary ciliary dysfunction might be present in severe asthma

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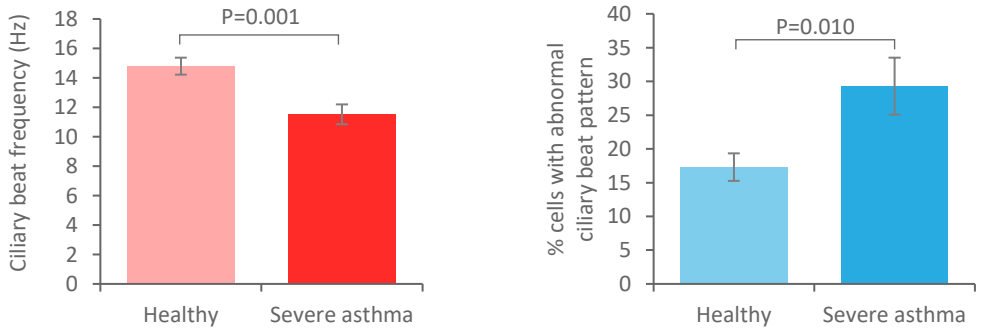
- To determine whether the ciliary dyskinesia of bronchial epithelial cells of patients with asthma is primary or secondary to chronic inflammation, ciliary function analysis was performed on respiratory ciliated epithelium using an in-vitro, air-liquid interface cell-culture model
- The ciliary beat frequency and beat pattern of cells from nasal brushings of healthy adults (n=14) and patients with severe asthma (n=10) were analysed using digital high speed videomicroscopy

Kempeneers C
University Hospital of Liège, Liège, Belgium

- As has been previously shown, ciliary dyskinesis was increased in patients with severe asthma compared with healthy controls

Ciliary function in respiratory epithelial cells from healthy adults and those with severe asthma



- Ciliary dyskinesis persisted in epithelial cells from patients with severe asthma (n=4) after cell culture; there was no significant difference before and after culture in ciliary beat frequency (11.4±2.9 vs 12.9±1.8; P=0.269) or proportion of cells with an abnormal beat pattern (32.7±15.8% vs 24.4±13.2%; P=0.378)

Key takeaways: *Impaired ciliary function of bronchial epithelium may contribute to impaired mucociliary clearance in patients with severe asthma, and this ciliary dyskinesis may be primary to chronic inflammation*

CB Kempeneers C, Bricmont N, Bonhiver R, Pirotte M, Guissard F, Moermans C, Seghaye MC, Louis R, Schleich F. Abstract presented at ERS 2022

Nasal airway epithelium differentiates between asthma and healthy subjects and identifies clinically relevant asthma endotypes

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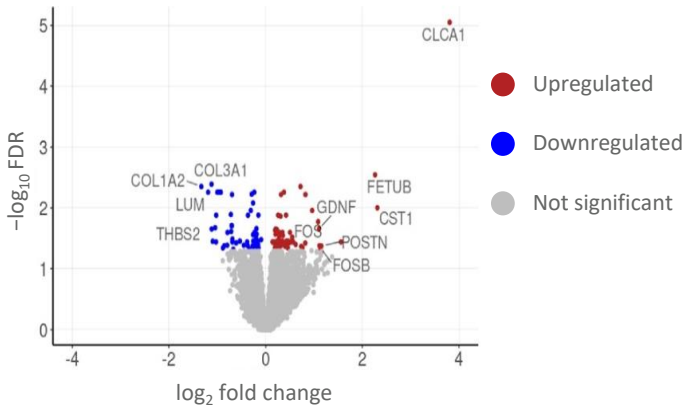
- Differential gene expression analysis of nasal brushings from patients with asthma (n=361) and healthy controls (n=57) was performed, to establish whether the nasal epithelium reflects lower airway changes in asthma

Karp T
University of Groningen, Groningen, The Netherlands

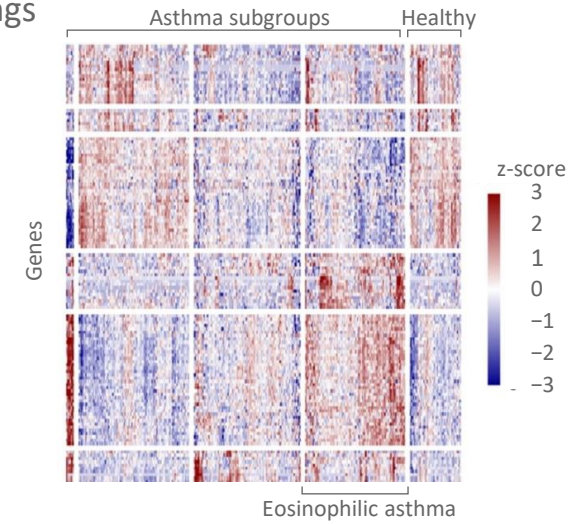
- Nasal brushings from patients with asthma revealed 67 genes that were upregulated and 59 genes that were downregulated (FDR <0.05) versus healthy controls; of these, several known asthma-associated genes were found to be upregulated, including *CLCA1*, *POSTN* and *CST1*

Differential expression analysis from patients with asthma versus healthy controls



- Hierarchical clustering identified known asthma endotypes, including eosinophilic and non-eosinophilic, and the nasal asthma-associated gene signature was replicated in independent cohorts of nasal and bronchial epithelial brushings

Heatmap representation of normalised level of differentially expressed genes in patients with asthma and healthy controls



Key takeaways: *Changes to the lower airway epithelium in patients with asthma are reflected in the upper airways, and gene expression is reflective of asthma endotypes based on clinical characteristics*

CLCA1, chloride channel accessory 1; CST1, cystatin SN; FDR, false discovery rate; POSTN, periostin

Karp T, Faiz A, Kerstjens HAM, Boudewijn MI, Kraft M, Vonk JM, Nawijn MC, Heijink HI, Fabbri LM, Rabe KF, Nicolini G, Papi A, Brightling C, Singh D, Van Der Molen T, Siddiqui S, Christenson S, Guryev V, Van Den Berge M. Poster PA3562 presented at ERS 2022

Altered airway epithelial cell landscape in U-BIOPRED severe asthma

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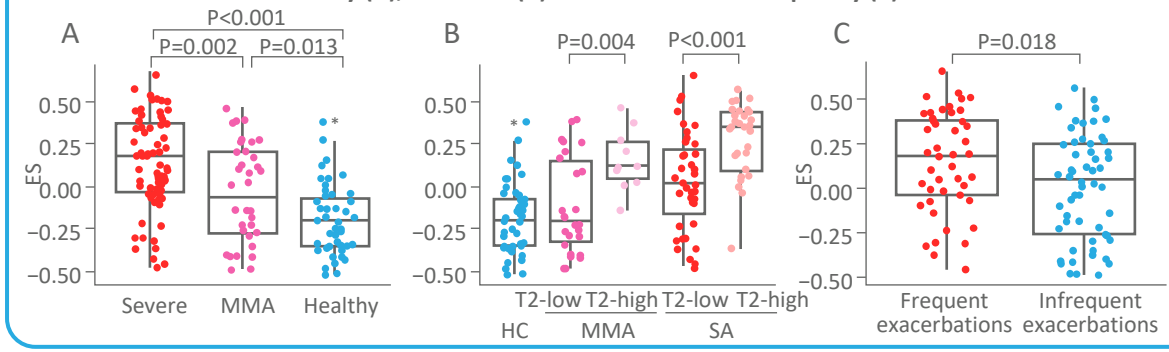
- Single cell signatures of bronchial brushings from patients with severe asthma (n=49), mild-to-moderate asthma (n=36), smokers with severe asthma (n=18) and healthy non-smoking controls (n=44) were analysed using cellular deconvolution and gene set variation analysis of RNA sequencing and microarray data

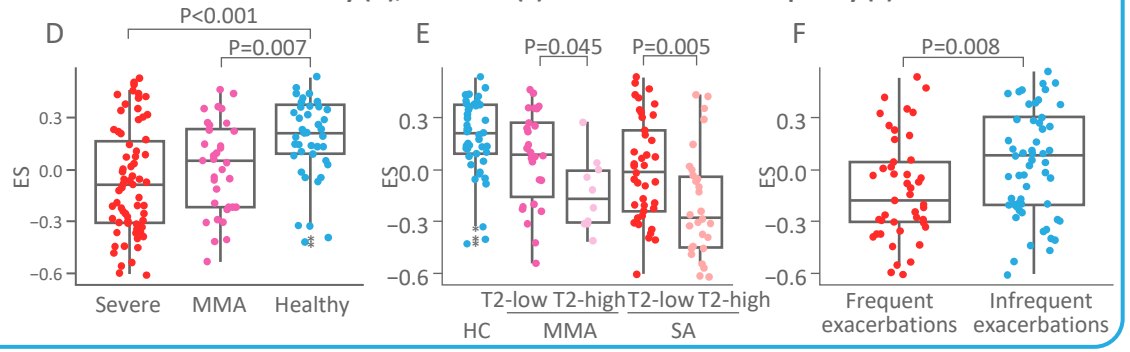
Shi Y
The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

- Cellular deconvolution identified high relative proportions of mucociliary lineage cells in patients with severe asthma versus healthy controls
- Enrichment scores showed that asthma severity, T2-high status, frequent exacerbations, permanent airflow limitation and current smoking were associated with higher secretory cell signatures and lower ciliated cell signatures
- A higher ionocyte enrichment score was associated with asthma severity, permanent airflow limitation and current smoking

Association between enrichment score of secretory cell signatures and asthma severity (A), T2 status (B) and exacerbation frequency (C)



Association between enrichment score of ciliated cell signatures and asthma severity (D), T2 status (E) and exacerbation frequency (F)



<u>Key takeaways:</u> *The airway epithelial cell landscape reflects asthma severity, and is modified by T2 biology and smoking*

ES, enrichment score; GSVA, gene set variation analysis; HC, healthy controls; MMA, mild-to-moderate asthma; SA, severe asthma; T2, type 2

Shi Y, Vidova L, Zounemat-Kermani N, Faiz A, Chung KF, Hansbro PM, Yao X, Van Den Berge M, Nawijn M, Adcock IM. Poster PA351 presented at ERS 2022

ILC2s in the transition from paediatric wheezing to asthma

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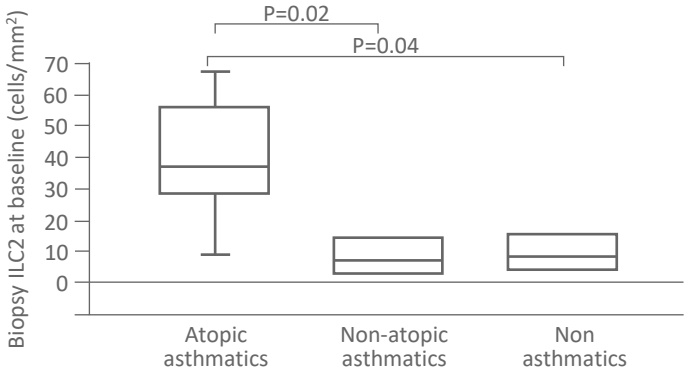
- To investigate ILC2 and T2 inflammation mechanisms involved in the development of asthma, bronchial biopsies from children undergoing clinically indicated bronchoscopy (n=61 in children with wheeze, n=48 in children without wheeze) were studied,* and patients were followed prospectively for a median of 5 years

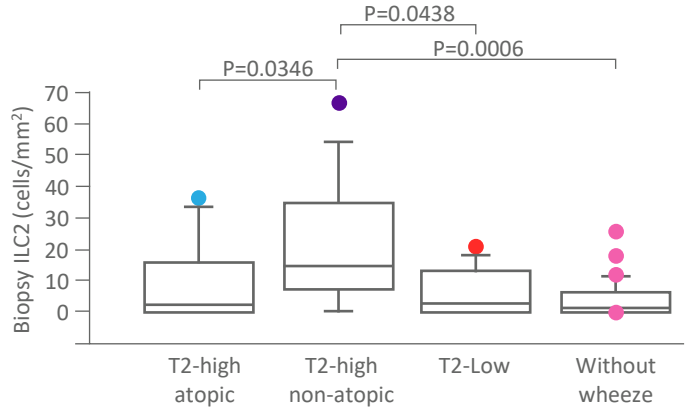
Bonato M
University of Padova, and Ospedale Ca' Foncello, Treviso, Italy

- Levels of ILC2 were higher in preschool children than school-age children (P=0.02) and in non-atopic compared with atopic children (P=0.03)†; levels of ILC2 also correlated with IL-5+ cells (r=0.64; P=0.02) and biopsy eosinophils (r=0.27; P=0.03), but not with IL-4+ cells, blood eosinophils, serum IgE or remodelling
- ILC2 levels were higher in children who developed atopic asthma compared with those without atopy† (P=0.02) or without asthma (P=0.04)

Bronchial biopsy ILC2 number in T2-high non-atopic children with wheeze, by asthma status at follow up†‡



Bronchial biopsy ILC2 number in children with and without wheeze, by T2 and atopy status†§



Key takeaways: *ILC2 may drive T2 inflammation in early life and be associated with non-atopy; ILC2 may also promote development of atopic asthma later in life*

*Paraffin-embedded biopsies were stained with haematoxylin and eosin to quantify epithelial loss and basement membrane thickness, and inflammatory cells, ILCs, eosinophils, IL-4 expression and IL-5 expression were quantified via immunohistochemical techniques;
†atopy status defined by total IgE higher than age-related normal levels and specific IgE >0.35 kU/L; ‡T2-high status at follow-up defined by FeNO >23 ppb; §T2-high status defined by biopsy eosinophils >23 cells/mm² and T2-low status by biopsy eosinophils ≤23 cells/mm²
FeNO; fractional exhaled nitric oxide; IgE, immunoglobulin E; IL, interleukin; ILC2, type 2 innate lymphoid cell; ppb, parts per billion; T2, type 2

Bonato M, Bazzan E, Maes T, Brusselle G, Turato G, Padrin Y, Turrin M, Cosio MG, Saetta M, Baraldo S. Poster PA2814 presented at ERS 2022

Epithelial cytokines and the inflammatory cascade

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Alarmin release in the bronchial epithelium of allergic asthma patients varies in response to different aeroallergens

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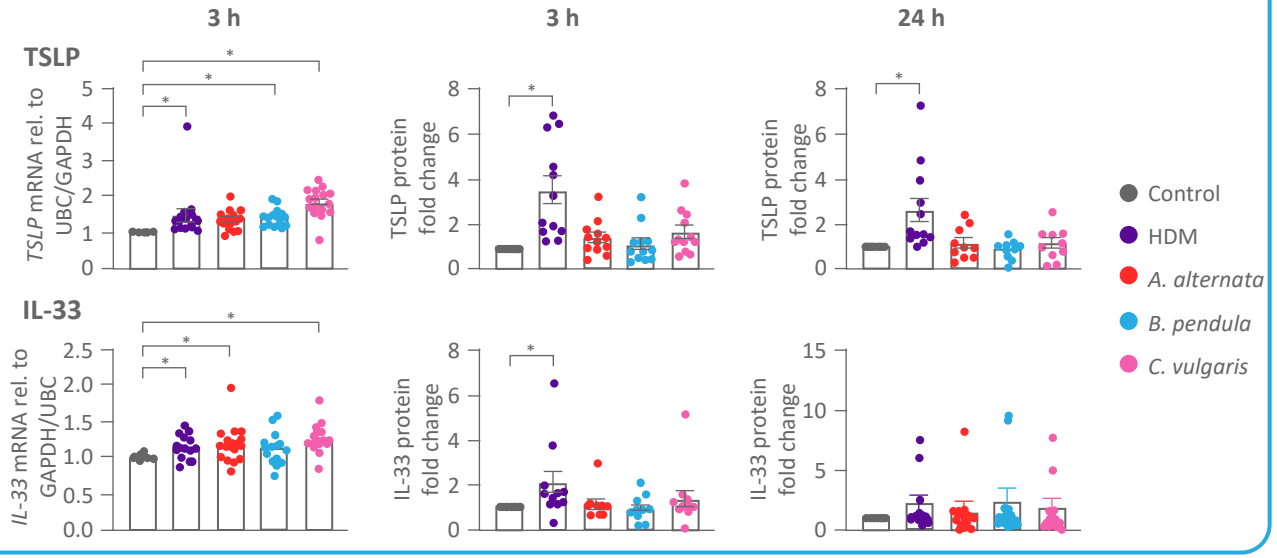
- The expression and release of alarmins TSLP and IL-33, as well as release of metabolite danger-associated molecular patterns, ATP and uric acid, following exposure of human bronchial epithelial cells from patients with allergic asthma (n=16) to allergens (HDM, *A. alternata, A. vulgaris* and *B. pendula*) was investigated†

Ramu S
Lund University, Lund, Sweden

- ATP levels in cell-free supernatants were raised 1 h after exposure of bronchial epithelial cells to *B. pendula* (P<0.05); there was no significant change for other allergens
- Both HDM (P<0.05) and *A. vulgaris* (P<0.05) induced uric acid release from bronchial epithelial cells, 24 h after exposure
- Only HDM was found to induce TSLP release at 3 h (mRNA: P<0.05; protein: P<0.05) and 24 h (protein: P<0.05) after exposure; a *TSLP* mRNA response to *B. pendula* and *A. vulgaris* was noted after 3 h of exposure (P<0.05)
- HDM also induced IL-33 release after 3 h; increased *IL-33* mRNA was seen 3 h after exposure to *A. alternata* and *A. vulgaris*

Alarmin gene and protein release from allergic asthma human bronchial epithelial cells in response to four allergens‡



Key takeaways: *Environmental triggers have differential effects on epithelial cells, and exposure to the common allergen HDM can cause release of epithelial cytokines*

*P<0.05; †Protein levels were measured by ELISA, mRNA expression was analysed using RT-qPCR and ATP and UA release was measured by biochemical assay; ‡data presented as mean ± SE, n=12
ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; h, hours; HDM, house dust mite; IL, interleukin; mRNA, messenger RNA; ns, not significant; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SE, standard error; TSLP, thymic stromal lymphopoietin; UBC, ubiquitin C

Ramu S, Woehlk C, Nieto-Fontarigo JJ, Cerpes S, Vazquez Mera S, Menzel M, Akbharshahi H, Porsbjerg C, Uller L. Poster PA3226 presented at ERS 2022

FD exposure decreases TNF-α/IFN-γ-mediated IL-33 expression in primary bronchial epithelial cells in part by interfering with IFNGR-JAK-STAT1 signalling

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- Early-life exposure to traditional farming is associated with protection against allergies and asthma
- To determine whether FD imparts a protective effect on epithelial barrier function, the induction of IL-33 was examined in primary bronchial epithelial cells following exposure to FD extract

Schrumpf JA
Leiden University Medical Center, Leiden, the Netherlands

- FD extract suppressed the TNF-α/IFN-γ-induced expression of IL-33 by bronchial epithelial cells
- Exposure to FD partly reduced phosphorylation of STAT-1 following IFN-γ-induction

Effect of pre-treatment with either FD extract or inhibitors of IFN-γ signalling pathways on IFN-γ-induced *IL33* mRNA expression and STAT1 phosphorylation in submerged primary bronchial epithelial cells

Pre-treatment	FD extract	JAK inhibitor	p38 MAPK inhibitor	EGFR inhibitor	NF-κB inhibitor
Induction of *IL33* mRNA expression by IFN-γ	↓ Reduced*	↓ Reduced	↓ Reduced	↓ Reduced	↓ No effect
STAT1 phosphorylation	↓ Partly reduced				

Key takeaways: *The protective effect of FD exposure on bronchial epithelium may be mediated by inference with the IFNGR-JAK-STAT1 signalling pathway and resulting inhibition of IL-33 expression*

*A reduction of IL-33 protein and *IL33* mRNA induction by TNF-α/IFN-γ was also reported for epithelial cells pre-treated with FD extract
EGFR, epidermal growth factor receptor; FD, farm dust; IFN, interferon; IFNGR, interferon-gamma receptor; IL, interleukin; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; STAT1, signal transducer and activator of transcription 1; TNF, tumour necrosis factor
Schrumpf JA, Tham M, Ninaber DK, von Mutius E, Smits HH, Hiemstra PS. Abstract presented at ERS 2022

Effect of bronchial thermoplasty on alarmin (S100A7/A8/A9) expression in severe asthma

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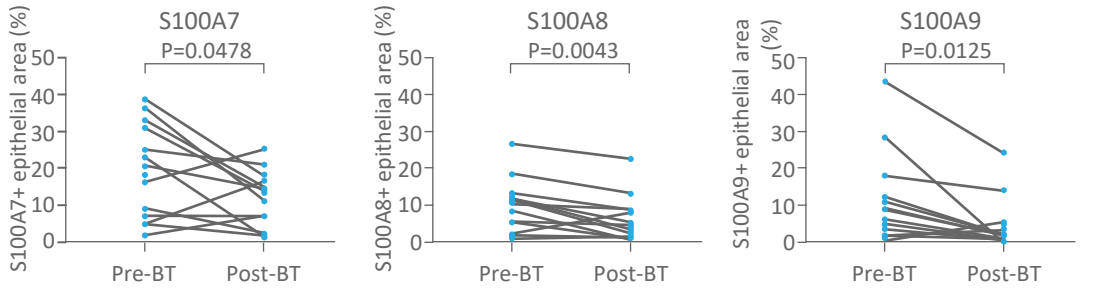
- To explore the molecular mechanisms by which BT results in improved asthma control and reduced exacerbations, gene and protein expression were analysed to identify novel markers associated with severe asthma and the response to BT*

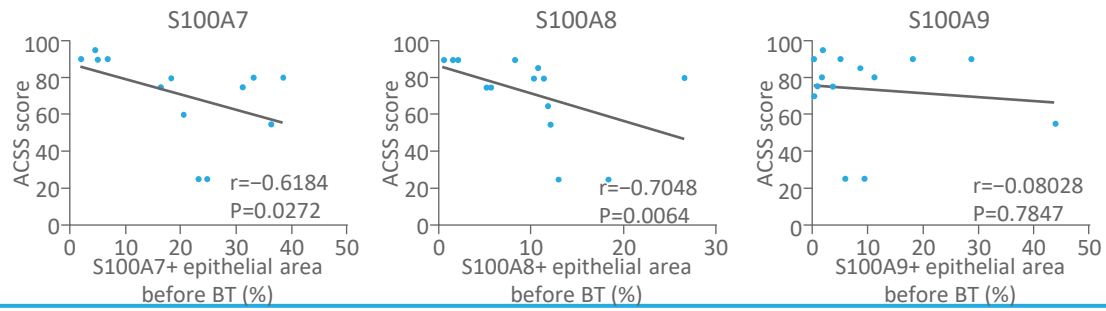
Gagnon PA
Université Laval, Quebec, Canada

- Gene and protein expression of S100A7/A8/A9 were elevated in patients with severe asthma compared with healthy controls and were downregulated following BT
- The change in S100A7/A9 protein expression post-BT negatively correlated with pre-BT expression (P<0.001)
- Pre-BT expression of S100A7/A8 negatively correlated with scores of asthma control

Alarmin gene expression in bronchial biopsies taken pre- and 1 year post-BT from patients with severe asthma



Correlation of S100A7/A8/A9 expression in pre-BT bronchial biopsies from patients with severe asthma



Key takeaways: *Alarmins S100A7/A8/A9 are elevated in the epithelium of patients with severe asthma and are associated with poor asthma control; expression of these alarmins is reduced following BT*

*Gene and protein expression were measured in bronchial epithelial cells from healthy controls (n=7) and patients with severe asthma (pre-BT: n=10; 1 year post-BT: n=6) by qPCR and Western blot and from patients with severe asthma by immunohistochemistry of bronchial biopsies (pre-BT and 1 year post-BT: n=20)

ACSS, Asthma Control Symptom Score; BT, bronchial thermoplasty; qPCR, quantitative polymerase chain reaction; SA, severe asthma

Gagnon PA, Assou S, Salem M, Biardel S, De Vos J, Lampron N, Martel S, Boulet LP, Laviolette M, Laprise C, Chakir J. Poster PA3583 presented at ERS 2022

Alarmin expression in the upper and lower airways of asthmatics with allergic rhinitis

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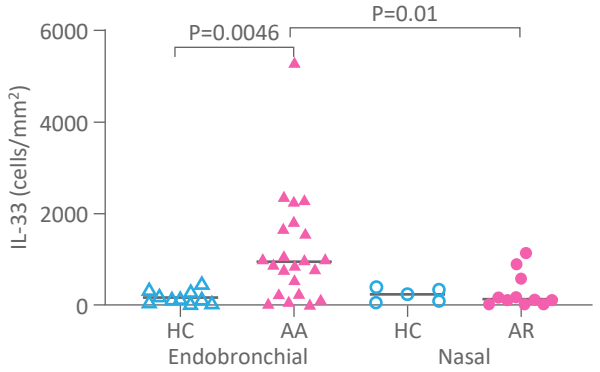
- The epithelial alarmins IL-33 and TSLP are key drivers of the allergic response and lead to downstream production of T2 cytokines and cellular infiltration
- In order to investigate alarmin expression in both the upper and lower airways during allergic inflammation, immunofluorescent staining was used to study endobronchial biopsies from HC (n=11) and patients with mild AA* (n=23), as well as inferior nasal biopsies from HC (n=5) and patients with AR (n=10)

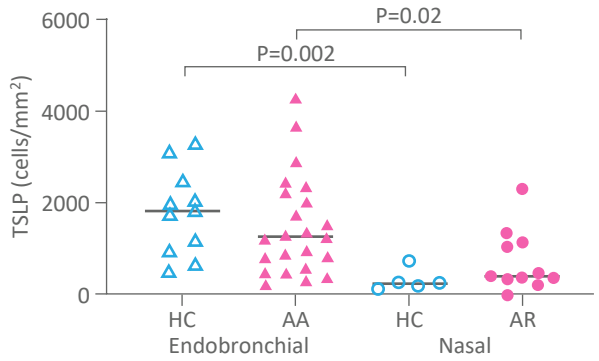
Whetstone CE
McMaster University, Hamilton, Ontario, Canada

- In patients with AA, lower airways contained more IL-33- and TSLP-positive cells than upper airways (P=0.01 and P=0.02, respectively)
- In HC, more TSLP-positive cells were observed in the lower airways compared with upper airways (P=0.002)
- In the lower airways, more IL-33-positive cells were observed for patients with AA, compared with HC (P=0.0046), but there was no difference in TSLP expression, possibly indicating constitutive expression under baseline conditions

IL-33+ cell numbers in bronchial and nasal biopsies from patients with allergic asthma, without and with comorbid rhinitis, respectively, and compared with healthy controls



TSLP+ cell numbers in bronchial and nasal biopsies from patients with allergic asthma, without and with comorbid rhinitis, respectively, and compared with healthy controls



Key takeaways: *In patients with AA, higher levels of alarmin-expressing cells in lower airways compared with upper airways may reflect heightened sensitivity to stimuli in the lungs*

*Mild allergic asthma defined as patients with positive skin prick test and methacholine PC20 <16 mg/mL
AA, patients with allergic asthma; AR, patients with allergic asthma with comorbid allergic rhinitis; HC, healthy controls; IL, interleukin; PC20, provocative concentration causing a 20% drop in forced expiratory volume in 1 second; T2, type 2; TSLP, thymic stromal lymphopoietin

Whetstone CE, Ranjbar M, Alsaji N, Al-Sajee D, Wiltshire L, Wattie J, O'Byrne PM, Cusack R, Sehmi R, Gauvreau GM. Poster PA933 presented at ERS 2022

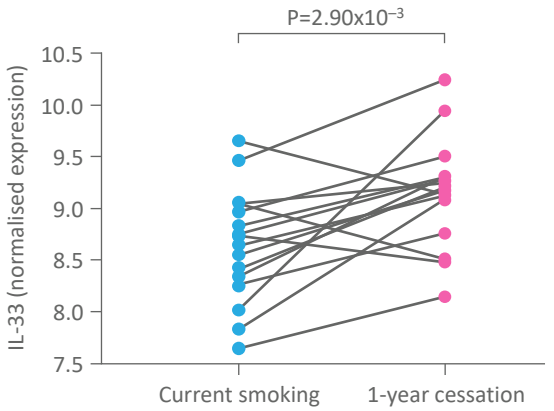
The regulation of IL-33 following smoking cessation

- To examine the effect of smoking on bronchial *IL-33* expression, gene expression and cellular deconvolution was carried out on four human bronchial datasets;* *IL-33* expression was also studied in bronchial biopsies from a 12-month smoking-cessation study (n=16)
- Single-cell RNA sequencing analyses were performed on ALI-cultured airway epithelial cells with and without exposure to whole gaseous smoke (n=7)

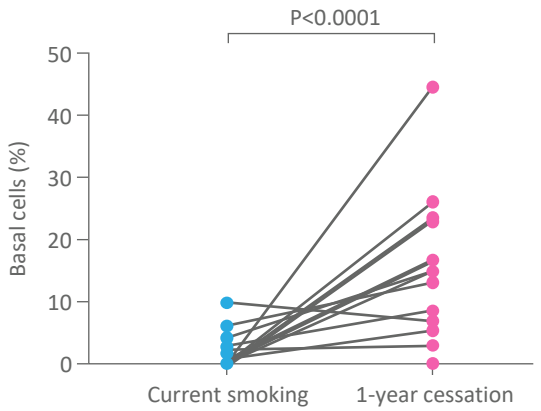
Faiz A
University of Technology, Sydney, Australia

- Across datasets, *IL-33* gene expression was consistently lower in bronchial biopsies of current smokers, when compared with non-smokers
- *IL-33* expression was largely restricted to resting basal cells, and expression decreased during airway epithelial differentiation
- Biopsies from current smokers compared with never- and ex-smokers, and primary cells cultured in the presence of smoke, showed a reduction in the resting basal cell population
- Cessation of smoking was associated in a recovery of *IL-33* expression and basal cell population

***IL-33* expression in bronchial biopsies taken before and after smoking cessation**



Predicted basal cell percentages based on cellular deconvolution of bronchial biopsies taken before and after smoking cessation



Key takeaways: *Smoking reduced numbers of IL-33-expressing resting basal cells within the bronchial epithelium; recovery of the basal cell population and IL-33 expression occurs following cessation of smoking*

*GLUCOLD: current smokers (n=36) and ex-smokers (n=22) with COPD, NORM: healthy never-smokers (n=40) and current smokers (n=37), CRUKPAP: current (n=77), ex- (n=151) and never- (n=8) smokers, and GSE47147: current smokers (n=30) and ex-smokers (n=57) with COPD

ALI, air-liquid interface; COPD, chronic obstructive pulmonary disease; IL, interleukin

Faiz A, Boedijono F, Timens W, Nawijn M, Hansbro P, Mahbub R, Johansen M, Brandsma C, Heijink I, Massip F, De Biase M, Schwarz R, Adcock I, Chung K, Hiemstra P, Goulaouic H, Xing H, Abdulai R, De Rinaldis E, Cunoosamy D, Harel S, Lederer D, Nivens C, Kerstjens H, Hylkema M, Van Den Berge M. Poster PA4262 presented at ERS 2022

Airway remodelling

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Screening and verification of differentially expressed genes for airway remodelling in asthma

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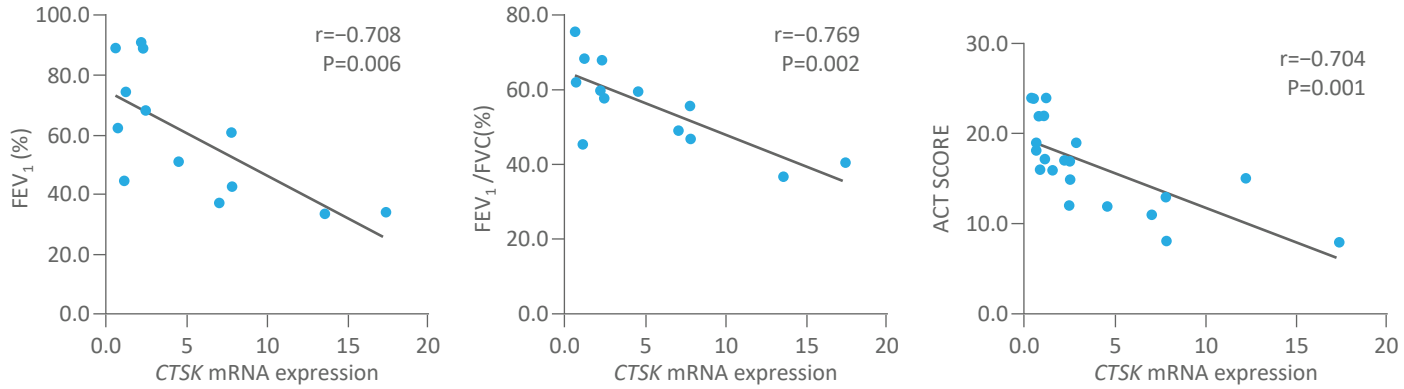
- Differentially expressed genes associated with airway remodelling were identified by RNA sequencing of primary airway epithelial cells in an asthma model of airway remodelling in response to HDM stress
- Genes of interest were measured by qPCR in the sputum of healthy controls and patients with asthma, and correlated with clinical outcomes

Qin L
Xiangya Hospital and Central South University, Shangsha, China

- Six genes were identified as differentially expressed during airway remodelling in response to HDM stress: *ACP5*, *CTSK*, *CTSS*, *CXCL5*, *CXCL12* and *ITGB2*
- *CTSK* gene expression in induced sputum from patients with asthma was associated with disease severity, as shown by negative correlations between *CTSK* mRNA levels and FEV_1, FEV_1/FVC, ACT scores and CT parameters of airway remodelling (outer diameter, cross-sectional area of the airway lumen and average wall thickness)
- Inhibition of *CTSK* was also found to reduce *TGF-β1* expression in airway epithelial cells

Expression of *CTSK* in induced sputum negatively correlated with lung function and asthma control in patients with asthma



Key takeaways: *CTSK may be involved in airway remodelling in asthma, possibly acting through the upregulation of TGF-β1, and sputum CTSK expression may be a useful marker of airway remodelling in patients with asthma*

ACP5, acid phosphatase 5; ACT, Asthma Control Test; BEC, bronchial epithelial cell; CT, computed tomography; CTSK, cathepsin K; CTSS, cathepsin S; CXCL, chemokine (C-X-C motif) ligand; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HDM, house dust mite; ITGB2, integrin subunit β 2; mRNA, messenger RNA; qPCR, quantitative polymerase chain reaction; TGF, tumour growth factor

Qin L, Liu C, Wu M, Yao Y. Poster PA916 presented at ERS 2022