effects of prednisolone to mepolizumab, on molecular mechanisms in the airways and blood are poorly understood.

**Aim** Determine the transcriptomic and proteomic effects of prednisolone versus placebo in the airways and blood in patients with SEA treated with mepolizumab.

**Methods** MAPLE was a randomized, double-blind, placebo-controlled crossover trial of prednisolone at stable state in adults with SEA after mepolizumab (Yang F, JACI Pract 2022;10:2925–34.e12). Prednisolone had a minor effect on FEV₁ but not on symptoms. Sputum and blood samples were taken before and after high dose prednisolone and placebo in patients treated with mepolizumab. These underwent O-link expression analysis of 1536 proteins. A paired comparison of normalised protein expression for 1536 proteins in sputum and serum were compared in a linear mixed effects model, with Benjamini-Hochberg correction for multiple testing.

Nasal scrape samples were taken for transcriptomic analysis after prednisolone and placebo in patients treated with mepolizumab. RNA was extracted (Qiagen) and good quality samples sequenced (Illumina Novaseq). We identified differentially expressed genes with paired t-tests with Benjamini-Hochberg correction for multiple testing.

**Results** 21 participants had paired serum, and 14 had paired sputum, before and after both prednisolone and placebo. Prednisolone significantly downregulated 173 and 229 proteins and upregulated 63 and 140 proteins in sputum and serum respectively. Downregulated proteins in sputum included IL-4, IL-5, IL-13, chemokines, and signatures of mast cells, prostaglandin synthesis, and alternatively activated macrophages. Up-regulated proteins included FKBP5, typical of steroid treatment.

6 people had paired nasal epithelial samples comparing prednisolone to placebo. 28 genes were down-regulated by prednisolone included leukocyte chemotaxis, mast cell tryptase and the 15-lipoxygenase pathway.

**Conclusions** Prednisolone in addition to mepolizumab suppresses type-2 pathways unaffected by IL-5 inhibition in the sputum and blood proteome, and nasal transcriptome. These findings support the notion that the type-2 airway epithelium remains active in mepolizumab-treated patients. The relationship of these additional effects to longer term clinical outcome is unknown.

Please refer to page A286 for declarations of interest related to this abstract.

**COMPUTED CARDIOPULMONOGRAPHY: AN INNOVATIVE ASSESSMENT OF LUNG FUNCTION BEFORE AND AFTER STARTING BIOLOGIC THERAPY FOR TH-2 HIGH ASTHMA**

1,2A Alamoudi, 1L Petralia, 1N Smith, 1X Xu, 1C Fullerton, 1G Richmond, 1D Sandhu, 1N Talbot, 1G Ritchie, 1P Favord, 1P Robbins, 1N Petousi. 1University of Oxford, Department of Physiology Anatomy and Genetics, Oxford, UK; 2Prince Sultan Military College of Health Sciences, Department of Respiratory Care, Damman, Saudi Arabia; 3University of Oxford, Department of Chemistry, Oxford, UK; 4University of Oxford, Nuffield Department of Medicine, Oxford, UK

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**Background** Asthma is a chronic inflammatory disease, commonly featuring an eosinophilic allergic-type signature. Patients with asthma experience diurnal variation in symptoms, with notable worsening in lung function around 04:00, coinciding with peak sputum eosinophilia. A better understanding of the rhythmic inflammatory pathways underpinning asthma may identify novel therapeutic targets.

**Experimental Design** Female C57BL/6 mice were exposed intranasally to House Dust Mite (HDM) or Phosphate Buffered Saline (PBS) 3x per week for 5 weeks. Tissues were harvested at 6hr intervals over a 72hr period. Immune cell populations in lung, bronchoalveolar lavage fluid (BALF), spleen, and blood were characterised (flow cytometry) and BALF and serum cytokines quantified (bioplex). qPCR and Immunohistochemistry assessed expression of tight junction components found in the airway epithelium. T-helper 2 cells we further analysed to show varying expression of the immune modulatory transcription factor E4BP4 in BALF, lung tissue, and peripheral blood.

**Results** Immune cell populations in HDM-exposed mice showed time-of-day variations. Eosinophils and TH2 cells peaked at 07:00 in BALF and reached a nadir at 19:00. Similarly, serum IL-5 peaked at 07:00. Blood eosinophils however displayed a consistent daily peak at 13:00 (figure 1), in both HDM and PBS exposed mice. Diurnal variation in the expression of tight junction genes zo-1 and ocln was lost in HDM-exposed mice indicating airways may be leakier, especially at 07:00. T-helper 2 cells, exhibit a reversed pattern of expression of the E4BP4 protein in the lung tissue compared to in the blood and BALF, perhaps driving the cells towards a more proinflammatory phenotype.

**Perspectives** Our murine model of chronic allergic inflammation demonstrates time of day variation in the cellular milieu of the lung. During the rest phase (when asthma symptoms are typically worse) levels of the eosinophil chemottractant IL5, lung eosinophils and barrier permeability are heightened. Ongoing work will address mechanisms facilitating rhythmic eosinophil accumulation and clock gene function in T-helper 2 cells when present in the inflamed lung.

Please refer to page A286 for declarations of interest related to this abstract.

**CIRCADIAN PATTERNS IN IMMUNE CELL TRAFFICKING IN CHRONIC ALLERGIC AIRWAYS DISEASE**

1JC Ain, 2JE Gibbs, 1HJ Durrington. 1The University of Manchester Faculty of Biology, Medicine, and Health, Manchester, UK; 2Centre for Biological Timing, Faculty of Biology Medicine and Health, University of Manchester, Manchester, UK

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**Background** Asthma is a chronic inflammatory disease, commonly featuring an eosinophilic allergic-type signature. Patients with asthma experience diurnal variation in symptoms, with notable worsening in lung function around 04:00, coinciding with peak sputum eosinophilia. A better understanding of the rhythmic inflammatory pathways underpinning asthma may identify novel therapeutic targets.

**Experimental Design** Female C57BL/6 mice were exposed intranasally to House Dust Mite (HDM) or Phosphate Buffered Saline (PBS) 3x per week for 5 weeks. Tissues were harvested at 6hr intervals over a 72hr period. Immune cell populations in lung, bronchoalveolar lavage fluid (BALF), spleen, and blood were characterised (flow cytometry) and BALF and serum cytokines quantified (bioplex). qPCR and Immunohistochemistry assessed expression of tight junction components found in the airway epithelium. T-helper 2 cells we further analysed to show varying expression of the immune modulatory transcription factor E4BP4 in BALF, lung tissue, and peripheral blood.

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**Perspectives** Our murine model of chronic allergic inflammation demonstrates time of day variation in the cellular milieu of the lung. During the rest phase (when asthma symptoms are typically worse) levels of the eosinophil chemottractant IL5, lung eosinophils and barrier permeability are heightened. Ongoing work will address mechanisms facilitating rhythmic eosinophil accumulation and clock gene function in T-helper 2 cells when present in the inflamed lung.

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Results Biologic therapy significantly improved both FEV1% pred and $\sigma$lnCL (p<0.01 and p<0.005, respectively), as did bronchodilation (p<0.001 for both), regardless of the specific biologic used (linear mixed-effects modelling). When considering BEC, FeNO, and ACQ5 as predictors in the models, BEC and ACQ5 significantly influenced FEV1%pred (F-ratio 22.7 and 22.6, respectively, both p<0.001). However, only BEC strongly affected $\sigma$lnCL values (F-ratio 34.1, p<0.001), while ACQ5 had a weaker effect (F-ratio 5.4, p<0.05). FeNO did not show statistical significance.

The change in post-bronchodilator $\sigma$lnCL following biologics followed a bimodal distribution (Akaike information criterion). Patients responding with a fall in $\sigma$lnCL also had a significant increase in FEV1%pred compared to those without $\sigma$lnCL changes (t-test, 13.1% vs. -1.6%, p<0.001, figure 1).

Conclusions CCP categorized patients into two groups: responders and non-responders to biologics in terms of lung function changes. Although the effectiveness of anti-IL5 therapy may not rely on direct lung function changes, a subgroup of patients experienced early and significant lung function improvement. Additionally, our findings revealed a strong association between $\sigma$lnCL and systemic eosinophilic inflammation levels (BEC) in type-2 high asthma, both at baseline and after biologic treatment.

REFERENCE

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