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Objective To investigate this relationship by correlating FeNO and blood Eos to inflammatory mediators in the airway and peripheral blood in severe asthma.

Methods Induced sputum Eos and 11 sputum supernatant plus 9 serum inflammatory proteins were analyzed by electrochemiluminescence or ELISA in a pooled cross-sectional analysis of patients with severe asthma and healthy controls. We recruited patients in whom we had a high degree of confidence in treatment adherence to high-dose inhaled corticosteroids (ICS) and/or systemic corticosteroids. Spearman correlations were computed between FeNO, blood Eos and sputum/serum analytes plus clinical measurements, controlling for a false discovery rate (FDR)<0.05. Significant correlations (*i.e.*: FDR<0.05) were translated in median-fold differences across biomarker categories (FeNO: <25, 25<50, ≥50 ppb; blood Eos: <0.15, 0.15-<0.30, ≥0.30×10⁹/L) with Jonckheere-Terpstra tests used to assess the ordinal trends (*p*<0.05 significant).

Results Correlation of FeNO and sputum/serum analytes in 74 patients with severe asthma showed significant correlations with sputum Eos (*r*=0.51), interleukin (IL)-4 (*r*=0.48), IL-5 (*r*=0.47), IL-33 (*r*=0.35), thymic stromal lymphopoietin (TSLP: *r*=0.41), eotaxin-3 (CCL26: *r*=0.55), thymus associated regulated cytokine (TARC or CCL17: *r*=0.32), and asthma attacks in the previous year (*r*=0.25). Blood Eos correlated with serum IL-5 (*r*=0.41). In effect, FeNO non-suppression was associated with higher sputum Eos (fold-difference in median values, FeNO <25 to ≥50 ppb: 17-fold, *p* for trend =0.001), IL-4 (7.6-fold, *p*=0.0006), IL-5 (8.9-fold, *p*=0.006), IL-33 (1.8-fold, *p*=0.02), TSLP (5-fold, *p*=0.002), eotaxin-3 (10-fold, *p*=0.00003), TARC (3.5-fold, *p*=0.005), and asthma attacks in the past year (3-fold, *p*=0.03). Greater blood Eos (<0.15 to ≥0.3×10⁹/L) was associated with higher serum IL-5 (1.9-fold, *p*=0.04). Results were similar when removing patients on systemic steroids. The highest FeNO and blood Eos categories generally had greater sputum Eos, sputum/serum type-2 cytokine, chemokine and alarmin levels than healthy controls.

Conclusion Raised FeNO despite adherence to high-dose ICS identifies corticosteroid-resistant airway type-2 cytokine (IL-4,

-5), chemokine (eotaxin-3, TARC), alarmin (IL-33, TSLP) and sputum eosinophilia, while blood eosinophils correlate with systemic IL-5. These biomarkers provide complementary information on distinct immunological compartments.

Please refer to page A190 for declarations of interest related to this abstract.

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CORRELATION OF EOTAXIN-3 GENE EXPRESSION AND OTHER IL-13-INDUCED GENES IN PATIENTS WITH ASTHMA

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Background Eotaxin-3 is an eosinophilic chemokine. We investigated how its expression in the bronchial epithelium correlates with other IL-13 signature genes in asthma.

Methods We performed a *post-hoc* analysis of data in patients with asthma and healthy controls (PMID#28933920). Asthma inflammatory phenotype was determined by the induced sputum differential: eosinophilic >3%; neutrophilic >61%. Bronchial epithelial gene expression was measured using RNA microarrays on endobronchial brushes. Eotaxin-3 gene expression was compared between phenotypes by one-way ANOVA corrected for 10 comparisons. Pearson correlations (*p*<0.05 significant) were computed between eotaxin-3 and fourteen other IL-13-induced genes reported in *in vitro* epithelial cell studies.

Results Data from 38 asthmatics and 18 controls were included. Eotaxin-3 gene expression was highest in eosinophilic asthma (figure 1A). The correlation matrix (figure 1B) shows strong associations between eotaxin-3 gene expression and IL-13-induced-genes in patients with asthma. The 3 most correlated genes were Serpin Family B Member 10

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(SERPINE1: $r=0.85$), periostin (POSTN: $r=0.79$) and nitric oxide synthase 2 (NOS2: $r=0.72$). Correlations in controls were not significant (not shown).

Conclusion Eotaxin-3 gene expression is upregulated in the airway epithelium in eosinophilic asthma and highly correlates with IL-13 signature genes – including biomarker genes POSTN and NOS2.

Please refer to page A190 for declarations of interest related to this abstract.

From bench to lung: scientific advances in respiratory research

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ELITE ATHLETES SUSCEPTIBLE TO RESPIRATORY TRACT INFECTION ARE CHARACTERISED BY REDUCED CIRCULATING MEMORY T REGULATORY CELLS, UPPER AIRWAY MICROBIAL DYSBIOSIS AND DYSREGULATION OF SPHINGOLIPID METABOLISM

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Rationale Respiratory tract infection (RTI) is a major issue in athlete health and is the leading cause of training and competition time-loss. The host-defence immunomodulatory factors associated with heightened RTI susceptibility remains unclear.

Objective This prospective study aimed to characterise host immune factors in international athletes exhibiting heightened RTI susceptibility. **Methods/measurements:** Comprehensive clinical and physiological phenotyping was prospectively undertaken in a cohort of 121 elite athletes. Athletes were characterised using objective retrospective electronic medical record analysis as highly susceptible (HS) (≥ 5 confirmed RTI over last 18 months) (N=22) or non-susceptible (NS) to RTI (< 2 confirmed infections over last 18 months) (N=23). Peripheral blood lymphocyte population phenotyping of HS and NS athletes was performed by flow cytometry, with validation of findings by mass cytometry. The immune response to microbial stimuli was analysed by peripheral blood mononuclear cell (PBMC) stimulation assays. Further immuno-metabolic phenotyping was performed through 16S rRNA microbial sequencing of oropharyngeal swabs and global untargeted plasma metabolomic profiling. Findings were compared to data from a non-athletic healthy control group (N=10).

Main Results HS athletes had a persistently reduced memory T regulatory cell compartment compared to NS athletes ($p=0.005$) and healthy controls ($p=0.002$) (see figure 1) with a T helper 2 skewed PBMC immune response to microbial stimuli additionally seen in HS athletes. 16S rRNA microbial sequencing revealed a reduced bacterial biomass of the oropharyngeal microbiome in athletes compared to healthy controls ($p=0.032$), with plasma metabolomic profiling showing significant differences in sphingolipid pathway metabolites in HS athletes compared to NS athletes and healthy controls. Immune phenotypic differences were not related to sporting discipline or evidence of underlying asthma or atopy.

Conclusion Elite athletes have evidence of upper airway microbial dysbiosis, with a reduction in circulating memory T regulatory cells, and metabolic dysregulation of the sphingolipid pathway evident in those HS to RTI. Further prospective longitudinal work is needed to explore this novel

Abstract S94 Figure 1 CD4⁺ FoxP3 populations in elite athlete cohort. The CD3⁺ CD4⁺ T cell population identified using CyTOF was subjected to tSNE analysis using Cytobank. Using heat maps overlaid on tSNE plots, 2 FoxP3⁺ populations were identified and termed as FoxP3 P1 and FoxP3 P2 (a). Heatmaps were further used to examine differences in CD4 and Treg markers, with FoxP3⁺ population 2 seen to be CD45RO^{hi}CD45RA^{dim}CD95^{hi} identifying it as a memory Treg subset (b). FoxP3 P2 populations as a total of total CD3+CD4+ T cells were calculated (c) and compared for total athletes (TA), highly susceptible (HS), non-susceptible (NS) athletes and healthy controls (HC)