Regarding epithelial dysregulation in obese severe asthmatics with gastro-oesophageal reflux

To the Editor:

We read with interest the recent article by Perotin *et al.* [1], which investigated epithelial dysregulation in obese severe asthma patients with gastro-oesophageal reflux. The researchers found that bronchial epithelial gene expression, sampled by airway brushing, identified an endotype defined by epithelial dysregulation associated with obesity, gastro-oesophageal reflux and use of proton pump inhibitors (OGP cluster). Relative to non-asthmatic healthy controls, pathway signature analysis indicated that the wingless tail (WNT)/β-catenin pathway was the top epithelial pathway dysregulated in the OGP cluster. The cluster was also associated with paucigranulocytic sputa, lower numbers of biopsy lymphocytes, but no thickening of the basement membrane.

The reported findings of the Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes (U-BIOPRED) initiative is an encouraging example of research between leading centres, and we congratulate the investigators for coordinating the demanding sampling of different airway compartments. With human airway epithelial brushing samplings taken, we wondered why differentially expressed genes in the epithelium were compared to genes regulated by proton pump inhibitors (PPIs) and bile acids through *in vitro* studies of alveolar A549 cells. The cancer origin of the A549 cell line might also interact with pathways such as WNT/β-catenin signalling [2]?

Transfer of low levels of micro-organisms has been described in radionuclide studies of normal volunteers at night during sleep [3] and we have previously shown that in gastric juice, with pH >4, that viable micro-organisms, including *Pseudomonas aeruginosa*, can be cultured [4, 5]. PPI use might be expected to lower gastric juice pH and we therefore wondered if the investigators had any data regarding the sputum microbiome detected in the OGP endotype cluster? In subjects with high body mass index we were also interested whether there were any subjects who had obstructive sleep apnoea (OSA), which can be under-diagnosed? We speculate that OSA and a potentially dysregulated aerodigestive microbiome could also be part of the complex interactome relevant in the OGP endotype very usefully highlighted by J.M. Perotin and the U-BIOPRED investigators.

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From the authors:

We thank C. Ward and Z.J. Kharaba for their interesting comments on the OGP endotype of severe asthma that we recently described [1].

Regarding their query about the use of the A549 cell line, the impact of proton pump inhibitors (PPI) and bile acids on the airway epithelium transcriptome has not been described previously. Therefore, in order to estimate the potential impact of PPI and bile acids on epithelial dysregulation, we exploited information obtained from a freely accessible database listing the transcriptional responses of different cell lines to more than 27 000 genetic or chemical perturbagens [2]. We chose to analyse the transcriptional responses of A459 lung epithelial cells to PPI and bile acids, as this was the most suitable cell line available in the database. Using these data, we identified high connectivity scores, suggesting a direct impact of bile acids and PPI on immune mechanisms in severe asthma. We agree that caution needs to be taken when interpreting data from the A549 cell line, which was established from a human lung carcinoma, and this is why we stated that our study should be viewed as exploratory. The hypotheses generated from this study are currently being tested in our laboratory, this time using primary bronchial epithelial cells cultured *in vitro*.

C. Ward and Z.J. Kharaba rightly highlight the potential for modification of the airway microbiome as a mechanism involved in epithelial dysregulation associated with severe asthma, gastro-oesophageal reflux disease (GORD) and obesity. GORD is, indeed, associated with changes in oesophageal microbiota [3]. Furthermore, Goleva *et al.* [4] recently described a change in the bronchoalveolar lavage microbiome of severe asthmatics treated with PPI, with a unique pattern characterised by expansion in *Streptococcus* species, including *S. mitis* and *S. pseudopneumoniae* which were found to be able to alter epithelial response to corticosteroids *in vitro*. While the airway microbiome was analysed in a subset of participants in the U-BIOPRED study, unfortunately, not enough participants provided both transcriptomic and microbiome data so we were unable to correlate the microbiome with the effects of GORD, obesity and PPI treatment.

The complexity of severe asthma management includes co-morbidities such as obstructive sleep apnoea syndrome (OSAS), which has been associated with poor asthma control, increased burden of asthma independent of any association with obesity and increased hospitalisation length of stay after exacerbation [5]. The impact of OSAS treatment on asthma outcomes is unclear [6, 7]; the mechanisms might involve nuclear factor-κB-dependent pro-inflammatory cytokine production and hypoxia-inducing factor 1 pathway [8]. OSAS was evaluated in the U-BIOPRED study using the Epworth sleepiness score (ESS). Our analysis shows that the OGP (obesity, gastro-oesophageal reflux and use of proton pump inhibitors) phenotype does not differ from the severe asthma clusters C2 and C3 in terms of total ESS score (9.82±4.13 in OGP *versus* 8.24±4.28 in C2 and 7.7±5.16 in C3) and number of subjects with ESS >10 (mild excessive daytime sleepiness; 4/21 in OGP, 5/23 in C2, 7/22 in C3).

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OGP phenotype is not characterised by obstructive sleep apnoea http://bit.ly/2kv2CKE

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