Reply: U-BIOPRED/BIOAIR proteins: inflammation or infection?

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Reply to D.L. Hahn and W. Webley:

We thank D.L. Hahn and W. Webley for their interesting interpretation of the data in our original article, in which we reported on plasma proteins associated with asthma severity in two independent cohorts, U-BIOPRED and BIOAIR [1].

These two studies were not designed to address the effect of chronic infection on plasma protein levels in asthma patients, but we have performed additional analysis of our findings in relation to previous history of respiratory infections and baseline sputum microbiome data.

In BIOAIR, the recording of relevant variables in the electronic case report form, reflecting pathogenic infections, was not included. Therefore, this study cannot be used in support of this response. In U-BIOPRED, inclusion criteria permitted only participants with a confirmed absence of ongoing upper or lower respiratory tract infection, or such symptoms. However, we were able extract data on the proportion of participants with a past history of physician-diagnosed bronchitis, chronic bronchitis or pneumonia. We combined these into the single definition of previous history of respiratory infections, a group that could be compared to those with no history of bronchitis, chronic bronchitis or pneumonia (figure 1a). In addition, sputum sample data were available from a subset of 115 of participants, in which the microbiome has previously been analysed [2], and where allocation of participants into one of two clusters, a commensal and a commensal-deficient with more pathogenic bacteria, has been demonstrated.

In this reply, we have focused on further examining the top 10 proteins that showed a difference between mild-to-moderate (MMA) and severe asthma (SA) in both U-BIOPRED and BIOAIR in our original publication in question.

Previous history of respiratory infections was more common in SA (65%) compared to MMA (21%) (figure 1a). There was no difference with respect to smoking status (figure 1a). When comparing history of previous infections with no previous history in each asthma severity group separately, complement component 9 (C9) in the non-smoking SA group was the only protein associated with slightly elevated levels in the previous infection group (figure 1b). To control for potential effects of previous infections, association with asthma severity was tested separately in those with and those without any prior respiratory infections. Here, all 10 proteins remained associated with asthma severity when comparing SA (irrespective of smoking status) and MMA in participants with no history of infections (figure 1b). Importantly, all proteins showed elevated levels in SA, reproducing findings in our original article.

In the smaller sputum microbiome subset analysed (115 of 525 study participants), 68% of SA (61% non-smokers, 82% smokers) and 96% of MMA belonged to the commensal sputum microbiome cluster. In this cluster, eight out of 10 proteins were different between SA and MMA (data not shown).

Our results show that patients with severe asthma were also more likely to have suffered past infections compared to those with milder disease. Accordingly, the presence of a pathogenic sputum microbiome was also more common in the severe asthma groups, with the vast majority of mild-to-moderate asthmatics having a commensal sputum microbiome. However, our main finding regarding the 10 plasma proteins in question [1], was that it was not possible to attribute increased levels in severe *versus* mild asthma to either history of pulmonary infections, or the presence of a pathogenic sputum microbiome.

Shareable abstract (@ERSpublications)
Protein profiles in plasma remained associated with asthma severity in the European asthma cohort U-BIOPRED when controlling for previous history of respiratory infections
https://bit.ly/3rdEL0O

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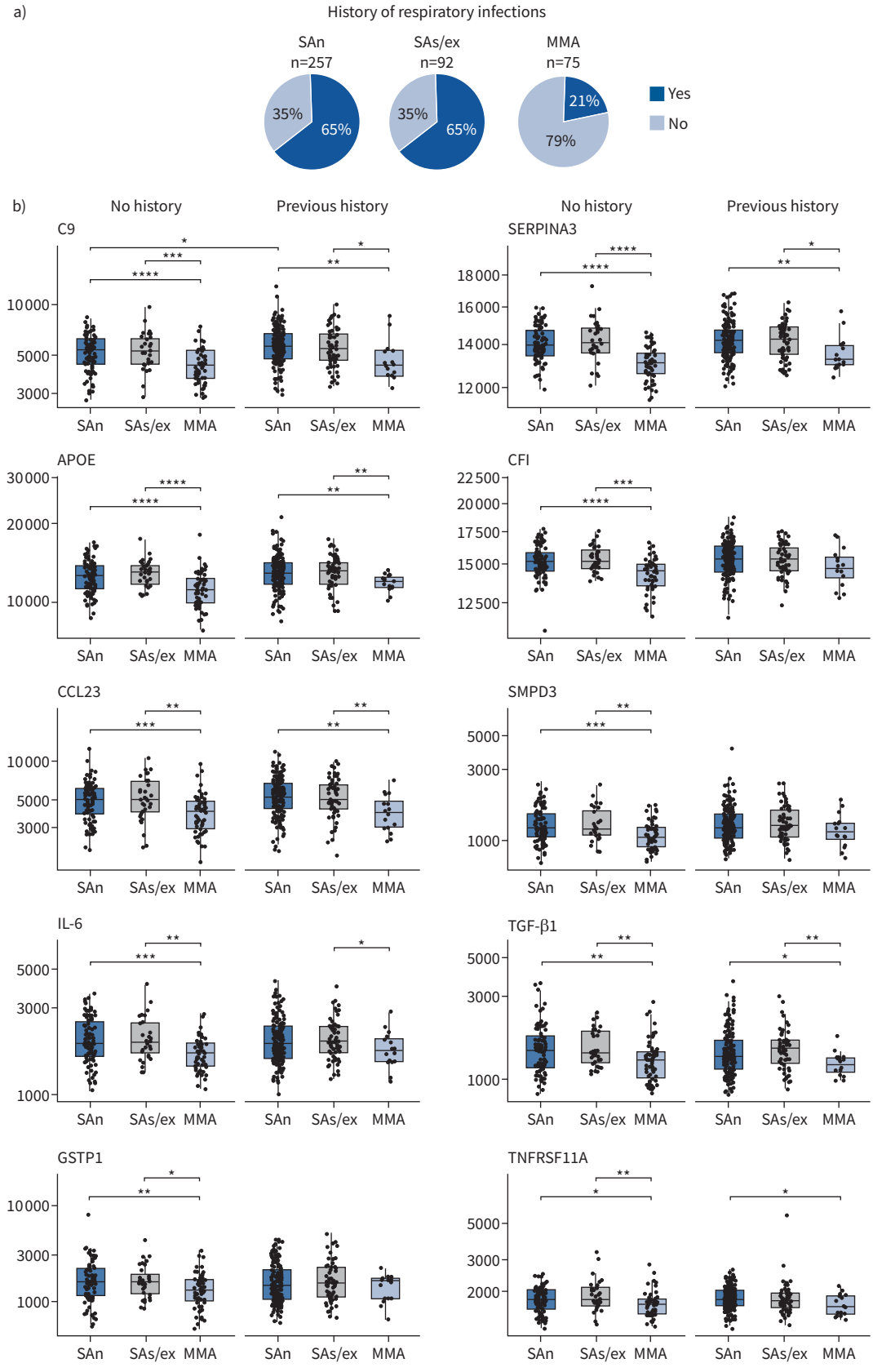

FIGURE 1 a) Previous history of respiratory infections among asthmatics in the U-BIOPRED cohort. Respiratory infections were defined as at least one of bronchitis, chronic bronchitis or pneumonia. b) Association with respiratory infections per asthma group and with asthma severity given no

history or previous history of respiratory infections (bronchitis, chronic bronchitis and/or pneumonia). Relative signal intensity depicted on *y*-axis. *: p<0.05, **: p<0.01, ***: p<0.001, and ****: p<0.0001 from Wilcoxon rank-sum tests. MMA: non-smokers with mild-to-moderate asthma; SAn: non-smokers with severe asthma; SAs/ex: smokers or ex-smokers with severe asthma; APOE: apolipoprotein E; C9: complement component 9; CCL23: macrophage inflammatory protein-3; CFI: complement factor I; GSTP1: glutathione S-transferase P; IL-6: interleukin-6; SERPINA3: alpha-1-antichymotrypsin; SMPD3: sphingomyelin phosphodiesterase 3; TGF-β1: transforming growth factor β1; TNFRSF11A: tumour necrosis factor receptor superfamily member 11a.

Interestingly, the observation of greater levels of the protein C9 among severe asthmatics with a history of infection, is in line with the well-known role of the complement system in our defence against bacteria, where C9 specifically is involved in the membrane attack complex (forming pores into the cell membrane) causing cell lysis [3].

The aim of this *post hoc* investigation in response to the letter of D.L. Hahn and W. Webley was to determine whether pulmonary infections may be the underlying reason why certain plasma proteins showed associations with disease severity in patients with asthma. Our results did not support that hypothesis, acknowledging the limitations of this analysis due to the original U-BIOPRED study design and main aims. For example, neither past history of respiratory infections, nor sputum microbiome data was collected from healthy controls. Several comparisons became subject to power issues, for example only one MMA patient belonged to the pathogenic sputum microbiome cluster. Furthermore, data regarding past or current colonisation with specific species as mentioned by D.L. Hahn and W. Webley (rhinovirus, respiratory syncytial virus, adenovirus, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Chlamydia trachomatis*) were not available. Nevertheless, we are thankful for the opportunity to examine our data from a different perspective and will consider the role of respiratory infections in more detail in future investigations.

Maria Sparreman Mikus [1,2,3], Johan Kolmert [3,4], Lars I. Andersson [1,2,3], Sven-Erik Dahlén [1,2,3,4] and Anna James [3,4]

[1]Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden. [2]The Clinical Laboratory for Lung and Allergy Research, Department of Respiratory Medicine and Allergy, Karolinska University Hospital Huddinge, Stockholm, Sweden. [3]Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden. [4]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

Corresponding author: Maria Sparreman Mikus (maria.mikus@ki.se)

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